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[Authors]. [Abstract Title]. Program No. XXX.XX. 2021 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2021. Online.

Digital Abstract Session

P001. Proliferation

Program #/Poster #: P001.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: R01MH113257

Title: Temporal medio-lateral patterning of neurogenesis in the thalamus: new developmental insights from MacBrainResource, a macaque brain resource

Authors: *T. SPADORY, A. DUQUE, L. D. SELEMON;
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Abstract: Maternal injections of ^{3}H-thymidine during gestation in the non-human primate have been used to determine the time of neurogenesis for various brain areas. Seminal analyses of two caudal thalamic nuclei revealed overlapping periods of neurogenesis in the lateral geniculate nucleus (E36-E43) and the pulvinar (E36-E45) (Rakic, 1977; Ogren and Rakic 1981). Here we examine neurogenesis in the rostral thalamus with focus on two higher order relay nuclei, the mediodorsal (MD) and the anterior (ANT) that have been implicated in the pathology of schizophrenia. The search function of MacBrainResource (macbrainresource.org) identified archived cases (N=12) of ^{3}H-thymidine-labeled slides (Collection 1) with maternal injection dates ranging from embryonic day 25 (E25)-E50 and postnatal sacrifice dates. Slides containing the rostral thalamus were scanned on an Aperio ScanScope at 20X magnification. Digital files (svs) of these scans were transferred to a MicroBrightField system, and Stereo Investigator software was used to map ^{3}H-thymidine labeled neurons within a contour that encompassed the entire rostral thalamus. In Adobe Illustrator, brain mappings (tif files) were superimposed onto closely corresponding sections from the online BrainMaps macaque atlas to facilitate analysis of the nuclear distribution of the label. Our novel analytic approach, i.e. mapping the entire rostral thalamus, uncovered topographic patterning in the temporal progression of neurogenesis in the thalamus. At E30, ^{3}H-thymidine labeled neurons were located in a compact medial band; by E38, labeling was more laterally and diffusely distributed, and at E40-E43 thalamic labeling predominated ventrolaterally. Peak neurogenesis in the MD spans E30-E43 and in the ANT occurs during a similar time frame (E31-E43). Because the time of neurogenesis represents a period of heightened neurodevelopmental susceptibility to prenatal insult, determining when neurons in the MD and ANT are born may provide insight into the etiology of neurodevelopmental pathologies such as schizophrenia. Moreover, discovery of a previously undetected temporal mediolateral patterning of neurogenesis in the thalamus advances our understanding of the sequentially staggered generation of thalamic nuclei.

Disclosures: T. Spadory: None. **A. Duque:** None. **L.D. Selemon:** None.

Digital Abstract Session

P001. Proliferation

Program #/Poster #: P001.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSF IOS-1656103

Title: Effects of astakine and serotonin on adult neurogenesis

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Abstract: Neurogenesis, the generation and integration of neurons into brain circuits, occurs throughout the lives of numerous organisms, ranging from mammals to crustaceans. Unlike mammals, the first-generation precursor cells in crayfish, which are housed in a neurogenic niche, are not self-renewing but rather are replenished by the immune system. Previous studies have shown that hemocytes (blood cells) are attracted to the niche and can differentiate into neurons. To better understand the link between the immune and the nervous systems, the influences of the neurotransmitter serotonin and the cytokine astakine on neurogenesis were tested. 1) Increased levels of 5-HT result in an attraction of hemocytes to the neurogenic niche, and also stimulates the expression of astakine in hemocytes. 2) Astakine then encourages the release of semi-granular cells, a particular type of hemocyte thought to be responsible for renewing the niche precursor cells. 3) Adoptive transfer experiments have shown that labeled hemocytes transferred from donor to recipient crayfish are found in recipient neurogenic niches and generate offspring that express appropriate neurotransmitters. 4) Further, exposure of recipient crayfish to increased serotonin levels increases the incorporation of donor hemocytes into recipient niches. With this knowledge, we designed an experiment to test whether serotonin and astakine serve as links between the immune and nervous systems. In adoptive transfer experiments, hemocyte donors are treated with astakine to provide maximal numbers of labeled hemocytes for transfer to recipient crayfish, and recipients are treated with serotonin. We hypothesize that these treatments will result in higher hemocyte counts in donor crayfish and more cells in the neurogenic niche compared to controls.

Disclosures: S.C. Baldwin: None. **B.S. Beltz:** None. **J.L. Benton:** None.

Digital Abstract Session

P001. Proliferation

Program #/Poster #: P001.03

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant AA025380
University of Houston Department of Psychology

Title: Developmental exercise effects on adulthood brain and behavior

Authors: *K. H. GEHM, E. C. PEREZ, L. LEASURE;
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Abstract: Sedentary behavior is an increasing concern in Western nations, particularly for children and adolescents. Aerobic exercise has many positive effects on brain plasticity, and research on early life positive stimulation (environmental enrichment) demonstrates lifelong brain benefits. But whether early life exercise benefits carry over into adulthood remains largely unexplored. Exercise conditions the stress circuitry without harmful psychological stress. The hippocampus, part of the stress circuitry, is a site of neurogenesis, a form of structural plasticity that is increased with exercise. In the hippocampal dentate gyrus (DG), there is a pool of quiescent (BLBP+) stem cells capable of dividing in response to a stimulus such as stress or exercise. This project assessed long-term effects of adolescent exercise: 1) whether it buffers the behavioral and cellular effects of chronic stress in adulthood, 2) whether a history of exercise increases neurogenesis after reintroduction of exercise in adulthood. Beginning at 5 weeks of age, male and female Long Evans rats voluntarily ran for 2hr daily, 5d/week for 6 weeks. Females were matched to male running distance. All rats then rested for 6 weeks, and then half underwent 10 consecutive days of chronic restraint stress (2hr/day). Rats were then tested in the open field and elevated plus maze, and then sacrificed 24hr post-stress for quantification of proliferating quiescent stem cells (Ki67+/BLBP+). Another group of rats exercised for 3 weeks (5d/week) after the rest period and were sacrificed immediately after the last exercise session for quantification of newly generated neurons (DCX+ cells). Chronic restraint stress increased anxiety-like and depressive-like behavior in males only. Males with a history of exercise escaped more quickly (although not significantly) from the restraint tube. Interestingly, in both sexes, brain size was increased by a history of exercise. Cellular quantification of BLBP+/Ki67+ cells in the DG demonstrated that stressed males had fewer proliferating stem cells compared to non-stressed males, regardless of exercise history. Again, females did not show any change. Quantification of DCX+ cells after the reintroduction of exercise in adulthood is ongoing. We conclude that adolescent exercise may impact stress-related behaviors, but not cellular changes in adulthood in response to chronic stress. Increased escape behavior suggests that coping strategies may be altered by exercise history in males. Additionally, given brain size increase, research on other structural elements of the brain that may be enhanced by a history of exercise is needed.

Disclosures: K.H. Gehm: None. **E.C. Perez:** None. **L. Leasure:** None.

Digital Abstract Session

P001. Proliferation

Program #/Poster #: P001.04

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH/NIDA Grant P50DA044123

Title: D-cysteine is an endogenous regulator of neural progenitor cell homeostasis in the mammalian brain

Authors: *E. R. SEMENZA, M. M. HARRAZ, M. M. GADALLA, S. H. SNYDER, R. ROYCHAUDHURI;
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Abstract: The D-stereoisomers of amino acids are increasingly recognized as important signaling molecules in the mammalian central nervous system. However, the D-stereoisomer of the amino acid with the fastest *in vitro* spontaneous racemization rate, cysteine, has not been examined. Using high-performance liquid chromatography and a stereoisomer-specific luciferase assay, we establish the presence of endogenous D-cysteine in the mammalian brain. We identify serine racemase, which generates the NMDA receptor co-agonist D-serine, as a candidate biosynthetic enzyme for D-cysteine. Levels of D-cysteine are enriched over twentyfold in the embryonic mouse brain compared to the adult. Treatment with 1 mM D-cysteine reduces the proliferation of cultured mouse embryonic neural progenitor cells (NPCs) by approximately 50% as assessed by Ki-67 expression and 5-ethynyl-2'-deoxyuridine incorporation. This effect is not shared with D-serine or L-cysteine. The antiproliferative effect of D-cysteine is associated with a dose-dependent inhibition of Akt and corresponding activation of the transcription factor FoxO3a, which is negatively regulated by Akt. Accordingly, D-cysteine treatment enhances the expression of FoxO3a target genes including the cyclin-dependent kinase inhibitors $p21^{Cip1}$ and $p27^{Kip1}$, which mediate cell cycle exit, and the pluripotency factor Sox2, which is essential for the maintenance of NPC identity. Collectively, these phenotypes resemble the quiescent state adopted during the late embryonic stage by the subset of NPCs that persist throughout life as adult neural stem cells and suggest that D-cysteine may function to prevent premature exhaustion of the NPC pool. Finally, we perform an unbiased screen for D-cysteine-binding proteins in NPCs by immunoprecipitation with a D-cysteine-specific antibody followed by mass spectrometry. This approach identifies myristoylated alanine-rich C-kinase substrate (MARCKS) and its relative MARCKSL1 as putative D-cysteine-binding proteins. Together, these results establish the existence of mammalian D-cysteine and implicate it as an endogenous regulator of neural progenitor homeostasis in the developing brain.

Disclosures: E.R. Semenza: None. **M.M. Harraz:** None. **M.M. Gadalla:** None. **S.H. Snyder:** None. **R. Roychaudhuri:** None.

Digital Abstract Session

P001. Proliferation

Program #/Poster #: P001.05

Topic: A.01. Neurogenesis and Gliogenesis

Support: R35 GM126994-01
R35 GM118137
Harvard Medical School Fix Fund Postdoctoral Fellowship

NINDS P30 Core Center grant NS072030

Title: The transcription factor CLAMP is required for neurogenesis inDrosophila melanogaster.

Authors: *M. A. TSIARLI[1,2], A. M. CONARD[1], L. XU[1], E. NGUYEN[1], E. N. LARSCHAN[1]; [1]Dept. of Mol. Biology, Cell. Biol. and Biochem., Brown Univ., Providence, RI; [2]Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Neural stem cell (NSC) differentiation is controlled by cell-intrinsic and external signals from the stem cell niche including niche surface glia (SG). However, the mechanisms by which transcription factors drive NSC differentiation within the niche remain largely unknown. Here, we show that the transcription factor, Chromatin-linked adaptor for MSL proteins (CLAMP) is required for NSC differentiation. CLAMP promotes transcription of genes involved in stemness, proliferation, and glial development and represses transcription of genes involved in neurogenesis and niche survival. Consistent with transcriptional changes, CLAMP promotes NSC proliferation and SG production. Furthermore, glial-specific knock-down of *clamp* causes similar phenotypes to *clamp* null mutants. CLAMP motifs are present at many target genes including the glial-determining gene, *glial cells missing*, and *Notch*, a key regulator of neurogenesis. Collectively, our results suggest that CLAMP regulates a transcriptional program which drives NSC proliferation and differentiation *via* cell-intrinsic and niche-dependent mechanisms that involve niche glia.

Disclosures: M.A. Tsiarli: None. **A.M. Conard:** None. **L. Xu:** None. **E. Nguyen:** None. **E.N. Larschan:** None.

Digital Abstract Session

P001. Proliferation

Program #/Poster #: P001.06

Topic: A.01. Neurogenesis and Gliogenesis

Support: PHS Grant MH104800
PHS Grant MH108994
Rutgers Brain Health Institute

Title: Prenatal Maternal T Cell Activation with Staphylococcal Enterotoxins Induces Postnatal Behavioral and Microglial Alternations in Mouse Offspring

Authors: *M. NISSENBAUM[1], R. GLASS[1], N. FOX[1], S. STEIGMAN[1], D. PRADO DE MAIO[2], L. COVEY[2], J. E. PINTAR[3], H. ZHANG[4], A. KUSNECOV[1]; [1]Psychology, [2]Cell Biol. and Neurosci., Rutgers Univ., Piscataway, NJ; [3]Dept Neurosci & Cell Biol, Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ; [4]Neurosci. and Cell Biol., Robert Wood Johnson Med. School, Rutgers Univ., Piscataway, NJ

Abstract: Maternal immune activation (MIA) during pregnancy has been shown to perturb normal neurobehavioral development. Recently, we found that maternal responses to Staphylococcal enterotoxins A (SEA) and B (SEB), induce deviations in normal offspring behavior. These toxins induce pronounced T cell proliferative and cytokine responses. To determine if genetic background influences the postnatal consequences of T cell activation, we used two strains of pregnant female mice (C57BL/6 and BALB/c). Pregnant mice (N=4-6/treatment) from each strain received saline, SEA or SEB (200 μg/Kg) on embryonic day 12.5 (E12.5). By 2hr, enterotoxin treatment induced increased maternal IL-2, IL-4, IL-6 and IFNγ levels, but no changes in IL-17A. Moreover, in additional studies in C57BL/6 pregnant females challenged with SEA or Saline (N=4/treatment) at E12.5, SEA-selective T cell expansion and cellular expression of IL-2 and IFNγ were increased by 48hr, suggesting that T cell responses to SEs are active over several days of embryonic development. Further experiments involving postnatal behavioral assessments of male and female juvenile offspring from SEA/SEB challenged mothers revealed strain-dependent differences in social behavior, in that BALB mice showed an increase, while C57BL/6 mice showed a decrease in social investigation. Both strains showed delayed spatial learning (using the Morris water maze). SEA treatment of pregnant C57BL/6 mice on E12.5 impaired acquisition of the water-based radial arm maze in male and female offspring. To determine if these behavioral effects were associated with changes in postnatal microglial density and morphology, pregnant C57 wildtype mothers mated with cx3cr1$^{gfp/gfp}$ males were given SEA or Saline on E10.5, E12.5 or E14.5. Quantitation of microglia across postnatal days P7-P30 were initiated in the hippocampus and prefrontal cortex. For P30 offspring of E10.5 injected mothers, SEA offspring (N=9), when compared to Saline offspring (N=9), had more microglia ($p<0.05$) in the CA1, CA3 and dentate gyrus (DG) of the hippocampus, and the prelimbic and infralimbic areas of the prefrontal cortex. At P15, SEA offspring (N=7) were significantly elevated only in the DG, compared to saline offspring (N=6). Offspring from E12.5 SEA-injected mothers did not show significant changes in microglia numbers. These data suggest that the gestational timing of SEA challenge can cause a postnatal deviation in the population dynamics of microglia in a brain region-specific manner. This might affect synaptic development and other characteristics of neuronal function. Analyses are ongoing to assess microglial morphological parameters.

Disclosures: M. Nissenbaum: None. **R. Glass:** None. **N. Fox:** None. **S. Steigman:** None. **D. Prado De Maio:** None. **L. Covey:** None. **J.E. Pintar:** None. **H. Zhang:** None. **A. Kusnecov:** None.

Digital Abstract Session

P001. Proliferation

Program #/Poster #: P001.07

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01DA041529

Title: Effects of early life opioid exposure on brain development and its later life consequences.

Authors: *A. HURST, H. HARDER, L. HANUS, C. SEARLES, A. Z. MURPHY;
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Abstract: Individuals exposed to opioid drugs *in utero* tend to have deficits in long-term learning and memory. Cognitive disorders have been previously shown to have cellular manifestations in the dentate gyrus of the hippocampus. Gestational morphine exposure may cause alterations in dentate gyrus development resulting in long term cognitive disorders. With the recent increase in infants born with an opioid use disorder, information on how gestational opioid exposure affects brain development is essential. To model the effects of gestational morphine on hippocampal development, we used an early life morphine exposure paradigm. Experimental male and female rat pups on the first day of life (P0) were given 2 subcutaneous injections of morphine (2 mg/kg) spaced 6 hours apart. Since the rat dentate gyrus forms postnatally, morphine injections on P0 should expose the pups to morphine at the onset of dentate gyrus formation. Male and female rats were sacrificed at P7, P14, or adulthood (P 60-100) and the dentate gyrus was assessed immunohistochemically for neuroblast proliferation and microglial colonization using antibodies targeting PCNA, doublecortin, and Ibal. Differences were detected in the number of proliferating cells and microglia numbers in morphine exposed rats, suggesting a cellular developmental delay. To determine the impact of early life morphine on microglia activation, adult rats received an injection of lipopolysaccharide (LPS) (250 ug/kg; i.p.). Brains were collected 6 hours post-LPS and histologically analyzed for number and morphology of proliferating cells, microglia, and presence of young neurons in the dentate gyrus. Animals exposed to morphine neonatally show differentially reactive microglia, alterations in the number of proliferating cells, and differences in young neuron maturation when compared with controls. Information from this study can be used to infer the consequences of *in utero* morphine exposure in humans.

Disclosures: A. Hurst: None. **H. Harder:** None. **L. Hanus:** None. **C. Searles:** None. **A.Z. Murphy:** None.

Digital Abstract Session

P001. Proliferation

Program #/Poster #: P001.08

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSERC Grant
BrainsCAN Accelerator Grant

Title: Exercise and metformin to enhance neurogenesis and memory in dietary obesity

Authors: *O. GHOSH-SWABY[1], A. C. REICHELT[3], T. J. BUSSEY[4], L. M. SAKSIDA[2];
[1]Schulich Sch. of Med. and Dent., [2]Western Univ., London, ON, Canada; [3]Adelaide Med. Sch., The Univ. of Adelaide, Adelaide, Australia; [4]Robarts Res. Inst., London, ON, Canada

Abstract: Excessive consumption of high fat and high sugar (HFHS) foods can cause weight gain and obesity, reduce adult hippocampal neurogenesis, and impair learning and memory. We examined whether two interventions known to boost hippocampal neurogenesis, aerobic exercise, or the diabetes drug metformin, can reverse HFHS diet-induced cognitive impairment in male and female mice. To study the impact of exercise, mice were fed either an HFHS diet (n=32) or a control diet (n=32) for 28 days and randomly assigned to either running wheel access (n=16/diet group) for 3 hours, 5 days a week, or no access (n=16/diet group). To study the effects of metformin, mice were fed either an HFHS diet (n=40) or a control diet (n=40) and administered metformin (200mg/kg, i.p.; n=20/diet group) or saline (n=20/diet group) for 28 days. Memory was then tested on a spontaneous location recognition (SLR) task, shown to be sensitive both to the consumption of a high sugar diet and changes in hippocampal neurogenesis. During a sample phase, mice explored an arena containing identical landmarks in 3 locations arranged in a triangular formation. After 3 hours, memory was tested by assessing the extent that mice can discriminate and remember the locations presented during sample. Cognitive load was varied across 3 conditions under which the similarity of the to-be-remembered locations was manipulated parametrically: dissimilar (ds-SLR), similar (s-SLR), or extra similar (xs-SLR). Following SLR, brain sections were immunohistologically examined using doublecortin as a marker of neuroproliferation (DCX+ cells). A two-way ANOVA was used to analyze each measure of interest by sex. HFHS diet led to impairments in memory under s-SLR & xs-SLR conditions. In contrast, control-fed mice spent more time exploring the novel location during ds-SLR and s-SLR conditions. Mice that exercised exhibited enhanced memory and novelty exploration during xs-SLR. Both exercise and metformin reversed cognitive decline in HFHS-fed mice tested on ds-SLR and s-SLR. Interestingly, both exercise and metformin enhanced performance in xs-SLR in female mice fed an HFHS diet. However, the relationship between exercise- and metformin-induced increases in neurogenesis, and cognitive improvement, was not straightforward. Physiologically, exercise and metformin reduced HFHS diet-induced weight gain and adiposity. Our results indicate that exercise and metformin can reverse diet-induced cognitive impairment in a hippocampal-dependent task, but the neural mechanism by which metformin improved memory may not be associated directly with increased neuroproliferation.

Disclosures: O. Ghosh-Swaby: None. **A.C. Reichelt:** None. **T.J. Bussey:** None. **L.M. Saksida:** None.

Digital Abstract Session

P002. Neuronal Cell Type Development

Program #/Poster #: P002.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: K99NS111731
U01MH114825

Title: Understanding cell types in the developing human brain

Authors: *A. BHADURI, C. SANDOVAL ESPINOSA, M. OTERO, U. EZE, A. KRIEGSTEIN;
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Abstract: The human cortex is organized into distinct functional areas with unique physical structure, connectivity, and function relevant to human cognition and perception. How these cortical areas emerge, and when they are specified, have long been of interest as the timing and mechanism of arealization has important implications in the etiology of neurodevelopmental disease, modeling of cortical structures, and comparison of developmental cell types across species. To characterize the emergence of human cortical arealization, we performed single-cell sequencing on 14 individuals across 6 emerging cortical regions. These data validated previous observations that early specification exists in establishing frontal and occipital identities, and additionally highlight the role for presence or absence of occipital gene signatures in regulating radial glia and early neuronal spatial identities. We also observed a large number of differentially expressed genes within radial glia, noting that the area-specific gene signatures for each cortical region are substantially remodeled across differentiation and maturation events. Comparisons of this dataset to adult datasets across cortical regions highlighted some similarities, though substantial changes appear to exist between area-specific gene signatures during developmental periods and adult time points. To validate the patterns we observed in our profiling data, we used an adaptation of sequential fluorescent in situ hybridization (FISH) methods in conjunction with automated probing and high content imaging to produce a snapshot spatial atlas across cortical regions. This approach validated the strong frontal to occipital exclusion patterns in our data and identified new clusters based upon the FISH signal. This dataset provides a valuable resource for understanding the identity and development of distinct human cortical regions.

Disclosures: A. Bhaduri: None. **A. Kriegstein:** None. **C. Sandoval Espinosa:** None. **U. Eze:** None. **M. Otero:** None.

Digital Abstract Session

P002. Neuronal Cell Type Development

Program #/Poster #: P002.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant EY017916
Leon Levy Foundation

Title: Terminal selector genes link neuronal fate with wiring specificity in the Drosophila visual system

Authors: *M. OZEL[1], C. SKOK GIBBS[2], I. HOLGUERA[1], R. BONNEAU[2], C. DESPLAN[1];
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Abstract: Cascades of transcription factors (TF) in neural stem cells are responsible for generating the enormous diversity of cells types in nervous systems, from flies to humans.

However, the gene-regulatory mechanisms that establish and maintain these cell fates in postmitotic neurons and instruct their specific morphology, connectivity and physiology remain unclear. The *Drosophila* optic lobes provide an excellent model system to address these questions with around 200 morphologically distinct neuronal types whose connectome has been almost completely characterized. In order to understand the cell-type specific transcriptional programs employed at different stages of neuronal differentiation, we have generated a very large single-cell RNA sequencing atlas spanning all stages of optic lobe development (Ozel, Simon et al. 2020, *Nature*). We found that unique combinations of 113 TFs, which are strongly enriched in homeobox proteins, are sufficient to define each of the 175 optic lobe neuronal types in our dataset (median: 8 TFs per cell type) throughout development as well as in adults. We hypothesized that these TFs represent terminal selectors that are activated in each neuron immediately after their birth and function as top-level regulators of their cell-type specific gene expression throughout their life. Accordingly, we show that modification of these TF 'codes' with knock-down and ectopic expression experiments in postmitotic neurons is sufficient to induce complete *in vivo* transdifferentiation between neuronal types. We identified a bistable loop formed by the mutually repressing TFs Mef2 and Aop in closely related transmedullary neurons. This ensures that transformations between them are all or none, with no intermediate phenotypes. We also performed computational network inference analysis using the *Inferelator* pipeline to understand the downstream effectors of these terminal selectors. This revealed that terminal selectors interact with other TFs that are transiently activated in response to extrinsic signals at specific time points to activate the cell-type specific cell-surface molecules and other effectors that instruct distinct morphological features and the synaptic partners of each neuron. Our results provide a unified framework of how specific fates are maintained in postmitotic neurons, and reveal the regulatory mechanisms that ensure the robustness of these cell fate choices. They open up new avenues to understand synaptic specificity through gene regulatory networks.

Disclosures: **M. Ozel:** None. **C. Skok Gibbs:** None. **R. Bonneau:** None. **C. Desplan:** None. **I. Holguera:** None.

Digital Abstract Session

P002. Neuronal Cell Type Development

Program #/Poster #: P002.03

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS114545-01
Sigrid Juselius Foundation, postdoctoral fellowship
Leon Levy Foundation, postdoctoral fellowship

Title: Cell type specific characterization of the epigenomic landscape during CNS neuronal development and maturation

Authors: *K. MÄTLIK[1], E.-E. GOVEK[1], M. R. PAUL[1], E. KORB[2], C. D. ALLIS[1], M. E. HATTEN[1];
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Abstract: Developing neurons undergo a complex series of dynamic, morphological changes to become highly specialized, polarized cells that relay information within brain circuitry. These transitions are accompanied by changes in chromatin conformation and gene expression. However, the epigenetic mechanisms that control neuronal differentiation and maturation are largely unknown. Using mouse cerebellar granule cells (GCs) as a model, we studied changes in epigenetic modifications during key stages of neurodevelopment: neuronal progenitor proliferation (postnatal day 7, P7), postmitotic neuron migration (P12) and synaptogenesis (P21). Importantly, observed epigenetic changes were correlated with changes in gene expression to assess their potential impact on the transcriptome. For each stage of development, we investigated specific histone modifications and bivalency using a combination of chromatin immunoprecipitation with sequencing (ChIPseq) assays for H3K4me3, H3K9me3, H3K27me3, H3K36me3 and H3K27ac, and imaging of a fluorescent H3K4me3 and H3K27me3 bivalency probe in GCs in ex vivo cerebellar slices. Chromatin accessibility was further assessed using an Assay for Transposase-Accessible Chromatin using sequencing (ATACseq). Accompanying changes in gene expression were evaluated using RNA sequencing. We found that specific changes in the distribution of histone modifications and their combinations are associated with gene expression changes that accompany cell cycle exit and neuronal maturation. In addition, we identified developmentally regulated histone bivalency at genes involved in GC proliferation and synaptogenesis. Visualization of bivalent chromatin domains using the fluorescent bivalency probe in GCs in *ex vivo* cerebellar slices revealed developmental stage specific differences between progenitors and differentiated GCs. Furthermore, by studying the distribution of histone modifications associated with cell identity, as well as enrichment of transcription factor motifs at developmentally regulated enhancers, we identified novel transcription factors predicted to be required at different stages of GC development. In conclusion, we present a comprehensive characterization of the epigenomic landscape and identification of novel mechanisms that regulate neuronal development in a specific, developing CNS neuron type *in vivo*.

Disclosures: K. Mätlik: None. **E. Govek:** None. **M.R. Paul:** None. **E. Korb:** None. **C.D. Allis:** None. **M.E. Hatten:** None.

Digital Abstract Session

P002. Neuronal Cell Type Development

Program #/Poster #: P002.04

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R24-MH114815
NIH Grant R24-MH114788

Title: ¬Single-cell transcriptomic atlas of the developing human hypothalamus reveals gene regulatory networks driving neuronal diversity

Authors: *B. R. HERB[1], H. GLOVER[2], A. BHADURI[3], A. CASELLA[1], T. L. BALE[1], A. R. KRIEGSTEIN[4], C. DOEGE[2], S. A. AMENT[5];
[1]Univ. of Maryland Baltimore, Baltimore, MD; [2]Columbia, New York, NY; [4]Eli and Edythe Broad Ctr. for Regeneration Med. and Stem Cell Res., [3]Univ. of California San Francisco, San Francisco, CA; [5]Inst. for Genome Sci. and Dept. of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The hypothalamus is critically important for regulating many autonomic, metabolic, and behavioral traits, yet a comprehensive understanding of neuronal subtypes and their development in the human brain is lacking. Here, we characterized the prenatal human hypothalamus by sequencing the transcriptomes of 56,278 single-cells from 12 individuals, spanning gestational weeks 4 through 25. Clustering these cells revealed a temporal trajectory from proliferative stem cell populations in first trimester samples to maturing excitatory and inhibitory neurons and glia in the second trimester. Sub-clustering of 11,349 post-mitotic neurons revealed 42 neuronal subtypes. Gene regulatory network modeling predicted transcription factors (TFs) that may be responsible for neuronal development and lineage differentiation within distinct hypothalamic nuclei. A merged analysis with paired samples from the cortex and ganglionic eminences (GE) revealed two distinct neurogenesis pathways emanating from a common progenitor pool, one unique to cortex and another shared between GE and hypothalamus. This study greatly expands the number of genes that potentially play a role in hypothalamus development, which will allow researchers to consider additional gene candidates in neurodevelopmental or neuroendocrine disorders. Indeed, we found significant association between GWAS candidate genes for circadian regulation (morningness) and schizophrenia, both previously linked to hypothalamus function, to specific neuronal subtypes expressing these target genes during development. Together, these results provide the first comprehensive transcriptomic view of human hypothalamus development at cellular resolution.

Disclosures: B.R. Herb: None. **H. Glover:** None. **A. Bhaduri:** None. **A. Casella:** None. **T.L. Bale:** None. **A.R. Kriegstein:** None. **C. Doege:** None. **S.A. Ament:** None.

Digital Abstract Session

P002. Neuronal Cell Type Development

Program #/Poster #: P002.05

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant UL1TR001866
NIH Grant 1R21NS093540-01
DoD USAMRAA Grant W81XWH1510189
DoD USAMRAA Grant W81XWH1910110
A-T Children's Project Grant HATTEN2018

Starr Tri-I Stem Cell Grant 2016-001

Title: Novel genetic features of human and mouse Purkinje cell differentiation defined by comparative transcriptomics.

Authors: *D. E. BUCHHOLZ[1], T. S. CARROLL[1], A. KOCABAS[1], X. ZHU[1], H. BEHESTI[1], P. L. FAUST[2], L. STALBOW[1], Y. FANG[1], M. E. HATTEN[1];
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Abstract: Comparative transcriptomics between differentiating human pluripotent stem cells (hPSC) and developing mouse neurons offers a powerful approach to compare genetic and epigenetic pathways in human and mouse neurons. To analyze human Purkinje cell (PC) differentiation, we optimized a protocol to generate hPSC-PCs that formed synapses when cultured with mouse cerebellar glia and granule cells and fired large calcium currents, measured with the genetically encoded calcium indicator jRGECO1a. To directly compare global gene expression of hPSC-PCs with developing mouse PCs, we used translating ribosomal affinity purification (TRAP). As a first step, we used *Tg(Pcp2-L10a-Egfp)* TRAP mice to profile actively transcribed genes in developing postnatal mouse PCs, and used metagene projection to identify the most salient patterns of PC gene expression over time. We then created a transgenic *Pcp2-L10a-Egfp* TRAP hESC line to profile gene expression in differentiating hPSC-PCs, finding that the key gene expression pathways of differentiated hPSC-PCs most closely matched those of late juvenile, mouse PCs (P21). Comparative bioinformatics identified classical PC gene signatures as well as novel mitochondrial and autophagy gene pathways during the differentiation of both mouse and human PCs. In addition, we identified genes expressed in hPSC-PCs but not mouse PCs and confirmed protein expression of a novel human PC gene, CD40LG, expressed in both hPSC-PCs and native human cerebellar tissue. This study therefore provides the first direct comparison of hPSC-PC and mouse PC gene expression and a robust method for generating differentiated hPSC-PCs with human-specific gene expression for modeling developmental and degenerative cerebellar disorders.

Disclosures: D.E. Buchholz: None. **T.S. Carroll:** None. **A. Kocabas:** None. **X. Zhu:** None. **H. Behesti:** None. **L. Stalbow:** None. **Y. Fang:** None. **M.E. Hatten:** None. **P.L. Faust:** None.

Digital Abstract Session

P003. Neuronal Differentiation

Program #/Poster #: P003.01

Topic: A.01. Neurogenesis and Gliogenesis

Title: The long-noncoding RNA Pantr2 affects radial glia differentiation timing through regulation of Nfix and Rgcc expression

Authors: *J. J. AUGUSTIN, L. A. GOFF;
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Abstract: Long-noncoding RNAs (lncRNAs) are defined as an RNA transcripts of length 200nt or longer with no protein coding potential. While functions for individual lncRNA transcripts remain largely unresolved, recent evidence has revealed critical roles for specific lncRNAs in both development and disease. The largest diversity of lncRNA expression in any somatic tissue is found within the mammalian central nervous system. As such, lncRNA biology has become a focus of neurodevelopment and neurodegenerative studies. Here, we characterize the mouse lncRNA Pantr2 and describe its function in the regulation of radial glia differentiation and neurogenic potential through interactions with the RNA binding protein Elavl1/HuR. Previous work has demonstrated that ablation of Pantr2 results in microcephaly and cortical mislamination in our knockout mouse model. This phenotype arises, in part, from a significant reduction in intermediate progenitor cells in the absence of Pantr2. Here we conduct an ATAC-seq study and identify regions of significant chromatin accessibility changes in Pantr2 knockout cortical neurospheres. We specifically identify an upstream regulatory element of the gene Rgcc with significant differential accessibility which contains a binding motif for the transcription factor Nfix. Single cell RNA sequencing analysis indicates that Pantr2 knockout-derived cortical neurospheres express both Nfix and Rgcc at significantly increased levels compared to wildtype controls, implicating these genes as downstream effectors of Pantr2 activity. We further test this association by demonstrating the ability for the Pantr2 transcription to function ectopically in Neuro2a (N2a) cells. N2a cells over-expressing Pantr2 exhibit significantly lower levels of both Nfix and Rgcc upon stimulation to differentiate. Moreover, a reduction in the percentage of differentiating Pantr2 overxpression N2a cells were observed in G2/ M phase of the cell cycle indicating a potential cell cycle block at M phase. Ex vivo analysis indicates ectopic expression of Nfix is sufficient to induce Rgcc expression in N2a cells and that expression of these two genes slows the progression of M phase. A block in M phase is consistent with the observed differentiation defect in the cortex of developing Pantr2 deficient mice. To confirm these results in vivo, in utero electroporation was used to demonstrate that ectopic expression of Nfix and/or Rgcc phenocopy the Pantr2 KO mice. Our results indicate that both Nfix and Rgcc are sensitive to Pantr2 RNA expression levels and that changes in the expression of these two genes recapitulates the Pantr2 KO phenotype.

Disclosures: J.J. Augustin: None. **L.A. Goff:** None.

Digital Abstract Session

P003. Neuronal Differentiation

Program #/Poster #: P003.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: Hill foundation
Zvi Meitar research prize

Title: The first neurons of the human brain: towards a policy for extending the 14-day limit

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Abstract: *Devastating brain disorders affect more than one billion people worldwide. In spite of recent large-scale initiatives in neuroscience there are very few effective cures for neurological and neuropsychiatric disorders. A variety of studies have already highlighted differences between human brain development and that of other mammals.*
Using high-resolution volume rendering of 3D confocal data sets from a Zeiss LSM 710 microscope we have examined the initial stages of neurogenesis in the forming neural tube and emerging trigeminal, otic, pituitary placodes and olfactory placodes. Human embryos from Carnegie stages (CS) 10-12 (29-31 days post-conception) were obtained from the Human Developmental Biology Resource UK. The rostral end of the neural tube, which becomes the future telencephalon, closes at CS 11. We found bipolar TU-20-positive neurons scattering through the several regions of the early cephalic epithelium adjacent to the forming neural tube. Such cells, not seen in other mammalian species, delaminate from the epithelium to coalesce within the forming mesenchyme. Our discovery of the the first neurons migrating into the optic cup from diencephalon at CS11 and the first neurons of the human presumptive cortex at CS12, raises important questions on the origin and role of these unique neuronal populations in human brain development. That can be clarified only by research on tissue currently unavailable to the scientific community because of the difficulties of getting tissue obtained by termination of pregnancy during the formation of the neural tube at CS 8-11, and the limitations of the "14-day rule" on human embryo research in vitro. For a number of reasons, including being able to trace the origin of the first neurons in the human brain, a new policy is needed for the use of human embryos created through in vitro fertilisation. Such a policy should seek to maximize best use of donated embryos, which currently get discarded even before the formation of the neural plate, leading to unnecessary waste of precious tissue. The 14-day limit on embryo research should be re-examined considering the latest knowledge of the scientific and medical opportunity costs entailed by such a restriction.
As an alternative to the 14-day rule, we propose extending the limit to CS11. Such an extension based on wide public consultation, is essential if we are to understand how the structure that makes us human begins to form.

Disclosures: I. Bystron: None. **A. Erler:** None.

Digital Abstract Session

P003. Neuronal Differentiation

Program #/Poster #: P003.03

Topic: A.01. Neurogenesis and Gliogenesis

Title: Determining the Metabolic Requirements of Electrically Active Rodent Primary Neurons in Long-Term Culture

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Abstract: Neurons have a high metabolic demand with the brain accounting for 20% of total body energy consumption. Most neuronal cell culture media contain energetic substrates in excess to ensure this demand is met. We investigated whether media with physiologic (e.g. BrainPhys™) or supraphysiologic (e.g. Neurobasal™) glucose levels supported the metabolic requirements and sustained activity of primary neurons in high-density cultures. E18 rat cortices were dissociated into single-cell suspensions and plated in NeuroCult™ Neuronal Plating Medium supplemented with NeuroCult™ SM1 Neuronal Supplement (SM1) in 96-well microelectrode array (MEA) plates. After 5 days of incubation, cultures were transitioned to BrainPhys™ (BP) with SM1 by performing half-medium changes every 2 - 3 days for up to 8 weeks. Increasing levels of glucose were added to BP, ranging from 2.5 to 25 mM final concentration. Cells were also cultured in high-glucose media (Neurobasal™ [NB] and Neurobasal™ Plus [NBPlus]) following the supplier's protocols. Before each half-medium change, media were collected for glucose measurements using a YSI Biochemistry Analyzer. Spontaneous neuronal activity was recorded three times weekly using the Axion Biosystems MEA system. Neuronal activity was first detected in BP-cultured neurons around day 9, with mean firing rate (MFR) recorded at 3.1 ± 0.3 Hz (mean ± SEM, n = 3). The activity of neurons in NB remained low throughout culture, with < 1.3 Hz of MFR recorded over time. For NBPlus-cultured neurons, activity was not detected until day 14 at 1.6 ± 0.3 Hz and increased to 8.6 ± 2.3 Hz on day 18, but dropped significantly to 2.1 ± 0.9 Hz on day 24 (mean ± SEM). MFR was maintained below 2.7 Hz thereafter and decreased to 0.8 ± 0.3 Hz on day 54 (mean ± SEM). The same "peak and drop" activity pattern was also observed in BP when MFR dropped to 0.05 ± 0.02 Hz (mean ± SEM) around day 14. Glucose measurements of media on day 14 showed that glucose was depleted in BP but not in NB and NBPlus. With 12.5 mM glucose added to BP, MFR was measured at 3.8 ± 1.5 Hz on day 14, 3.1 ± 0.4 Hz on day 28 (mean ± SEM), and maintained at this level until the end of culture, with 3.3 ± 0.9 Hz recorded on day 54 (mean ± SEM). Notably, both NB and NBPlus had higher levels of glucose throughout culture but both displayed lower activity than BP after week 4. Glucose supplementation in BP at a higher (but not hyperglycemic) level was the only condition that could support neuronal activity in long-term culture. These data demonstrate that BrainPhys™ is not only optimized for energetic substrates but its performance can be further enhanced by supplementation with glucose to meet higher metabolic demands of electrically active neurons in vitro.

Disclosures: C.K.H. Mak: None. **K. McCormack:** None. **A.C. Eaves:** None. **S.A. Louis:** None. **E. Knock:** None.

Digital Abstract Session

P004. Glial Development

Program #/Poster #: P004.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP (30-5; 1-5)

Title: Hypoxia-inducible factor 1 alpha promotes peripheral nerve myelination

Authors: *T. ARAKI, Y. UJIIE-KOBAYASHI, S. WAKATSUKI;
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Abstract: Schwann cells (SCs) generate myelin sheath in the peripheral nervous system (PNS), and their differentiation plays an important role in PNS myelination. Mature SCs are formed via a stepwise differentiation during development from neural crest cell-derived SC precursors to immature SCs and pro-myelinating SCs, followed by myelinating/non-myelinating SCs. Characteristic expression of genes, including several transcriptional factors, have been described in each developmental stage. However, underlying mechanisms of SC differentiation remain unclear. Recent studies have shown that hypoxia-inducible factor-1 alpha (HIF-1α) plays a role in myelination in the central nervous system. Here, we investigated the role of HIF-1α in PNS myelination. We found that HIF-1α can be stabilized in nuclei of SCs cultured under hypoxia (1% O_2). Culturing in hypoxia or overexpression of HIF-1α bearing mutation to be resistant to proteasomal degradation resulted in upregulation of myelin related gene expressions in SCs. By immunohistochemistry, HIF-1α was localized in S100β-positive SCs in murine sciatic nerve during development. HIF-1α expression in protein level was higher during development than in adulthood in mice, while HIF-1α mRNA expression was almost constant. Moreover, HIF-1α stabilizing drug that inhibits prolyl hydroxylation was able to upregulate myelin protein expression and promoted myelination in culture. Transient hypoxic incubation also facilitated in vitro myelination. These finding suggest that HIF-1α induces SCs differentiation and promotes PNS myelination during development.

Disclosures: T. Araki: None. **Y. Ujiie-Kobayashi:** None. **S. Wakatsuki:** None.

Digital Abstract Session

P004. Glial Development

Program #/Poster #: P004.02

Topic: A.01. Neurogenesis and Gliogenesis

Title: Cortical interlaminar astrocytes have unique developmental trajectory and molecular markers in primates

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Abstract: Interlaminar astrocytes (ILAs) are a cortical astrocyte subtype that have a soma in layer I and long interlaminar processes that run perpendicular to the pia into deeper cortical layers. We previously performed a comparative study of ILAs in 46 species of therian mammals. We described *rudimentary ILAs* with short GFAP$^+$ processes confined to layer I, and *typical ILAs* with long GFAP$^+$ processes exiting layer I and extending into deeper cortical layers. We found that ILAs are present in all mammals, and what makes them special in primates is their highest number and highest morphological complexity.ILAs have been described in postnatal animals, but exactly when they appear during development has not been determined. We studied ILA developmental origin and differentiation of ILAs in prenatal and postnatal cortex by analyzing GFAP$^+$ and S100b$^+$ ILAs in fixed postmortem brains of mouse, rhesus macaque, chimpanzee and human. We found that ILAs are present in the prenatal brain and increase in number and morphological complexity throughout development. We compared the expression of specific markers in ILAs across development in mouse and macaque and found some similarities in protein expression by mouse rudimentary ILAs and macaque typical ILAs, but noted key differences that may indicate distinct functions across species. These data provide new information on ILA astrogenesis and function in the developing cerebral cortex.

Disclosures: **C. Falcone:** None. **E. Penna:** None. **T. Hong:** None. **A. Tarantal:** None. **P.R. Hof:** None. **W. Hopkins:** None. **C. Sherwood:** None. **S. Noctor:** None. **V. Martínez-Cerdeño:** None.

Digital Abstract Session

P004. Glial Development

Program #/Poster #: P004.03

Topic: A.01. Neurogenesis and Gliogenesis

Support: UABC to T.N.O.B.
Consejo Nacional de Ciencia y Tecnología (Conacyt-México) and Soluciones para un México Verde, S.A. to A.O.

Title: Gene expression of the glutamate transporters GLAST in glial cells of larvae, postalarvae, and adults of the Pacific sea biscuit Dendraster excentricus

Authors: *T. N. OLIVARES-BAÑUELOS[1], G. OSUNA-CORTEZ[1], F. CORREA-SANDOVAL[1], L. ENRIQUEZ-PAREDES[2], A. ORTEGA[3];
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Abstract: Echinoderms are a phylum within marine invertebrates characterized by having a pentaradial secondary symmetry. Marine invertebrates are strategic study models for biological sciences, helping to understand the functioning of the nervous system for the diagnosis and treatment of neurological disorders. Similarities have been found between invertebrates and vertebrates in their nervous system, which is made up of two main types of cells: neurons and

glia, whose nerve cells have similarities in biochemistry and functionality. Although the presence of glial cells in echinoderms has already been demonstrated, it is not yet known at what point in larval development these cells begin to develop. Considering this, the present work aims to analyze larval and adult tissue of the Mexican Pacific sea biscuit *Dendraster excentricus* to know the expression of GLAST transporters at each stage of larval and postlarval development. These information let us know in which stage these organisms have functional glial cells. *Dendraster excentricus* sea biscuit is a common echinoderm on the shores of the Pacific Ocean. Adult organisms of *D. excentricus* were collected from the Punta Banda estuary in Baja California, Mexico. Larvae cultures were carried out and larvae with four, six or eight-arms, competent and metamorphosed larvae, were collected. Quantitative PCR was carried by triplicate with each of the developmental stages, as well as with adults, to measure GLAST gene expression; ribosomal 18S housekeeping gene expression was measured too as internal control. Results showed statistically significant differences in GLAST expression between sea biscuit development stages and adults. The higher relative expression of GLAST was observed in adult samples, followed by the expression in both metamorphosed and six-arms larvae. Low expression of GLAST was detected in larvae with four- and eight-arms, besides in competent larvae. Based on these results, it seems that glia cells in the sea biscuit *Dendraster excentricus* are present from the six-armed stage. As was reported for other echinoderms, the nervous systems of adult organisms of *D. excentricus* present functional glial cells. Knowing the development and functional characteristics of a simpler nervous system is important to implement techniques that lead to the identification and development of new treatments for neurodegenerative diseases.

Disclosures: T.N. Olivares-Bañuelos: None. **G. Osuna-Cortez:** None. **F. Correa-Sandoval:** None. **L. Enriquez-Paredes:** None. **A. Ortega:** None.

Digital Abstract Session

P005. Cell Lineage

Program #/Poster #: P005.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: Natural Sciences and Engineering Research Council of Canada (NSERC) Canada Graduate Scholarship-Master's
Canadian Institutes of Health Research (CIHR) Project Grant PJT – 162108

Title: Exploring the combinatorial functions of proneural genes Neurog1 and Neurog2 in neocortical stem cell maintenance

Authors: *A.-M. OPROESCU[1,2], S. HAN[1,3], D. ZINYK[1], C. SCHUURMANS[1,3];
[1]Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada; [2]Lab. Med. and Pathobiology, [3]Biochem., Univ. of Toronto, Toronto, ON, Canada

Abstract: Proneural genes encode basic-helix-loop-helix transcription factors that promote neural stem cell (NSC) differentiation in the developing nervous system. In the embryonic

neocortex, proneural genes *Neurog1* and *Neurog2* are redundantly required to specify a glutamatergic neuronal identity. Paradoxically, our lab previously found that *Neurog1* tampers the pace of early-stage neurogenesis when co-expressed with *Neurog2*. In the present work, we observe precocious depletion of NSCs in *Neurog1*$^{-/-}$;*Neurog2*$^{-/-}$ but not in *Neurog1*$^{-/-}$ or *Neurog2*$^{-/-}$ single mutant control cortices. Therefore, the aim of this study is to test the hypothesis that *Neurog1* and *Neurog2* operate together to maintain the neocortical NSC pool. We utilize a novel split-Cre system to examine only those NSCs that co-express *Neurog1* and *Neurog2*, generating two new knock-in lines, *Neurog1CCre*^{KI} and *N2NCre*^{KI}, in which the C- and N-terminal regions of Cre are knocked-in to *Neurog1* and *Neurog2* loci, respectively. When both genes are co-expressed, functional full-length Cre is re-constituted. By crossing *Neurog1CCre*^{KI};*N2NCre*^{KI} mice with a *Rosa-ZsGreen* reporter line, we performed long-term lineage tracing of double-positive NSCs throughout embryonic corticogenesis and into the postnatal period. *Neurog1-Neurog2* co-expressing cells give rise to neurons throughout the deep and superficial layers of the cortical plate, and to cells that populate the entire neocortex and thalamus in postnatal day 150 brains. To assess the functional requirement for *Neurog1-Neurog2* cells in NSC pool maintenance, *Neurog1CCre*^{KI};*N2NCre*^{KI} mice are now being crossed to *Rosa-DTA* mice in which cell death is achieved by the expression of Diphtheria toxin fragment A only in double-positive NSCs. With these studies, we aim to determine whether *Neurog1-Neurog2* co-expressing cells have a unique role in maintaining the neocortical NSC pool.

Disclosures: S. Han: None. **D. Zinyk:** None. **C. Schuurmans:** None.

Digital Abstract Session

P006. Mechanisms of Cell Fate

Program #/Poster #: P006.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: "Research Cooperability" Program of the Croatian Science Foundation funded by the European Union from the European Social Fund under the Operational Programme Efficient Human Resources 2014-2020 PSZ-2019-02-4710 (ZK) Scientific Centre of Excellence for Basic, Clinical, and Translational Neuroscience (project "Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain"; GA KK01.1.1.01.0007 funded by the EU Fund

Title: Sox2 reveals a stem cell potential in the human fetal subplate

Authors: *J. KOPIC, T. MISKIC, A. JUNAKOVIC, I. KOSTOVIC, Z. KRSNIK;
CIBR, Sch. of Medicine, Univ. of Zagreb, Zagreb, Croatia

Abstract: Human neocortical organization is established by orchestrated spatio-temporal histogenetic and neurogenetic processes regulated by differential gene expression. During fetal corticogenesis, critical step happens in the proliferative ventricular (VZ) and subventricular

(SVZ) zone, where neurons are born from stem cells and other progenitors. SOX2 is a transcription factor responsible for upholding the identity of neural stem cells, therefore represents a key player during corticogenesis. The aim of our study was to identify stem cells and cells with proliferative capacity in the human non-proliferative, synaptic subplate during midgestation, by utilizing double labelling immunofluorescence on postmortem human fetal material. Interestingly enough, our results show shifts in the SOX2 expression pattern from proliferative zones at midgestation. Furthermore, during the late mid-fetal period, SOX2 was sparsely distributed throughout the subplate, whose neurodevelopmental role remains elusive.

Disclosures: J. Kopic: None. **T. Miskic:** None. **A. Junakovic:** None. **I. Kostovic:** None. **Z. Krsnik:** None.

Digital Abstract Session

P006. Mechanisms of Cell Fate

Program #/Poster #: P006.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: FAPESP 2015/13345-1
FAPESP 2018/07366-4

Title: Atp and calcium oscillations in huntington's disease: targeting neural stem cells

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Abstract: Background: Purinergic receptors have been attributed with developmental functions including gastrulation and neural differentiation. Upon activation, P2 purinergic receptors trigger intracellular calcium transients controlling cellular processes. Huntington’s disease (HD) is a genetic neurodegenerative disease caused by the loss of GABAergic neurons (GABANs) from the basal ganglia and the symptoms onset start around the third decade of life spam. Thus recent studies propose that the disease actually starts much earlier, and HD is a neurodevelopmental disease. For clarifying this correlation, we induced GABAN differentiation of neural stem cells derived from a HD patient and recorded the intracellular calcium oscillations during the process of differentiation and compared to ATP-promoted response and cell death rates. Methods and results: First of all, P2Y2 receptor stimulation along differentiation of mouse embryonic stem cells increases the efficacy of GABANs differentiation (27±5 vs 42±2.2, n=3) by boosting the frequency of spike-like calcium oscillations. Using a technique that couples the imaging of alterations in cytosolic calcium concentration by Fluo4-AM (calcium imaging) and Ascl-1 or Neurogenin 2 by luciferase activity (stable transfected cells with Ascl-1 or Ngn2 promoter-protein fusion to luciferase reporter construct), we observed the effectiveness improvement was due to prolonged expression of Ascl-1. In view of that, we investigated HD-NPC and control NPC differentiation patterns. HD-NPCs differentiated from patient iPS cells did not reveal any

spike-like oscillations, while they showed increased cell death / apoptosis rates when compared to the healthy donor (30±8.3 vs 8.6±4.1, n=3), despite of caspase3/7 activation. Moreover, HD NPCs challenged with ATP showed higher amplitude of cytosolic calcium transients if compared to the healthy donor (4.2±0.4 n=51 vs 2.4±0.06 n=35). Blockade of intracellular calcium mobilization by P2Y2 receptors using thapsigargin could not prevent cell death. Thus, NPC differentiation status was changed, by decreasing the pool of undifferentiated NPCs when calcium oscillations were blocked, indicated by nestin expression detected by flow cytometry (90.7±1.1vs 48.4±10.4, n=3). Conclusion: Altogether these data suggest that P2Y2 receptor activation or inhibition modulates spontaneous calcium oscillations during neural differentiation and consequently changes the expression pattern of Ascl-1, thus controlling the cell fate decision to GABAergic neurons. This process is altered in HD patients' cells, compromising the pool of NPCs during early nervous system development.

Disclosures: T. Glaser: None. **Y.D. Teng:** None. **R. Kageyama:** None. **H. Ulrich:** None.

Digital Abstract Session

P007. Mechanisms of Stem Cell Quiescence

Program #/Poster #: P007.01

Topic: A.03. Stem Cells and Reprogramming

Support: RGCB Intramural Funds

Title: Is non-canonical Notch independent Hes-1 expression responsible for maintenance of adult neural stem cell of embryonic origin in the SVZ?

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Abstract: It is known that the quiescent neural stem cells in the adult sub ventricular zone (SVZ) are maintained through notch signaling. There are also reports in adult rodent cortex that show activation of Notch-1 receptor only in the activated neural stem cells and not in the quiescent state. Previous reports from our lab have show that Notch receptor independent Hes-1 (NIHes-1) expression is responsible of maintaining the embryonic neural stem cells in the developing embryonic neocortex *(Dhanesh et.al., Cerebral Cortex, (8)27, 2017, 3943–3961)*. Reading the above two reports together we hypothesized that NIHes-1 could be responsible for maintenance of the quiescent neural stem cell in adult SVZ.To prove this we generated a plasmid construct that expressed GFP upon activation of NIHes-1. This plasmid construct was electroporated into adult mouse SVZ. Further, to show the quiescent nature of the neural stem cells we injected BrdU into E14 pregnant mouse. The pups that were delivered we allowed to grow for 2-3 months. The quiescent neural stem cells in the SVZ was identified by the fragmentation if BrdU. The neural stem cells with minimum fragmentation were considered as quiescent since they had undergone minimum number of cell division. Whereas, cells that have highly fragmented BrdU

was considered as actively proliferating cell. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Rajiv Gandhi Center for Biotechnology and carried out as per CPCSEA guidelines. Our results showed the existence of both notch dependent (NDHes-1) and NIHes-1 expression in adult SVZ. Out of this a majority of quiescent neural stem cells with unfragmented BrdU in the SVZ also showed GFP expression (NIHes-1 expression). Further interpretation of the data showed the embryonic origin of the quiescent neural stem cells in adult SVZ. These observations showed that the quiescent neural stem cells in the adult SVZ is of embryonic origin and is maintained through NIHes-1 expression.

Disclosures: **V. Meera:** A. Employment/Salary (full or part-time):; Rajiv Gandhi Center for Biotechnology. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NA. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Rajiv Gandhi Center for Biotechnology. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); NA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NA. F. Consulting Fees (e.g., advisory boards); NA. Other; Rajiv Gandhi Center for Biotechnology. **P.A. Riya:** None. **S. Surya:** A. Employment/Salary (full or part-time):; Rajiv Gandhi Center for Biotechnology. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NA. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Rajiv Gandhi Center for Biotechnology. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); NA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NA. F. Consulting Fees (e.g., advisory boards); NA. Other; Rajiv Gandhi Center for Biotechnology. **B. Basu:** None. **S. Parvathy:** None. **S. Lalitha:** None. **J. James:** None.

Digital Abstract Session

P007. Mechanisms of Stem Cell Quiescence

Program #/Poster #: P007.02

Topic: A.03. Stem Cells and Reprogramming

Support: Cancer Research UK Grant FC0010089
UK Medical Research Council Grant FC0010089
Wellcome Trust Grant FC0010089
Cancer Research UK Career Development Fellowship A14958

Title: Neural stem cells alter nucleocytoplasmic partitioning and accumulate nuclear polyadenylated transcripts during quiescence

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Abstract: Quiescence is a cellular state characterised by reversible cell-cycle arrest and diminished biosynthetic activity that protects against environmental insults, replicative exhaustion and proliferation-induced mutations. Entry into and exit from this state controls development, maintenance and repair of tissues, plus in the adult central nervous system, generation of new neurons and thus cognition and mood. Cancer stem cells too can undergo quiescence, which confers them resistance to current therapies. Despite clinical relevance, quiescence lacks understanding and is defined functionally given lack of molecular markers. Inhibition of the Target of Rapamycin pathway is a common denominator of quiescence across species and cell types. This pathway integrates diverse signals towards control of metabolism and cytoskeleton, and lowered pathway activity in quiescence ensures decrease of the most resource-intensive cellular process of protein synthesis. Here, we combine *Drosophila* genetics and a mammalian model to show that altered nucleocytoplasmic partitioning and nuclear bias of a fraction of polyadenylated RNAs are novel evolutionarily conserved mechanisms of quiescence regulation. Moreover, we observe widespread decreased in intron retention in the transcripts that accumulate in the nucleus upon quiescence induction. Altogether, we propose that alterations to mRNA export and differential subcellular partitioning of transcripts are novel and underappreciated mechanisms to inhibit protein translation in quiescent cells, whilst likely priming them for quick reactivation in response to appropriate cues.

Disclosures: A. Rossi: None. **A. Coum:** None. **M. Madelenat:** None. **L. Harris:** None. **A. Miedzik:** None. **S. Strohbuecker:** None. **A. Chai:** None. **H. Fiaz:** None. **R. Chaouni:** None. **P. Faull:** None. **W. Grey:** None. **D. Bonnet:** None. **E.V. Makeyev:** None. **A.P. Snijders:** None. **G. Kelly:** None. **F. Guillemot:** None. **R. Sousa-Nunes:** None.

Digital Abstract Session

P007. Mechanisms of Stem Cell Quiescence

Program #/Poster #: P007.03

Topic: A.02. Postnatal Neurogenesis

Support: R01GM112721

Title: Age-related neural pathologies and cognitive impairments of NRMT1 knockout mice are preceded by expansion of the neural stem cell population

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Abstract: Neural stem cell-based therapies show great promise for treatment of ischemic stroke and neurodegenerative diseases. While spontaneous neurogenesis occurs post-injury, this process is transient and inadequate for repair of significant damage. Identifying targets for therapeutic intervention that could increase and prolong endogenous neural stem cell (NSC) response could significantly diminish or reverse accumulated damage. Here we identify the N-terminal methyltransferase NRMT1 as a novel regulator of NSC proliferation and differentiation. N-terminal methylation by NRMT1 regulates protein/DNA interactions and plays a role in many cellular processes, including DNA damage repair, mitosis, proliferation, transcriptional regulation, and protein stability. Our previous generation of a constitutive NRMT1 knockout ($NRMT1^{-/-}$) mouse demonstrated that its loss results in sensitivity to DNA damaging agents, severe developmental abnormalities, and premature aging phenotypes. As accumulation of DNA damage and appearance of premature aging phenotypes often precede or accompany neurodegeneration, we began to more specifically examine how NRMT1 loss affects neural pathology and cognitive behaviors. We find that $NRMT1^{-/-}$ mice have postnatal enlargement of the lateral ventricles, significant reduction of striatal and corpus callosum volume, age-dependent hippocampal neurodegeneration, and short- and long-term memory impairments. Interestingly, these morphological and behavioral abnormalities are preceded by a significant increase in Sox2 positive (Sox2+) NSCs and Doublecortin positive (Dcx+) neuroblasts, indicating that early misregulation of the NSC pool may be altering postnatal neurogenesis and impeding replacement of aged or damaged neurons in $NRMT1^{-/-}$ mice. This is the first work showing a role for NRMT1 in neural development, neurodegeneration, and NSC regulation.

Disclosures: J. Catlin: None. **L.N. Marziali:** None. **B. Rein:** None. **Z. Yan:** None. **M.L. Feltri:** None. **C.E. Schaner Tooley:** None.

Digital Abstract Session

P007. Mechanisms of Stem Cell Quiescence

Program #/Poster #: P007.04

Topic: B.09. Network interactions

Support: CNPq
FAPERJ

Title: Modulation of mesenchymal stromal cells secretory activity from Wistar rats exposed to neural tissue segments

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Abstract: Bone marrow-derived mesenchymal stromal stem cells (BM-MSC) exert regenerative potential due to their paracrine activity and the modulation of the microenvironment of injured tissues. Activity occurs due to the capacity of these stem cells to release soluble factors with trophic activity. Studies have revealed the potential of the BM-MSC to promote regeneration of Peripheral Nervous System (PNS), but gaps are present regarding the behaviour of MSCs in the injured tissue microenvironment. In this study, we investigated the role of BM-MSC trophic activity through indirect co-culture with different neural tissue explants. BM-MSCs were obtained from bone marrow of male Wistar rats and characterized by their morphology immunolabeling (CD90). The indirect co-culture of BM-MSC was performed under different conditions based on the following explants: skin, sciatic nerve, cerebral cortex and spinal cord, during 48 hours. BM-MSCs co-culture conditioned medium was analyzed by Enzyme-Linked Immunosorbent Assay (ELISA). The viability and proliferation of BM-MSCs were evaluated by LIVE/DEAD assay and by selective markers. BM-MSCs in indirect co-culture showed no difference in cell viability, but the morphology of cells in co-culture with cerebral cortex and spinal cord fragments were altered. BM-MSCs culture density increased when exposed to fragments of both spinal cord or sciatic nerve when compared to fragments of cerebral cortex. Cell proliferation of BM-MSCs, evaluated by immunocytochemistry, showed no significant difference between conditions. Conditioned medium was evaluated for the presence of VEGF (Vascular Endothelial Growth Factor) and showed a small increase of VEGF levels when BM-MSCs were co-cultured with Central Nervous System (CNS) tissues. Moreover, we observed alterations in cytoarchitecture of BM-MSCs co-cultured with cerebral cortex fragments, by actin filaments stain. In conclusion, BM-MSCs exposed to CNS tissue fragments altered their morphology as well as VEGF expression, indicating that the interaction with these tissues may be modulating the behaviour of MSCs.

Disclosures: I. Santos: None. **M.B. Da Silva:** None. **R. de Melo Reis:** None. **V. Ribeiro-Resende:** None.

Digital Abstract Session

P008. Postnatal Neurogenesis: Molecular Mechanisms

Program #/Poster #: P008.01

Topic: A.02. Postnatal Neurogenesis

Support: NIH grant R35NS116843

Title: Single-cell RNA-sequencing reveals discrete steps in the transformation of developmental precursors to adult neural stem cells

Authors: *A. M. BOND, D. JIMENEZ-CYRUS, G.-L. MING, H. SONG;
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Abstract: The dentate gyrus region of the hippocampus is one of only two regions in the adult mammalian brain where substantial levels of neurogenesis occur throughout adulthood.

Quiescent adult neural stem cells in the subgranular zone of the dentate gyrus occasionally become reactivated in adulthood to generate new neurons which integrate into the existing network and contribute an added layer of plasticity to the hippocampus. Previously, our lab showed that embryonic neural stem cells which contribute to developmental cytogenesis in the dentate gyrus transition into quiescent adult neural stem cells, suggesting that a common lineage of neural stem cells contributes to developmental and adult neurogenesis. However, the molecular mechanisms regulating this developmental transition remain completely unknown. Here we used single-cell RNA-sequencing of $Hopx^{+}$ dentate gyrus progenitors across multiple stages of early postnatal development in mice to identify the molecular cascade associated with the transformation of developmental precursors to quiescent adult neural stem cells. We discover that early postnatal dentate gyrus neural stem cells exist in multiple heterogeneous states, and we identify a trajectory of cycling neural stem cells as they transition into a quiescent state. We also discover that dentate gyrus precursors gradually acquire an adult-like state after they exit cell cycle. This suggests that the developmental transition consists of two sequential steps: first precursors exit cell cycle to enter a quiescent state and then precursors acquire an adult-like state throughout a quiescence maturation period. We further identify transcription factor networks associated with each stage of the transition and biological processes that change over the course of the transition. Collectively, our work identifies molecular and cellular mechanisms associated with the transformation of developmental precursors to quiescent adult neural stem cells, and serves as a foundation for future studies investigating mechanisms that regulate formation of the adult neural stem pool.

Disclosures: **A.M. Bond:** None. **D. Jimenez-Cyrus:** None. **G. Ming:** None. **H. Song:** None.

Digital Abstract Session

P008. Postnatal Neurogenesis: Molecular Mechanisms

Program #/Poster #: P008.02

Topic: A.02. Postnatal Neurogenesis

Support: Tom and Elizabeth Long Excellence Fund for Honors
William W. and Ida W. Taylor Fellowship

Title: Understanding the Role of Eed Deletion in Medulloblastoma

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Abstract: Medulloblastoma is uniquely sensitive to DNA damage-inducing therapies with conventional treatment resulting in an 80% 5-year survival rate. By researching the apoptotic pathways that make treatment effective in some and how those pathways contribute to resistance in others, we can identify therapies that reduce the need for toxic radiation and chemotherapy. In cerebellar development, Sonic Hedgehog (SHH) signaling drives proliferation of cerebellar granule neuron progenitors (CGNPs), but hyperactivation leads to SHH-subgroup

medulloblastoma, making up 30% of medulloblastoma cases. In differentiated cerebellar neurons, where SHH signaling is low, the polycomb repressive complex 2 (PRC2) silences target genes by trimethylating H3K27 residues in regulatory regions. SHH signaling upregulates target genes by displacing the PRC2 and removing H3K27 trimethylation marks via the JMJD3/KDM6B demethylase complex.Based on its divergent roles in proliferating and differentiating cells, the PRC2 has varying effects in different tumors. We deleted Eed, a component of PRC2, in SHH medulloblastoma tumors in mice to see its effects on tumor development. While Eed deletion reduced proliferation and tumor size at postnatal day 12 (P12), the mice had poorer survival. This study aimed to elucidate how cell cycle dynamics are altered in Eed-deleted medulloblastomas by performing fluorescence-activated cell sorting (FACS).We bred Math1-Cre;SmoM2 mice, which develop tumors by constitutively expressing Smoothened allele (SmoM2) in a CGNP-specific manner (Math1+ population), with EedloxP/loxP mice. The resulting Math1-Cre; SmoM2;EedloxP/loxP (Eed cKO) mice developed tumors in which Eed was deleted. We then dissociated tumor tissue from 6 Eed cKO, 5 Eed het, and 5 control tumor mice at P12 as well as 3 Eed cKO, 4 Eed het, and 4 control tumor mice at P18. We subsequently stained for pRB, EdU, and FX cycle violet before performing FACS. We then measured the percentage of total cells in G0, G1, S, G2, and M; we also measured the percentage of cells that were in each of G1, S, G2, and M out of the total number of pRB-positive cells. Based on these quantifications, we found that at P12, there are more cells exiting the cell cycle, but of the ones that remain, they are cycling faster than in control tumors. By the time the mice reach P18, they are not significantly different from control tumors. Thus, we hypothesize Eed deletion slows tumor growth in early development, but the tumor later develops resistance to cause decreased survival. Our goal is to better understand how SHH signaling regulates target gene expression in order to identify novel therapeutics for SHH-driven medulloblastoma.

Disclosures: **M. Churiwal:** None. **A. Cleveland:** None. **T. Gershon:** None.

Digital Abstract Session

P008. Postnatal Neurogenesis: Molecular Mechanisms

Program #/Poster #: P008.03

Topic: A.02. Postnatal Neurogenesis

Support: OHRI Grant
NSERC Grant

Title: Beclin1 Regulates Hippocampal Adult Neural Stem and Progenitor Cell Dynamics

Authors: ***A. KALININA**[1], J. DHALIWAL[2], Y. XUE[1], D. MAISONNEUVE[1], M. MCCAMBLEY[1], M. VACULIK[3], D. C. LAGACE[1];
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Abstract: Background and Objective: The dentate gyrus of the hippocampus contains stem and progenitor cells that generate new neurons throughout the mammalian lifespan via neurogenesis. Our group and others have demonstrated that certain key members of the autophagy pathway are required for adult neurogenesis. This work specifically determines if and how Beclin1, a key member of the autophagy pathway involved in the initiation of the autophagosomes, dynamically maintains adult hippocampal neurogenesis
Methods: This project uses an inducible transgenic mouse model of Beclin1 deletion from adult nestin-expressing hippocampal neural stem cells and progeny to perform in vivo lineage tracing. Neurogenesis and cell cycle was measured using a variety of assays, including immunohistochemistry, flow cytometry, and neurosphere assay. Furthermore, 10x genomics single-cell RNA sequencing and machine learning were used to identify unique transcriptional changes associated with the removal of Beclin1-dependent autophagy.
Results: Beclin1-null neurogenic cells had a significant reduction in autophagic flux in vivo as measured using mCherry-EGFP-LC3 retroviral labeling. Two weeks after removal of Beclin1 the number of proliferating neural stem cells and progenitor cells was reduced, which led to a significant reduction in adult-generated NeuN-expressing neurons by one month. Single-cell RNA-sequencing of wildtype and Beclin1-null cells revealed the dynamic role of Beclin1 in driving the activation and proliferation of adult neural stem and progenitor cells by regulating cell cycle and differentiation.
Conclusions: Beclin1-mediated autophagy is essential for adult hippocampal neurogenesis with transcriptional profiling of Beclin1-null cells supporting Beclin1 as a regulator of proliferation that is essential for the coordination of the life-long addition of adult-generated dentate gyrus neurons.

Disclosures: **A. Kalinina:** None. **J. Dhaliwal:** None. **Y. Xue:** None. **D. Maisonneuve:** None. **M. McCambley:** None. **D.C. Lagace:** None. **M. Vaculik:** None.

Digital Abstract Session

P008. Postnatal Neurogenesis: Molecular Mechanisms

Program #/Poster #: P008.04

Topic: A.02. Postnatal Neurogenesis

Support: Emerald Foundation Grant
NIH R01 Supplement CA204127

Title: The role of DNA repair and replication in BRCA2-null medulloblastoma

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Abstract: Medulloblastoma is the most common metastatic pediatric brain cancer and can arise from cerebellar granule cell progenitors. Granule cell progenitors undergo a period of high proliferation during cerebellar development. We hypothesize that this high level of proliferation

will necessitate robust DNA repair mechanisms to avoid an accumulation of mutations during this crucial period in development. Over seventy percent of children that have mutations in the tumor suppressor BRCA2 develop medulloblastomas before the age of five. Thus, we hypothesize that BRCA2 will be necessary to preserve genome stability in granule cell progenitors during postnatal cerebellar development and prevent medulloblastoma formation. The main aims of this study are to investigate how the loss of BRCA2 affects cerebellar development and induces mutations that lead to medulloblastoma. We have been able to observe tumor formation within two to three months of age in BRCA2-deficient mice have analyzed these tumors through RNA and whole-genome sequencing, identifying the overexpression of genes important in DNA repair and the protection of replication. We have developed primary cell culture of medulloblastoma cells as well as *ex vivo* slice culture of tumor tissue to validate the role of these genes in providing a selective advantage for these tumor cells. We have also adapted methods to study DNA replication fork behavior and chromosomal abnormalities for granule cell progenitors as well as primary medulloblastoma cells. We will be able to use tools developed in the field of DNA repair and replication to provide a novel characterization of how mutagenesis occurs in granule cell progenitors and leads to tumor formation in childhood brain cancer.

Disclosures: A. Smogorzewska: None. **M. Hatten:** None.

Digital Abstract Session

P008. Postnatal Neurogenesis: Molecular Mechanisms

Program #/Poster #: P008.05

Topic: A.02. Postnatal Neurogenesis

Support: CIHR

Title: Adult-born neurons modulate activity in developmentally-born neurons during learning

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Abstract: Recent reports indicate that lateral inhibition plays a powerful role in selecting which dentate gyrus (DG) neurons are recruited during memory formation. This raises the question of whether developmentally-born and adult-born DG neurons have distinct roles for inhibition, particularly in vivo when neuronal ensembles are selected during memory encoding. To address this we combined chemogenetics and immunohistochemistry for BrdU+Fos to silence and measure activity in developmentally and adult-born neurons as rats learned a spatial water maze task. Specifically, retrovirus was injected into the DG of male rats at 6 weeks of age to express the inhibitory DREADD receptor, HM4Di, in neurons born in adulthood. The same rats were also injected with BrdU to label developmentally or adult-born neurons. At 10 weeks of age rats were injected with either the HM4Di agonist CNO or vehicle, then trained in the water maze. We found that silencing a subset of adult-born neurons (aged 4 weeks) increased activity levels in the

developmentally-born neuron population. However, silencing adult-born neurons did not affect activation in other adult-born neurons within the DG, suggesting limited interaction amongst the adult-born population. As well, we have examined activation of interneurons (PV+ and SST+) within each treatment group to determine if silencing neurons at different ages impacts downstream activity in inhibitory interneurons. Our recent findings implicate PV+ interneurons in the modulatory subcircuit between neuron populations within the DG.

Disclosures: **A. Ash:** None. **E. Regele-Blasco:** None. **D. Seib:** None. **E. Chahley:** None. **J.S. Snyder:** None.

Digital Abstract Session

P008. Postnatal Neurogenesis: Molecular Mechanisms

Program #/Poster #: P008.06

Topic: A.02. Postnatal Neurogenesis

Title: Modeling adult neurogenesis in vitro: morphometric and marker analysis of neural precursors from young and adult mouse hippocampus using the neurosphere assay

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Abstract: Adult neurogenesis in the dentate gyrus (DG) of the hippocampus is involved in many physiological functions such as learning and memory, as well as neurological disorders such as Alzheimer's disease and age-related cognitive decline. Most of the neurogenesis quantification and manipulation studies are done on rodents soon after they reach sexual maturity (around postnatal day 40), which is routinely used as an approximation of an adult-like stage of development. Such studies however do not provide much insight into long-term contribution of newly generated granule cells (GCs) to hippocampal function and disorders later in life, which underscores the need for more studies regarding neurogenesis in the adult brain across different ages. Additionally, hippocampal neurogenesis undergoes dynamic changes throughout life and is affected by numerous intrinsic and extrinsic factors that make studying this process in vivo challenging. Here, we show the neurosphere culture system that was developed to isolate neural progenitor cells from the subgranular zone of DG of adult mice (≥8 months) and to differentiate them to mature GCs *in vitro*. These studies would allow us to systematically examine the phenotypical and molecular properties of cells in transit. We were able to successfully isolate and establish multiple stable neurosphere cell lines from adult wild-type mice and compare them to neurosphere cell cultures derived from the young (≤3 months) animals. We found that the dynamics of neurosphere growth derived from the young and the adult animals were different. Neurospheres from the adult brain were consistently smaller in diameter than the ones from the young brain over the course of two weeks in culture. Differentiation of neural progenitors within neurospheres was studied in monolayer assay, where dissociated neurospheres were grown on an adherent substrate. Morphological and molecular marker analysis demonstrated that

neurospheres from young and adult brains generate mature hippocampal GCs in the cell culture environment within 21 days by the markers Prox1 and NeuN. Additionally, neural progenitors from neurospheres from both age groups are multipotent as immunocytochemical staining confirmed differentiation to astrocytes and neurons in monolayer assay. Overall, this approach for isolating and propagating neural progenitor cells from DG of the adult brain offers a novel *in vitro* model of adult neurogenesis and can serve as a platform for investigating important biological questions regarding the development and differentiation of hippocampal GCs generated throughout adult life.

Disclosures: O. Vafaeva: None. **P. Namchaiw:** None. **K.D. Murray:** None. **E. Diaz:** None. **H. Cheng:** None.

Digital Abstract Session

P009. Stem Cell Models of Neurodevelopmental Disorders

Program #/Poster #: P009.01

Topic: A.03. Stem Cells and Reprogramming

Support: Jordan's Guardian Angels Scientific Award

Title: Investigating how rare variants in PPP2R5D are implicated in Jordan's Syndrome

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Abstract: RESEARCH OBJECTIVE/RATIONALE: Jordan's Syndrome is a rare, neurodevelopmental intellectual disability (ID) caused by *de novo* missense mutations in protein phosphatase 2 regulatory subunit b'delta (*PPP2R5D)*. *PPP2R5D* encodes a regulatory subunit of protein phosphatase 2A (PP2A) and is involved in the proper development of and signaling between neurons. Advancements in neuronal disease modeling provide a starting point of investigation to elucidate the molecular underpinnings of *PPP2R5D*-related ID. The overall goal of this project is to evaluate four common variants of Jordan's Syndrome patient-derived fibroblasts that have been reprogrammed into iPSCs, differentiated into neural progenitor cells (NPCs), as well as differentiated to neurons to establish developmental alterations at the cellular and molecular levels. **METHODS/RESULTS:** The most common *PPP2R5D* variant—E198K—and its isogenic control were grown and cultured as induced pluripostent stem cells (iPSCs). RT-qPCR was performed to evaluate gene expression of *PPP2R5D* and western blotting was performed to semi-quantify protein expression. At the cellular level, immunocytochemistry was used to characterize morphology through differentiation from iPSC to neural stem and progenitor cells and mature neurons using antibodies targeting proteins

expressed in each cell type. Because PP2A is a negative regulator of AKT, we are also evaluating how downstream targets of the PI3K/AKT/mTOR signaling cascade is impacted by PPP2R5D variants. A comparison between multiple causative variants and their isogenic controls will determine how *PPP2R5D* influences cellular, molecular, and developmental function. **CONCLUSIONS:** The data obtained from this characterization of *PPP2R5D* variants and their isogenic controls in addition to identifying how downstream targets of PI3K/AKT/mTOR signaling cascade is impacted by these variants would be an important step toward development of biomarkers and could provide us with therapeutic targets for individuals diagnosed with this rare neurodevelopmental disorder.

Disclosures: C.J. Welch: None. **J.L. Carter:** None. **J.A.N.M. Halmai:** None. **N. Bellini:** None. **C. Gonzalez:** None. **J.J. Waldo:** None. **P. Deng:** None. **W. Cary:** None. **J. Nolta:** None. **K.D. Fink:** None.

Digital Abstract Session

P009. Stem Cell Models of Neurodevelopmental Disorders

Program #/Poster #: P009.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: Ricerca Corrente 2019/2020 Italian Ministry of Health
Project: #1758 - Cycle 2018A Lejeune Foundation

Title: Smith-magenis syndrome: patients' ipsc-derived human neural stem cells reveal a cellular deregulation

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Abstract: Copy number variations (CNVs) are often the cause of multiple neurodevelopmental diseases, among them there is Smith-Magenis syndrome (SMS; MIM 182290, incidence 1/15,000) which is linked to interstitial deletions in the 17p11.2 chromosome region spanning around 75 genes. Among them, RAI1 is considered the causative gene of the disease because its nucleotide mutations reproduce almost all SMS clinical features. SMS is a disorder characterized by dysmorphic craniofacial features, brachydactyly, hypotonia, developmental delay and sleep abnormalities. Most individuals present a moderate range of intellectual disability; however, the substantial deleterious behaviours leads to altered adaptive function and a lower perceived cognition. Autistic spectrum disorder has been reported in as many as 90% of SMS subjects. Little is known about RAI1 molecular function and its role in neural development. This gap of knowledge is likely due to limited availability of patient-derived cell models that recapitulate

disease and lack of information of the pathogenetic mechanisms underlying *RAI1* haploinsufficiency. METHODS: We obtained two fibroblast lines from two SMS patients, one carrying point mutation in *RAI1* gene (RAI1-S399P40fx), the other carrying the canonical SMS-CNV (RAI1 del). In order to avoid confounding effects due of the loss of several genes occurring in the CNV, and rather to identify which neurodevelopmental features are specifically altered by the loss of RAI1, Neural Stem Cells where generated upon RAI1-S399P40fx fibroblast reprogramming into induced Pluripotent Stem Cells (SMS-hiNSCs). RESULTS: Both fibroblast lines showed a loss of nuclear RAI1, altered cell proliferation and enhanced cell death. Gene expression analysis showed a series of dysregulated genes. We produced SMS-iPSCs and differentiated them into SMS- hiNSC in order to evaluate how the truncated form of RAI1 might influence the cell fate, the proliferation and differentiation pattern of these cells in vitro. The SMS-hiNSCs were transplanted into immunodeficient mice and differentiated in vitro: our results showed that hiNSC cells carrying RAI1 mutation showed a low ability to differentiate, compared to the control lines consistent with the SMS phenotype.

Disclosures: **J.D. Rosati:** None. **E. Turco:** None. **L. Sireno:** None. **D. Ferrari:** None. **G. Mazzoccoli:** None. **A. Nardone:** None. **R. Onesimo:** None. **L. Bernardini:** None. **M. Pennuto:** None. **A. Vescovi:** None.

Digital Abstract Session

P009. Stem Cell Models of Neurodevelopmental Disorders

Program #/Poster #: P009.03

Topic: A.03. Stem Cells and Reprogramming

Title: Chd8 haploinsufficiency alters the trajectories of human excitatory and inhibitory neurons linking autism phenotypes with developmental slopes

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Abstract: Among the hundreds of In recent years, genetic variants associated to autism, studies have identified tens of autism susceptibility genes. Among them the chromodomain helicase DNA-binding 8 (*CHD8*) is is one of the most frequently mutated bona fide causative genes leading autism-associated gene. While the accompanying macrocephaly *CHD8* haploinsufficiency leads to autism accompanied by macrocephaly, implicatesying cortical development abnormalities in *CHD8* autism, however, itsthe function of the human *CHD8* in human brain development remains unexplored. Here we employecombinedd human cerebral organoids and single cell sequencingtranscriptomics to studydefine the effect of ASD-linked *CHD8* mutations in aon human cortical developmenting brain model. We found thatWe found

that while wild type cerebral organoids recapitulate well the sequence of events of human brain development, C*HD8* haploinsufficiency causes a major disruption of-mutant samples show abnormalneurodevelopmental trajectories. with In particular, we report that C*HD8*-mutantions lead to an accelerated generation of inhibitory neurons and a delayed production of excitatory neurons alongside the ensuing protraction of the and an accelerated generation of inhibitory neurons. The delayed generation of excitatory neuron, and consequent sustained proliferation phase. This imbalance, leads to a significant cerebral organoids enlargement of cerebral organoids aligned to, resembling the macrocephaly observed in patients with *CHD8* mutations. Furthermore,By adopting an isogenic design of employing patient- specific mutations and a mosaic cerebral organoids, we model we show a define genotype-phenotype relationships and uncover their that the abovementioned phenotypes are governed by cell-autonomous nature defects. Finally, our resultsdata assign different *CHD8*-associateddependent molecular defects to particular cell types, pointing to abnormal ribosomal biogenesis and alternative splicing as specifically affected in, respectively, suggesting that inthe radial glial and mature cells *CHD8* mutations are associated with abnormal ribosomal biogenesis while in neuronal compartments.s By identifying linking temporally restricted with cell-type specific effects of human CHD8 mutations, our study uncovers transient developmental slopes as reproducibìlereproducible endophenotypes for neurodevelopmental disease modelling.they are linked to alternative splicing defects. Al together, our study reveals that *CHD8* mutations lead to transient molecular defects in a cell type-specific manner, thus impacting the normal course of human cortical development.

Disclosures: **C. Cheroni:** None. **E. Villa:** None. **A. Lopez Tobon:** None. **C. Sommer:** None. **R. Sacco:** None. **C. Dotter:** None. **B. Oliveira:** None. **A. Cerag Yahya:** None. **J. Morandell:** None. **M. Gabitto:** None. **G. Novarino:** None. **G. Testa:** None.

Digital Abstract Session

P009. Stem Cell Models of Neurodevelopmental Disorders

Program #/Poster #: P009.04

Topic: A.03. Stem Cells and Reprogramming

Title: Transcriptome changes mediated by human cytomegalovirus infection impact neural development

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Abstract: Human cytomegalovirus (HCMV) is a beta herpesvirus that upon infection during pregnancy can cause severe congenital birth defects including microcephaly, vision loss, and hearing loss. Currently, no approved treatment options exist for managing in utero infections. Our previous work has shown that HCMV infection in undifferentiated human induced pluripotent stem cell (iPSC) derived neural progenitor cells (NPCs) and iPSC derived cortical

organoids have changes in calcium signaling. Following infection using GFP-expressing HCMV, NPCs and organoids exhibited reduced baseline calcium levels and were unresponsive when evaluated 5 days post infection. To extend these studies, we have determined that it is feasible to conduct RNA sequencing on different NPC populations within an infected organoid labeled with GFP to understand how the infection impacts the transcriptome. These studies were again conducted using a GFP expressing HCMVTB40e strain. Which creates unique cell populations of varying viral load that can be sorted via FACs sorting and then sequenced. Our studies demonstrate that HCMV infection significantly impairs neural function and several developmental pathways. Interestingly, these developmental gene targets are significantly downregulated in all sorted populations within the infected organoid regardless of viral load. Many of these gene targets fall into the basic helix loop helix transcription factor family which performs crucial roles in neurodevelopment. Specifically, downregulation is observed in the NeuroD/Hes1 pathway which controls neural progenitor cell proliferation and differentiation. Additionally, from our RNA seq results we were able to identify, map, and quantify the expression of HCMV viral genes within our sorted infected organoid populations. In an attempt to understand what viral proteins and genes are responsible for the transcriptomic changes observed, we conducted additional experiments using a modified HCMV virus that allows us to control immediate early (IE) gene expression. These IE genes are demonstrated regulators of fibroblast gene expression, but little is known at this time about their involvement in NPC gene regulation. We found that on their own these genes were not responsible for developmental gene downregulation, so they must interact with other viral genes to cause these effects.

Disclosures: **B.S. O'Brien:** None. **A. Ebert:** None. **M. Schumacher:** None. **R. Mokry:** None. **S. Rao:** None. **S. Terhune:** None. **K. Pulakanti:** None.

Digital Abstract Session

P009. Stem Cell Models of Neurodevelopmental Disorders

Program #/Poster #: P009.05

Topic: A.03. Stem Cells and Reprogramming

Support: European Union's Horizon 2020 research and innovation programme EDC-MixRisk (grant n. 634880)
ENDpoiNTs (grant n. 825759)

Title: From cohorts to organoids: Endocrine disruption in human neurodevelopmental models

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Abstract: Endocrine disrupting chemicals (EDC) are compounds interfering with the human hormonal system. A growing body of evidence correlates early life EDC exposure with human disorders, but the triggered molecular events remain unknown. As opposed to previous studies

focusing on single compounds, we interrogated the impact of the EDC mixture associated to neurodevelopmental adverse outcomes, in the Selma epidemiological study. We integrated the pregnancy cohort results with in vitro exploration of the molecular effects of EDC exposure in human cellular systems. We used two complementary models that transcriptionally resemble the early stages of brain development: i) human fetal primary neural stem cells, and ii) 3-dimensional cortical brain organoids differentiated from human pluripotent stem cells. These models underwent both acute and chronic exposure to several EDC mixture dilutions, including the environmental concentration. Transcriptomic analysis highlighted differentially expressed genes that are significantly enriched for gene ontology categories related to epigenetic regulation, cell proliferation and neuronal maturation. Moreover those results were confirmed by immunofluorescence assays on cortical brain organoids. We also showed how key genes related to different hormonal pathways are affected by EDC exposure and finally that among the EDC dysregulated genes there is significant enrichment for genes with genetic causative role for autism spectrum disorders. In conclusion we showed that real life relevant EDC mixture affect gene networks linked to neurodevelopmental disorders, a finding that should guide EDC policy regulators.

Disclosures: **N. Caporale:** None. **C. Cheroni:** None. **G. Testa:** None.

Digital Abstract Session

P009. Stem Cell Models of Neurodevelopmental Disorders

Program #/Poster #: P009.06

Topic: A.03. Stem Cells and Reprogramming

Support: NIH/NINDS R01NS091616
The Georgia Clinical and Translational Science Alliance (GCTSA; NIH/NCATS)
NIH/RCMI (2U54) MD007602-31

Title: Neuregulin-1 attenuates Plasmodium falciparum histidine rich protein II-induced inflammation and neuronal injury in brain organoids

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Abstract: Brain disorders associated with exposure to *Plasmodium falciparum* (Pf) infection during fetal stages of life are responsible for significant morbidities, mortalities and disabilities globally. Exposure to malaria in pregnancy leads to significant rates of developmental sequelae in newborns but the factors mediating the brain injury during brain development are unclear. Previous *in vitro* studies showed that increased erythrocyte destruction during parasite multiplication release heme and histidine rich protein II (HRPII) into maternal/fetal circulation which disrupts the placental trophoblasts and exposes fetuses to the deleterious factors. Elevated plasma HRPII antigen, widely used as a marker of Pf infection for diagnosing malaria, correlate

with malaria severity and mortality. Previous attempts to assess HRPII-induced brain injury in 2D cell cultures and murine experimental cerebral malaria, did not adequately recapitulate the direct effects of hemolysis and HRPII on brain development, inflammation, and neuronal injury. However, the HRPII effects was attenuated by infusion of Neuregulin1 (NRG1), a neuroprotective factor. We used 3D brain cortical organoids to model the HRPII-induced brain neuronal damage associated with fetal exposure to Pf infection. We hypothesized that HRPII will induce brain inflammation and reduced neuronal viability that can be attenuated by NRG-1. We assessed the direct effects of HRPII on organoid growth, and brain injury (BDNF) marker expression. We tested the role of NRG-1 and its receptor ErbB4 in attenuating HRPII effects. For rigor and reproducibility, we utilized flow cytometry, immunohistochemistry, western blot and gene arrays to investigate neuronal apoptosis and brain organoid inflammation (CXCL10, Ang2 and Ang1) in response to exposure to HRPII during brain organoid development. Our results show that exposure of brain organoids to physiologically relevant concentrations of HRPII during malaria infection compromised their structural architecture and altered expression of cleaved caspase 3 as well as key astrocyte, neuron, and microglia marker genes. Pro-inflammatory factors including CXCL10 and its receptor CXCR3, as well as the ratio of Ang2:Ang1 were elevated. The HRPII effects were mediated by toll-like receptor signaling and were attenuated by NRG-1 via an ErbB4 mediated pathway. In conclusion, our brain organoid model has enabled us to investigate the role of HRPII in malaria-induced fetal brain damage and dysfunction as well as the attenuating effects of NRG1.

Disclosures: **A. Harbuzariu:** None. **A. Nti:** None. **K. Harp:** None. **J. Cespedes:** None. **A. Shaw:** None. **S. Asberry:** None. **J.K. Stiles:** None.

Digital Abstract Session

P010. Advances in Pluripotent Stem Cell Techniques

Program #/Poster #: P010.01

Topic: A.03. Stem Cells and Reprogramming

Support: NBAAD Grant

Title: Urine sample-derived cerebral organoids suitable for studying neurodevelopment and neuroregeneration

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Abstract: Purpose: Human cerebral organoids (COs) have yielded significant discoveries regarding developmental biology, disease mechanisms and pharmacological responses in the central nervous system. Here, we intend to establish cerebral organoids suitable for modeling brain development and neuroregeneration based on somatic cells that are isolated noninvasively. **Methods:** The development of such organoids began with the collection of urinary epithelial cells (UECs) from human urine samples, followed by reprograming them into induced

pluripotent stem cells (hUEC-iPSCs), which were further used for the generation of COs. To comprehensively characterize the cellular and molecular features of our COs, we examined samples at different developmental time points *in vitro*. To check their survival ability and the neuroregenerative potential, the COs were implanted into immunodeficient mouse brains and further examined.
Results: The hUEC-iPSC-developed COs exhibit normal development with neurogenesis and maturation of neuronal cells forming brain layers. These COs produce neurotropic and anti-inflammatory factors that are presumably critical for neurogenesis and neural repair. Several metalloproteases that may facilitate cell migration and microenvironment rearrangement are also present. After transplantation into the mouse cerebrum, vascularization quickly develops in the implanted COs, suggesting their viability and ability to interact with the environment.
Conclusions: Our work begins to reveal the promise of generating personalized COs from cells that are isolated from urine samples. With further adaptation, this human CO platform could form a unique and personalized model for the investigation of neural development and facilitate the innovation of novel therapies for treating neurological diseases.

Disclosures: J. Hu: None. **V. Lin:** None. **Y. Wang:** None.

Digital Abstract Session

P010. Advances in Pluripotent Stem Cell Techniques

Program #/Poster #: P010.02

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R01MH106056
NIH Grant U01DA048279-01
NIH Grant R01 DA04788

Title: Efficient production of pure midbrain dopaminergic neurons from human iPS cells by lineage-promoting transcription factors

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Abstract: Dopaminergic neurons are critical to many motor, motivational, affective, and cognitive systems in the brain and are dysregulated in several disease processes. Derivation of dopaminergic neurons from human induced pluripotent stem cells (hiPSCs) enables isogenic, in vitro modeling of both typical neurodevelopment and disease biology. Current techniques for generating hiPSC-derived dopaminergic neurons yield heterogenous cell populations with variable purity, have inconsistent reproducibility across donor lines and labs, and require extended maturation to achieve functional maturity. We report the efficient production of relatively homogenous induced dopaminergic neurons (iDANs) from hiPSC with lineage-

promoting transcription factor reprogramming combined with chemical selection. Throughout the technique, iDANs (n=5 donors; 3 female, 2 male) showed a gradual development of neuronal morphology and expressed key marker genes at markedly higher levels than in hiPSC at two weeks in vitro (WIV2), with some genes showing further increases in expression at WIV5. By WIV3, iDANs consisted of cells that stained ~90% positive for *Tyrosine Hydroxylase* across five donors with at least two replicate staining experiments each. Immunocytochemistry followed by confocal microscopy confirmed pervasive expression of several additional marker genes. We observed a dramatic maturation-dependent increase in dopamine synthesis from WIV0 to WIV3 by ELISA. Electrophysiologic recordings of individual neurons from two donors (n=15-18 cells each) at WIV5 revealed that iDANs possessed hallmark functional properties of in vivo midbrain dopaminergic neurons, including a-type potassium currents, slow-rising after hyperpolarization potentials, tonic sub-threshold oscillatory activity, and spontaneous burst firing (~1 Hz). Finally, transcriptome analysis with RNA-seq of isogenic (n=2 donors; 1 female, 1 male) hiPSC, iDANs, and induced GABAergic and glutamatergic neurons found that iDANs widely express genes involved in midbrain dopaminergic neuron functional identity and specifically expressed genes identified from previous scRNA-seq studies. For these reasons, iDANs may serve as a vital source of relatively homogenous, functionally mature neurons that can be generated reproducibly across multiple donor lines for modeling normal and disordered biological processes.

Disclosures: **S. Powell:** None. **C. O'Shea:** None. **I. Prytkova:** None. **K. Dobrindt:** None. **R. Elahi:** None. **S. Ho:** None. **P.A. Slesinger:** None. **S. Akbarian:** None. **K. Brennand:** None.

Digital Abstract Session

P010. Advances in Pluripotent Stem Cell Techniques

Program #/Poster #: P010.03

Topic: A.03. Stem Cells and Reprogramming

Support: 1R21NS093540-01
The Renate, Hans, and Maria Hofmann Trust

Title: Human pluripotent stem cell modeling of the ATOH1 lineage reveals a heterochronic shift in transcriptional regulators in the developing human cerebellum

Authors: *H. BEHESTI, A. KOCABAS, D. E. BUCHHOLZ, T. CARROLL, M. E. HATTEN; Rockefeller Univ., New York, NY

Abstract: Brain development is regulated by conserved transcriptional programs across species, but little is known about divergent mechanisms that create species-specific characteristics. Among brain regions, the cerebellum is now recognized to contribute to human cognitive evolution having a broad range of non-motor cognitive functions in addition to motor control. Emerging studies highlight the complexity of human cerebellar histogenesis, compared with non-human primates and rodents, making it important to develop methods to generate human cerebellar neurons that closely resemble those in the developing human cerebellum. Here we

report a rapid and simple protocol for the directed derivation of the human ATOH1 lineage, the precursor of all excitatory cerebellar neurons, from human pluripotent stem cells (hPSC), and strategies to decrease culture variability; a common limitation in hPSC studies. By Translational Ribosome Affinity Purification (TRAP)-seq, the ATOH1 lineage most closely resembled human cerebellar tissue in the second trimester. Unexpectedly, TRAP-seq identified a heterochronic shift in the expression of RBFOX3 (NeuN) and NEUROD1, which are classically associated with differentiated neurons, within granule cell progenitors (GCPs) in the human external granule layer. This molecular divergence may provide the mechanism by which the GCP pool persists into year two post birth in humans, but only lasts for two weeks in mice. Our approach provides a scalable *in vitro* model of the human ATOH1 lineage that yields cerebellar granule cells within 48 days as well as a strategy for identifying uniquely human cellular and molecular characteristics.

Disclosures: H. Behesti: None. **A. Kocabas:** None. **D.E. Buchholz:** None. **T. Carroll:** None. **M.E. Hatten:** None.

Digital Abstract Session

P010. Advances in Pluripotent Stem Cell Techniques

Program #/Poster #: P010.04

Topic: A.03. Stem Cells and Reprogramming

Support: NSF Grant OIA-1457888
Arkansas EPSCoR Program

Title: Tempo cellulose substratum for neural stem cell differentiation in vitro

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Abstract: Cellulose based materials are being studied extensively for applications in the biomedical field due to its abundance, amenability and biocompatibility. We created novel, stable TEMPO cellulose materials utilizing the TEMPO/NaOCl/Oxone® ($KHSO_5$) with microwave irradiation method. We further tested these TEMPO cellulose materials as an extracellular matrix substratum to support *in vitro* neural stem cell (NSC) differentiation into neurons/oligodendrocytes (ODCs). Rat NSCs harvested at E14 (embryonic day 14) were propagated and differentiated on a TEMPO cellulose-coated culture surface in neuron-favoring conditions for 7 days. At day 7, immunocytochemical staining was performed to determine the percentage of cells positive for neuronal marker βIII tubulin and astrocytic marker GFAP (glial marker glial fibrillary acidic protein) relative to the total cell count. About 33% and 25% of NSCs differentiated into neurons and astrocytes on TEMPO cellulose substratum, respectively,

comparable to the traditional poly-D-lysine surface. Separate batches of NSCs were propagated for 10 days on the TEMPO cellulose surface under ODC-favoring culture conditions. By Day 10, about 70% and 30% of NSCs differentiated into Rip (receptor interacting protein)+ ODCs and GFAP+ astrocytes, respectively, similar to those on the precursor micron cellulose surface. These results indicate that TEMPO cellulose is a viable material supporting stem cell survival and differentiation *in vitro*.

Disclosures: K.D. Sharma: None. **S. Dachavaram:** None. **S. Pandanaboina:** None. **P.A. Crooks:** None. **J.Y. Xie:** None.

Digital Abstract Session

P010. Advances in Pluripotent Stem Cell Techniques

Program #/Poster #: P010.05

Topic: A.03. Stem Cells and Reprogramming

Support: NIH/NIMH 1K99MH119327-01
Burroughs Wellcome Fund 1018707
NIH/NIMH U01MH105669
NIH/NIMH U01MH115727

Title: Exploration of human neural phenotypic diversity through mixed-donor cultures of stem cell-derived NGN2-accelerated progenitors

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Abstract: Our species is characterized by an immense diversity in neurological and psychological traits that partially originates from differences in the behaviors of cells present during the earliest stages of brain development, including neural progenitor cells (NPCs). We currently know little about the role of genetic variation on the cellular and molecular mechanisms underlying NPC diversity given the rarity of manipulatable human brain tissue and inefficiencies with conventional stem cell-derived NPC induction methods. Here, we describe a novel *in vitro* culture system that captures the influence of genetic diversity on specific NPC phenotypes on a large scale. Stem cell-derived NGN2-accelerated Progenitor cells (SNaPs) are human dorsal telencephalic NPCs created through a highly reproducible 48-hour induction protocol. We pooled SNaPs generated from dozens of unique donors into a shared culture environment known as a "village-in-a-dish," which enabled high-throughput and unbiased cell line comparisons under conditions that minimize technical noise. We then processed our SNaP villages through new whole genome ("Census-seq") and scRNA-seq ("Dropulation") analytical pipelines to reveal dramatic differences across cell lines in proliferation dynamics and gene expression. We detected around 900 expression quantitative trait loci (eQTL), including a single

nucleotide polymorphism in the promoter of the antiviral *IFITM3* gene. To assess functional impact, we infected a SNaP village with the neurotropic Zika virus and discovered that this *IFITM3* SNP could explain one-third of the variation in viral susceptibility across cell lines. Our work uncovers the impact of genetic diversity on certain neurodevelopmental processes and establishes a blueprint for future endeavors seeking to do the same.

Disclosures: **M.F. Wells:** None. **M. Salick:** None. **J. Mitchell:** None. **E. Hill:** None. **J. Nemesh:** None. **S. Ghosh:** None. **R. Nehme:** None. **O. Pietilainen:** None. **A. Kaykas:** None. **S. McCarroll:** None. **K. Eggan:** None.

Digital Abstract Session

P011. Transplantation

Program #/Poster #: P011.01

Topic: A.04. Transplantation and Regeneration

Support: R01DA044761

Title: Neovascularization for optimal survival of neural transplants

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Abstract: Neovascularization for optimal survival of neural transplants Authors: Krzyspiak, J., Yan, J., Galinski, B., Lituma, P., Zukin, S., Castillo, P., Weiser, D., Khodakhah, K., Hebert, J. Dominick P. Purpura Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, USA
Disclosures: None.
The transplantation of neural stem cells holds promise for improving function in the damaged brain. After transplantation, however, transplant-derived cells can be greatly reduced in numbers. There is growing evidence that supporting cell types may be required to facilitate the success of a transplant in contrast to transplanting pure populations of cells. Specifically, there is evidence that neovascularization may be critical for transplant cell survival. As in the fetus where vascularization must match the physiological demands of each growing tissue, vascularization of neural cell transplants might also need to occur rapidly to promote neuron survival, differentiation, and function. Here we test the hypothesis that combining vascular and neuronal precursors improves engraftment by transplanting mixed embryonic neocortical cells into adult mice with neocortical strokes. Our studies with transplants of dissociated embryonic 12.5 forebrain cells into the young adult neocortex of unaffected and stroke-affected male and female mice show that transplants generate large grafts (up to 15 mm^3) containing upper and lower cortical neuron identities. The neurons are often arranged in pseudo-layers, which can project to appropriate host targets and become synaptically integrated. Grafts also formed donor-derived

vessels that anastomosed with host vessels to perfuse blood. To show that donor-derived vessels are integrating with the host circulation, we used an intravenous fluorescent dye to confirm fusion with host vessels. Graft size increased with the proportion of total vasculature that came from the donor, with the contribution of donor-derived vessels within the graft increasing as a function of distance from the graft-host border. Interestingly, graft size was also greater when cells were transplanted at stroke sites than when transplanted in uninjured neocortex, suggesting that stroke sites are especially conducive to donor-derived vascularization of the graft. We observe that within transplants on the stroke- affected side, blood vessels primarily develop from donor-derived cells, in contrast to the control side. Indeed, excluding vascular cells from the donor cell population strictly limited graft size. Thus, inclusion of vessel-forming vascular cells with NSCs is required for more efficient engraftment and ultimately tissue repair.

Disclosures: J.E. Krzyspiak: None. **J. Yan:** None. **P.J. Lituma:** None. **P.E. Castillo:** None. **K. Khodakhah:** None. **J. Hebert:** None. **S. Zukin:** None.

Digital Abstract Session

P012. Regeneration: CNS

Program #/Poster #: P012.01

Topic: A.04. Transplantation and Regeneration

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No.: 07181356)
National Natural Science Foundation of China (NSFC) (Ref. no.: 81971149)

Title: Bioactive compounds induce long-distance axon regeneration for functional restoration

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Abstract: In central nervous system (CNS), the hostile extrinsic microenvironment and limited intrinsic regenerative capacity of adult neurons remained major obstacles for successful axon regeneration after injuries. By contrast, adult peripheral neurons are capable of regenerating their injured axons. However, the slow rate of regeneration (1-2mm/day) significantly limited the regain of motor functions as the regenerated axons fail to reinnervate their distal targets largely due to chronic denervation. Therefore, there is an urgent need to search for bioactive compounds which enable long-distance axon regeneration for clinical meaningful functional restoration. Wolfberry (*Lycium barbarum*) is a widely used traditional Chinese medicine known to exhibit a broad range of neuroprotective effects in various neurological disorders such as glaucoma and

spinal cord injury. In this study, we first demonstrated that *Lycium barbarum* polysaccharides (LBP, major bioactive constituent of wolfberry) promoted sensory and motor functional restoration after severe peripheral nerve injury (PNI). In CNS, intravitreal injections of LBP induced robust axonal regrowth 14 days after optic nerve crush (ONC) injuries. In an attempt to screen for bioactive small molecules that recapitulated the transcriptional change induced by LBP in peripheral neurons, we successfully identified two FDA-approved small molecules which promoted axon regeneration in both PNS and CNS. Similar to LBP, these two small molecules markedly enhanced the intrinsic growth capacity of axotomized dorsal root ganglion neurons *in vitro*. The distal extent of *in vivo* sensory axonal regrowth was also increased in mice treated with both small molecules 3 days after PNI. More importantly, we confirmed that both FDA-approved small molecules could facilitate long-distance axon regeneration after CNS injuries. While only limited axons can regenerate across the lesion in vehicle-treated mice, mice treated with both small molecules displayed remarkable axon regeneration 14 and 28 days after ONC. Some axons could even regenerate along the entire length of optic nerves and reached the optic chiasm 28 days after ONC in mice treated with these small molecules. In both time points, only 20% of RGCs were survived in vehicle-treated controls, which was dramatically increased in mice treated with both small molecules. Taken together, our study demonstrated the therapeutic potential of LBP and two FDA-approved small molecules in promoting axon regeneration after injuries. Further study is required for optimizing the dosage of these bioactive compounds in order to improve the clinical outcomes after nervous system injury.

Disclosures: **N.P. Au:** None. **G. Kumar:** None. **P. Asthana:** None. **D.H. Geschwind:** None. **G. Coppola:** None. **R.C.C. Chang:** None. **K.F. So:** None. **C.H.E. Ma:** None.

Digital Abstract Session

P012. Regeneration: CNS

Program #/Poster #: P012.02

Topic: A.04. Transplantation and Regeneration

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No.: 05160126)

Title: Overexpression of basic helix-loop-helix protein induces sustained long-distance axon regeneration and elicits neural activity at distal brain targets

Authors: ***C. H. E. MA**[1], N. P. B. AU[1], G. KUMAR[1], S. K. CHIU[2], D. H. GESCHWIND[3], G. COPPOLA[3];
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Abstract: Peripheral nerve injury (PNI) primes the peripheral neurons to an active growing state via reactivation of a transcription program that recapitulate some of cellular processes for robust axonal outgrowth during developmental stage. These neurons are capable of regenerating their

injured axons at an extremely slow rate (i.e. 1-2mm/day) after PNI. However, patients with proximal PNI, such as brachial plexus injuries, usually have poor clinical outcome even after immediate surgical repair after injury. Since long-distance axon regeneration is required for these patients, the regenerated axons fail to form functional synapses at the motor end plates largely due to chronic denervation. In contrast, the neurons in central nervous system (CNS) have limited intrinsic regenerative capacity, and virtually no axons can regenerate across the lesion for target reinnervation after CNS injuries. This leads to permanent loss of motor functions in patients with CNS injuries such as spinal cord injury and optic neuropathy. There is an urgent need to develop new therapeutic strategy to promote axon regeneration and functional recovery after injuries to our nervous system. Previously, we identified basic helix-loop-helix (bHLH) protein as a key regulator for successful axon regeneration. Overexpression of bHLH induced robust axonal regrowth, and accelerated sensory and motor functional recovery after PNI. Here, we tested if overexpression of bHLH could also induce robust axon regeneration after CNS injuries. Two weeks before optic nerve crush injuries, adeno-associated virus (AAV) overexpressing bHLH (i.e. AAV-bHLH) was intravitreally injected to the injured eye for transduction of bHLH in retinal ganglion cells (RGCs). The extent of axonal regrowth and the survival of RGCs was assessed at days 14 and 28 post-injury, respectively. Strikingly, we observed a marked increase in axon regeneration with increased RGC survival in AAV-bHLH-treated mice 2 and 4 weeks after optic nerve crush injury, respectively. In particular, some of the regenerating axons could even reach optic chiasm in AAV-bHLH-treated mice 4 weeks after injury. By combining bHLH overexpression with *Pten* deletion, a gene manipulation known to promote CNS regeneration, we successfully detected reliable eye-evoked local field potentials in superior colliculus (SC) upon optical stimulation in injured RGCs 6 weeks after injury, suggesting that a considerable amount of regenerating axons could reinnervate to distal brain target at SC. Screening of small molecules identified bioactive compounds that induce transcription program recapitulating the growth-promoting effects by bHLH overexpression.

Disclosures: **C.H.E. Ma:** None. **N.P.B. Au:** None. **G. Kumar:** None. **S.K. Chiu:** None. **D.H. Geschwind:** None. **G. Coppola:** None.

Digital Abstract Session

P012. Regeneration: CNS

Program #/Poster #: P012.04

Topic: A.04. Transplantation and Regeneration

Support: The Miriam and Sheldon G. Adelson Medical Research Foundation (LB)
NEI R01EY05690 (LB)
NIH IDDRC grant P30 HD018655 to Boston Children's Hospital

Title: Stromal cell-derived factor 1 is a macrophage-derived growth factor for non-α retinal ganglion cells

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Abstract: Although adult mammalian retinal ganglion cells (RGCs) lose the ability to regenerate injured axons, this loss can be partially reversed by factors expressed in cells of the innate immune system. Stromal cell-derived factor 1 (SDF1), one of the key factors mediating the effects of intraocular inflammation on optic nerve regeneration, is produced by infiltrative macrophages and acts synergistically with oncomodulin (Ocm), which is expressed by neutrophils and macrophages)[1]. Here we report that regeneration induced by SDF1 treatment arises from distinct populations of non-αRGCs. Using Kcng4-GFP mice (Kcng4-Cre:STOP$^{flx/flx}$ GFP) in which αRGCs express GFP, we found that, with SDF1 treatment (via AAV2-monomeric SDF1, AAV2-mSDF1), GFP^{+} axons (from αRGCs) were seen proximal to the injury site but failed to extend beyond this point. Thus, the regenerating axons do not arise from αRGCs. This result contrasts sharply with the effects of Pten inactivation, in which growing axons are GFP^{+} and thus from αRGCs[2]. When combined, treatment with AAV2-mSDF1 and AAV2-shPten induced high levels of regeneration, although most regenerating axons were GFP negative, and thus again not from αRGCs. By examining the survival of different RGC subtypes, we found that SDF1, either alone or combined with Pten inactivation, did not change the percentile of surviving αRGCs, ipRGCs and oo-dsRGCs among the total surviving RGC population after optic nerve injury. This is also different from another treatment (Sox11 overexpression) that stimulates non-αRGC regeneration but is toxic to αRGCs[3]. Our study demonstrates that the RGC subtypes that are stimulated to regenerate are treatment-specific. SDF1-induced regeneration is non-αRGC based, and when combined with Pten inactivation, which otherwise targets αRGCs, SDF-1 enhances strong regeneration from non-αRGCs. Thus, the regenerative RGC subtypes are not a simple addition of two pools of regenerating RGC subsets. These findings may help uncover important interactions among signaling mechanisms in RGCs. **References** 1. Y. Yin *et al.*, Annual Meeting, SfN, San Diego, CA, 2018. 2. X. Duan *et al.*, *Neuron* **85**, 1244-1256 (2015). 3. M. W. Norsworthy *et al.*, *Neuron* **94**, 1112-1120 e1114 (2017).

Disclosures: Y. Yin: None. **O. Strelko:** None. **M. Ma:** None. **L.I. Benowitz:** None.

Digital Abstract Session

P012. Regeneration: CNS

Program #/Poster #: P012.05

Topic: A.04. Transplantation and Regeneration

Support: Medical Research Council MRC Grant

Title: In vivo modelling of human cortical axon regeneration

Authors: *S. KHAN[1], M. TORTORA[1], I. ALIC[2], N. O'BRIEN[2], C. LUFF[3], N. GROSSMAN[3], D. NIZETIC[2], V. DE PAOLA[1];
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Abstract: Injured central nervous system neurons display limited axon regeneration. There is a pressing need for new experimental models that can mimic human-specific features and for strategies that can enhance axon regeneration of mature human neurons. To address these issues, we developed an approach to investigate the dynamics of injured human cortical synaptic networks using donor-derived cells transplanted into the mouse brain, intravital structural and functional longitudinal imaging and laser microsurgery. Being able to follow the fate of individual fluorescent axons over time *in vivo*, we have been able to observe unequivocal human axon regeneration occurring as early as 6-hours post-injury, with axons reaching and growing past the lesion site at 3 weeks post-transplantation. Most immature human axons (~ 70%) were able to regenerate past the lesion site within 24-hours, while fewer regenerated as early as 6-hours post-lesion (~12% of all regenerating axons). In contrast to mouse cortical axons, regenerating human axons followed their original trajectories. The maximum regrowth observed was ~2 mm over 7 days. Importantly, the proportions of regenerating axons significantly decreased with maturity of grafted human cortical neurons at 3 months post-transplantation. In addition, mature non-regenerating human axons progressively retracted over 7 days *in vivo*. To investigate the role of neural activity in regulating intrinsic human axon regenerative capacity, we measured axonal calcium signals before and after injury in regenerating and non-regenerating human cortical neurons, using the activity-dependent sensor GCaMP6s and an activity-independent cell-filling reporter tdTomato. Mapping calcium responses to human axonal injury at single-cell-resolution *in vivo* revealed significantly higher pre-lesion activity levels of mature regenerating human axons compared to mature non-regenerating and immature human axons. Ongoing experiments are aimed at using non-invasive temporal interference stimulation technology to enhance regeneration fractions of mature human cortical axons. Overall, this experimental system recapitulates many important features of the physiological axon response to injury, including proximal axon fragmentation, the loss of regeneration potential with maturity, progressive die-back and retraction of mature human axons from the injury site, as well as sprouting and branching. Together, these data establish a new *in vivo* model and method to study human cortical axon regeneration *in vivo*.

Disclosures: S. Khan: None. **M. Tortora:** None. **I. Alic:** None. **N. O'Brien:** None. **C. Luff:** None. **N. Grossman:** None. **D. Nizetic:** None. **V. De Paola:** None.

Digital Abstract Session

P013. Regeneration: PNS

Program #/Poster #: P013.01

Topic: A.04. Transplantation and Regeneration

Support: The General Research Fund grant from the Research Grant Council of the Hong Kong Special Administrative Region Government (Ref. No.: 11100519).

Title: Targeting mitochondrial dynamics as a therapeutic intervention to promote axon regeneration

Authors: *P. ASTHANA, N. P. B. AU, R. CHAND, W. Y. TAM, C. H. E. MA; Neurosci., City Univ. of Hong Kong, Kowloon Tong, Hong Kong

Abstract: Axon regeneration is an energy-demanding process which involves active polymerization of actin and tubulin (building blocks of microtubule) at the barbed end of a growth cone for proper axon elongation. After a peripheral nerve injury (PNI), axonal transport of mitochondria markedly increases in the regenerating axons to fulfil the increased energy demand at distal axons. In contrast, the axonal mitochondrial transport was significantly impaired after spinal cord injury (SCI; a form of CNS injury), leading to limited extent of axonal regrowth in SCI. This suggests that active mitochondrial transport plays a pivotal role in successful axonal regrowth after injuries. In the current study, we first examined the mitochondrial transport in peripheral neurons overexpressing a human heat shock protein 27 (hHsp27), a known regeneration-associated gene to promote axonal regrowth after PNI. In hHsp27-overexpressing neurons, we observed an increased number of mitochondria at the most distal axons with increased mitochondrial size. Kymograph analysis revealed a marked increase in the velocity of axonal mitochondrial transport in these neurons compared with the wild-type controls in the same culture. Blockade of axonal mitochondrial transport using Ciliobrevin D completely abolished the growth-promoting effects of hHsp27 overexpression in cultured dorsal root ganglion (DRG) neurons. Next, we tested if a bioactive small molecule known to promote mitochondrial fusion could recapitulate the growth-promoting effects of hHsp27 overexpression via modulation of mitochondria dynamics. In line with our hypothesis, this small molecule markedly enhanced mitochondrial clustering at the distal axons of axotomized DRG neurons, and promoted mitochondrial fusion (as indicated by larger mitochondrial size) via up-regulation of mitochondrial fusion proteins Opa1 and Mfn2. Also, this small molecule markedly enhanced axonal mitochondrial transport in cultured DRG neurons via up-regulation of the molecular machineries for both anterograde and retrograde axonal transport. Remarkably, this small molecule markedly enhanced the intrinsic growth capacity from axotomized DRG neurons, and accelerated the *in vivo* axonal regrowth after PNI. Taken together, our study shed new light for the development of mitochondria-based therapy to orchestrate mitochondria dynamics which enable long-distance axon regeneration for target reinnervation and functional recovery after PNS injuries. Further study is required to examine if modulation of mitochondrial dynamics using this small molecule could also induce long-distance axon regeneration after CNS injuries.

Disclosures: P. Asthana: None. **N.P.B. Au:** None. **R. Chand:** None. **W.Y. Tam:** None. **C.H.E. Ma:** None.

Digital Abstract Session

P014. Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Program #/Poster #: P014.01

Topic: A.05. Axon and Dendrite Development

Support: the National Research Foundation (NRF) of Korea (NRF-2020R1A5A1019023, NRF-2018R1A2B6004759
NRF- 2017M3C7A1029611)
Korea Health Technology R&D Project (HI18C0789) through the Korea Health Industry Development Institute (KHIDI)

Title: Pathogenic GRM7 mutations associated with neurodevelopmental disorders impair axon outgrowth and presynaptic terminal devleopment

Authors: *J. SONG, Y. SUH;
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Abstract: Metabotropic glutamate receptor 7 (mGlu7) is an inhibitory heterotrimeric G protein-coupled
receptor that modulates neurotransmitter release and synaptic plasticity at presynaptic terminals in the mammalian central nervous system. Recent studies have shown that rare mutations in glutamate receptors and synaptic scaffold proteins are associated with neurodevelopmental disorders (NDDs). However, the role of presynaptic mGlu7 in the pathogenesis of NDDs remains
largely unknown. Recent whole-exome sequencing studies in families with NDDs have revealed that several missense mutations (c.1865G>A:p.R622Q; c.461T>C:p.I154T; c.1972C>T:p.R658W and c.2024C>A:p.T675K) or a nonsense mutation (c.1757G>A:p.W586X) in the GRM7 gene may be linked to NDDs. In the present study, we investigated the mechanistic links between GRM7 point mutations and NDD pathology. We find that the pathogenic GRM7 I154T and R658W/T675K mutations lead to the degradation of the mGlu7 protein. In particular, the GRM7 R658W/T675K mutation results in a lack of surface mGlu7 expression in heterologous cells and cultured neurons isolated from male and female rat embryos. We demonstrate that the expression of mGlu7 variants or exposure to mGlu7 antagonists impairs axon outgrowth through the MAPK-cAMP-PKA signaling pathway during early neuronal development, which subsequently leads to a decrease in the number of presynaptic terminals in mature neurons. Treatment with an mGlu7 agonist restores the pathological phenotypes caused by mGlu7 I154T but not by mGlu7 R658W/T675K due to its lack of neuronal surface expression.
These findings provide evidence that stable neuronal surface expression of mGlu7 is essential for neural development and that mGlu7 is a promising therapeutic target for NDDs.

Disclosures: J. Song: None. **Y. Suh:** None.

Digital Abstract Session

P014. Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Program #/Poster #: P014.02

Topic: A.05. Axon and Dendrite Development

Support: NSF Grant #1826871
NSF Grant #1748523

Title: Investigating the effects of temperature on the regulation of the expression of neuronal GFP in C. elegans

Authors: *B. G. GARCIA-GONZALEZ, S. J. AVANT, A. E. CARRASCO-PENA;
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Abstract: Jeopardized genetic and environmental factors such as the disruption to the mTOR signaling pathway and prenatal stress are hypothesized to lead to the development of schizophrenia. Researchers Lee and colleagues observed decreased levels of DPYSL2 in the prefrontal cortex and hippocampus of rats exposed to prenatal stress. DPYSL2 is important for axonal outgrowth, cell differentiation, and promoting proper microtubule assembly in humans. The *C. elegans* homolog of DPYSL2, UNC-33, is also important for proper axonal formation and elongation. The purpose of this study is to monitor the effects of environmental stress, using the stressor of high temperatures, on the expression of GFP driven by the *unc-33* promoter. Preliminary studies from our laboratory have shown that neuronal GFP expression driven by the *unc-33* promoter is reduced at high temperatures. Herein we present results from a novel thermal tolerance curve investigating the gradual effect of increasing temperatures on GFP expression driven by the *unc-33* promoter. To this end, *C. elegans* hermaphrodites were incubated at various temperatures until they reached an L2/L3 stage. Using confocal microscopy, the head of *C. elegans* hermaphrodites was imaged to determine the GFP expression in the nerve ring and glial cells. The analysis of the quantification of thirty nematodes per condition shows that GFP expression in neurons decreases as temperature increases. While the neuronal expression is reduced due to this high temperature, glial expression is increased. This study provides insight into the role that high temperature has on regulating the expression of neuronal *unc-33*, one of the molecular players shown to be negatively impacted by prenatal stress in rats and by polymorphic variations in patients suffering from Schizophrenia.

Disclosures: B.G. Garcia-Gonzalez: None. **S.J. Avant:** None. **A.E. Carrasco-Pena:** None.

Digital Abstract Session

P014. Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Program #/Poster #: P014.03

Topic: A.05. Axon and Dendrite Development

Support: HRC Grant 9134-3712384
AMRF PhD Scholarship

Title: The role of extracellular matrix molecule hyaluronan in hippocampal neuron morphological development in vitro

Authors: *M. I. ABRAHAM, R. N. KARUNASINGHE, T. M. FOWKE, J. M. DEAN; Physiol., Univ. of Auckland, Auckland, New Zealand

Abstract: The brain's extracellular matrix provides key structural and functional support to neurons and glial cells. Hyaluronan is a glycosaminoglycan sugar, and a major component of the developing brain's extracellular matrix. Hyaluronan metabolism is regulated by the family of hyaluronan synthases (HAS1-3) and hyaluronidases (Hyal1-5, SPAM1, CEMIP, and TMEM2), which synthesise and degrade hyaluronan, respectively. Hyaluronan has a wide range of functions including both structural and functional, and in particular can activate signaling pathways important for non-neuronal cell growth. However, a specific role for hyaluronan in controlling neuronal development remains unclear. Herein, we examined the expression and function of hyaluronan, HASs, and Hyals in developing hippocampal neurons *in vitro*. Dissociated hippocampal neuron cultures (<5% glial cells) were generated from Sprague Dawley rat embryos (E18) and collected at 1-21 days *in vitro (DIV)*. Cultures were fixed for immunocytochemical localisation studies. Live-cell images were acquired with a Nanolive 3D Cell explorer, a holographic tomographic microscope that resolves lamellipodia, transient early developmental structures associated with cell motility and neurite outgrowth. Cultured cells exhibited robust mRNA (RT-qPCR) and protein (immunocytochemistry) expression of HAS2-3, Hyal1-3 (but not HAS1, Hyal4, 5, SPAM1, or CEMIP), and hyaluronan, from (DIV) 1 to 21 (n=4 independent cell cultures per time-point). Pharmacological HAS inhibition with 4-methylumbelliferone (4-MU), or Hyal inhibition with L-ascorbic acid 6-hexadecanoate (VCPAL), altered early neuron morphological development, and both treatment groups had significantly reduced lamellipodia number and area, and filopodia numbers at DIV2 compared to controls (n=10 cells for each treatment condition from 4 independent cell cultures). HAS inhibition also increased primary neurite number and average neurite length compared to controls, as well as an increase in axonal outgrowth from 24h in culture (n=10 cells for each treatment condition from 4 independent cell cultures). Conversely, Hyal inhibition reduced primary neurite number (DIV3) and average neurite length (DIV2 & 3). These data suggest that hippocampal neurons can endogenously metabolise hyaluronan, and that neuronally-regulated hyaluronan metabolism is important for the early stages of morphological development, including growth cones and neurite outgrowth.

Disclosures: M.I. Abraham: None. **R.N. Karunasinghe:** None. **T.M. Fowke:** None. **J.M. Dean:** None.

Digital Abstract Session

P014. Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Program #/Poster #: P014.04

Topic: A.05. Axon and Dendrite Development

Support: NIH SIG grant 1S10OD016328-01
NIH/NIDCD 010796
NIH 5T32DC010775

Title: Caspase-3 prefers a non-canonical consensus sequence associated with cytoskeletal substrates during auditory brainstem development

Authors: *F. WEGHORST[1], Y. MIRZAKHANYAN[2], P. D. GERSHON[2], K. S. CRAMER[1];
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Abstract: The auditory brainstem contains extremely precise circuitry responsible for determining sound source location based on interaural differences in sound arrival time. We previously showed that the apoptotic protease caspase-3 is necessary for development of the auditory brainstem projection from chick *nucleus magnocellularis* (NM) to *nucleus laminaris* (NL). Inhibition of caspase-3 prior to the period of developmental cell death in auditory brainstem nuclei resulted in NM axon mistargeting. However, the mechanisms by which caspase-3 facilitates the formation of the NM-NL projection are not known. To this end, we characterized proteins cleaved by caspase-3 in the chick auditory brainstem. Embryos were treated with intraventricular injection of the caspase-3 inhibitor z-DEVD-fmk or vehicle solution on embryonic days 9 and 10. Brainstems were harvested and subjected to proteomic mass spectrometry. Peptidomes were filtered for peptides likely generated by *in vivo* caspase-3 activity based on two criteria: the peptide terminated in a caspase-like cleavage site (i.e. with a C-terminal aspartate or glutamate residue) and was present in vehicle-injected brainstems but absent in caspase-3-inhibited brainstems. Proteins with at least one such peptide were considered likely auditory brainstem caspase-3 (ABC3) substrates. We generated an IceLogo of the ABC3 consensus cleavage site and found that the resulting tetrapeptide sequence N-terminal of the scissile bond (DHRD) bore some similarity to that of the canonical caspase-3 consensus site (DEVD). However, the substitution of "EV" with "HR" suggested a systematic change in the usual substrate preference of caspase-3. We used functional annotation enrichment analysis to determine whether ABC3 substrates with "HR" cleavage sites were associated with a specific protein category disproportionately targeted by caspase-3 activity. Compared to the background of all ABC3 substrates, substrates with an H or R at the same position as the consensus sequence were enriched for the the GO term "structural constituent of cytoskeleton." The observed consensus sequence therefore reflects a shift toward cleavage of cytoskeletal proteins, which are essential for axon guidance and synapse formation, suggesting a specialized, non-apoptotic function for caspase-3 in auditory brainstem circuit formation.

Disclosures: F. Weghorst: None. **Y. Mirzakhanyan:** None. **K.S. Cramer:** None. **P.D. Gershon:** None.

Digital Abstract Session

P014. Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Program #/Poster #: P014.05

Topic: A.05. Axon and Dendrite Development

Support: NORTE 2020 - Norte Portugal Regional Operational Programme, Portugal 2020; NORTE-01-0145-FEDER-028623
FCT - Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior; PhD Scholarship: SFRH/BD/143926/2019

Title: Microtubules as a determinant of DRG axon asymmetry and regenerative capacity

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Abstract: In dorsal root ganglion (DRG) neurons a single axon bifurcates into a peripheral and a central branch with different functions and properties. Whereas the peripheral DRG axon regenerates, the central DRG axon only gains regenerative capacity after lesion to the peripheral axon -conditioning lesion (CL) effect. We have previously shown that a CL induces a global increase in axonal transport that extends to DRG central axons, supporting their regeneration. To further understand the mechanisms underlying central-peripheral DRG asymmetry and the conditioning lesion effect, we analyzed the microtubule (MT) cytoskeleton of DRG axons. By live imaging of DRG explants from Thy1-EB3-GFP mice, we observed that MT dynamics is asymmetric in the DRG, with central axons displaying a more dynamic cytoskeleton. Importantly, peripheral axon injury, but not central axon injury, decreased MT dynamics in both peripheral and central axons, leading to a more stable MT cytoskeleton following injury. While the severing enzymes spastin and katanin control MT dynamics by destabilizing MTs, Tau binds to MTs increasing their stability and protecting them from the action these enzymes. We observed that in the DRG central branch the expression of katanin and spastin is dramatically increased, while the expression of Tau is higher in the DRG peripheral branch. Our results suggest that Tau in the DRG peripheral branch protects MTs from the action of severing enzymes and, consequently, increases the stability of this DRG branch. We next investigated if spastin is a specific regulator of DRG MT dynamics and axonal transport asymmetry, by crossing a spastin knock-out mice with either the Thy1-EB3-GFP mouse or Thy1-MitoRFP mouse. We observed that in the absence of spastin loss of asymmetry of MT dynamics occurs in DRG axons as well as disrupted mitochondria axonal transport, particularly in central axons. Finally, *in vivo*, the absence of spastin led to decreased regeneration of DRG peripheral axons following sciatic nerve crush and DRG central axons following a conditioning lesion. In summary, our results support that spastin is a specific regulator of MT dynamics in DRG neurons underlying their central-peripheral asymmetry, being an essential enzyme for optimal axon regeneration to occur.

Disclosures: **A. Costa:** None. **B. Murillo:** None. **T. Misgeld:** None. **M. Brill:** None. **M. Sousa:** None.

Digital Abstract Session

P014. Axon Growth and Guidance: Adhesion and Cytoskeletal Dynamics

Program #/Poster #: P014.06

Topic: A.05. Axon and Dendrite Development

Title: Neuron navigator 1 in early neural development

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Abstract: Proper regulation of the cytoskeleton is essential for neurodevelopment. Cytoskeleton associated proteins integrate extra- and intracellular signals to influence actin and microtubule dynamics in the developing neuron. These cytoskeleton dynamics are necessary for later circuit integration and brain development, but many of the proteins involved and mechanisms have yet to be elucidated. Neuron Navigator 1 (Nav1) is a microtubule +TIP protein highly expressed during brain development. Nav1 is necessary for neuritogenesis, but the underlying mechanisms are relatively unknown. Using RNAi and knockout strategies in primary neurons and cell lines, we find that Nav1 is important for coordinating the cytoskeleton of the cell periphery for proper cell morphogenesis. This includes an unexpected role in regulating F-actin in the growth cone, as well as a novel role in regulating actin-membrane coupling. These data highlight the importance of Nav1 in cell morphogenesis, as well as reveal new roles of this +TIP protein in regulating actin and plasma membrane behavior.

Disclosures: **R. Powers:** None. **B. Calabrese:** None. **E. Desai:** None. **S.L. Halpain:** None.

Digital Abstract Session

P015. Dendritic Growth and Branching

Program #/Poster #: P015.01

Topic: A.05. Axon and Dendrite Development

Support: The Cleon C. Arrington Research Initiation Grant Program (RIG-93) to AMM
The National Ataxia Foundation to AMM
Second century initiative graduate fellowship, Georgia State University, to DY
Molecular Basis of Disease graduate fellowship, Georgia State University, to DY

Title: A functional role for a hypothetical Linear ubiquitin chain Determining Domain of the RNF216/TRIAD3A Ubiquitin Ligase

Authors: *D. W. YAKOUT[1], J. KADIRVELU[1], R. LIU[2], A. CHARLES[1], J. YIN[2], A. M. MABB[1];
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Abstract: Ubiquitination is a type of posttranslational modification (PTM) that involves the covalent attachment of Ubiquitin (Ub) to various substrates. This PTM controls several cellular processes, which include proteasome-dependent substrate degradation. Ub conjugation requires the activity of three enzymes: A Ub activating enzyme (E1), Ub conjugating enzyme (E2), and the Ub ligase (E3). RBR (RING-Between-RING) is an E3 ligase subfamily which includes HOIP (HOIL-1-Interacting Protein), which is a subunit of LUBAC (linear ubiquitin chain assembly complex). HOIP contains an LDD (Linear ubiquitin chain Determining Domain) that transfers the Ub chain to the N-terminus of its target substrate and is involved in the catalytic activity and specificity of linear ubiquitin chain assembly. Linear ubiquitination promotes inflammation and regulates cell death, but there is no evidence for its role in the nervous system. Here, we show that the RNF216 E3 ligase isoform TRIAD3A (the most abundant isoform in the brain) has an amino acid sequence that shares homology with the LDD of HOIP. Deletion of this sequence decreases TRIAD3A autoubiquitination. Moreover, deletion of this domain alters the localization of the protein in primary hippocampal neurons, reduces dendritic complexity and alters dendritic spine morphologies. Our findings suggest that the Triad3A hypothetical LDD might act independently of its RBR domain to regulate hippocampal neuron development via ubiquitination of unique substrates.

Disclosures: D.W. Yakout: None. **J. Kadirvelu:** None. **R. Liu:** None. **A. Charles:** None. **J. Yin:** None. **A.M. Mabb:** None.

Digital Abstract Session

P015. Dendritic Growth and Branching

Program #/Poster #: P015.02

Topic: A.05. Axon and Dendrite Development

Support: Health Research Council of New Zealand Grant 3712384
University of Auckland Doctoral Scholarship

Title: Validation of neurite orientation dispersion and density imaging (NODDI) magnetic resonance imaging (MRI) for assessment of brain development in the neonatal rat

Authors: *P. B. WHITE[1], J. D. PRASAD[1], Y. VAN DE LOOIJ[2], S. SIZONENKO[2,3], S. RANASINGHE[1], L. BENNET[1], A. GUNN[1], J. M. DEAN[1];
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Abstract: Preterm birth increases a child's risk of brain injury and is associated with reduced cortical growth and poor neurodevelopmental outcomes. However, brain injury is seldom

diagnosed immediately. Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) model used clinically to identify preterm brain injury. Although, DTI parameters lack cellular specificity. Neurite Orientation Dispersion and Density Imaging (NODDI) is a type of diffusion MRI that aims to provide greater specificity than DTI for assessing the cellular components contributing to diffusivity changes. However, the histological correlates of NODDI parameters in the brain remain unclear, but are important for detecting the origin and evolution of preterm brain injury. Herein, we examined the histological correlates of NODDI parameters during normal brain development. Sprague Dawley (SD) rats were euthanized at postnatal day (PND) 1, 3, 7, 14, 21, and 35 (n=10, per age, male and female). Brain tissues were processed for Golgi-Cox staining (n=5 per age) or fixed for *ex-vivo* MRI analysis (n=5 per age, using an actively shielded 9.4T/31cm magnet). Data were fitted with the NODDI toolbox (Matlab) and spatially normalised DTI. MRI tissues were processed for immunohistochemistry (microtubule associated protein 2, glial fibrillary acidic protein), and cellular process density was calculated via the Spaceball method (radius =7.5µm, Stereoinvestigator software). Dendritic morphology was assessed in Golgi-Cox tissue via manual tracing (Neurolucida software), on a Zeiss Axio Imager M2 upright microscope. In both the motor (M1-M2) and somatosensory (S1HL-S2) cortices, the DTI parameter fractional anisotropy progressively decreased from PND1-PND7, while the NODDI parameter orientation dispersion index progressively increased from PND1-PND14. Both values plateaued thereafter. The DTI parameter mean diffusivity increased from PND1-PND3 and then decreased from PND3-PND7, while the NODDI parameter neurite density index decreased from PND1-PND3 and then increased from PND3-PND7. Histologically, there was a progressive increase in all dendritic complexity measures in the motor and somatosensory cortices from PND1-PND21. Correspondingly, dendritic density decreased at PND3. These data suggest that while MRI measurements may reflect changes in dendritic morphology and density with development, there may be other contributing factors to developmental cortical anisotropy. Nevertheless, NODDI may provide a novel technique for assessing cortical pathology in preterm-born infants.

Disclosures: **P.B. White:** None. **J.D. Prasad:** None. **Y. Van de Looij:** None. **S. Sizonenko:** None. **S. Ranasinghe:** None. **L. Bennet:** None. **A. Gunn:** None. **J.M. Dean:** None.

Digital Abstract Session

P015. Dendritic Growth and Branching

Program #/Poster #: P015.03

Topic: A.05. Axon and Dendrite Development

Support: ANID (Agencia Nacional de Investigacion y Desarrollo) FONDECYT grant Nº 1171137
Basal Center of Excellence in Science and Technology CONICYT (AFB170005)

Title: Mechanism of axonal TrkB signaling endosomes inducing long-distance dendritic growth in cortical neurons

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Abstract: Brain-Derived Neurotrophic Factor (BDNF) is broadly expressed in many circuits of the central nervous system (CNS). It binds TrkB and p75 to trigger different signaling pathways, including ERK1/2 and PI3K-mTOR, to induce dendritic growth and synaptic plasticity. When binding to BDNF, TrkB and p75 are endocytosed to signaling endosomes to continue signaling inside the cell. Whether BDNF/TrkB-p75 signaling endosomes in axons are regulating long-distance signaling in cell bodies to modify neuronal morphology is unknown. Here, we studied the functional role of BDNF signaling endosomes in long-distance regulation of dendritic growth using compartmentalized cultures of rat and mouse cortical neurons derived from $p75^{exonIII}$ knock-out or $TrkBF^{616A}$ knock-in mice. By applying BDNF to distal axons, we showed the capacity of axonal BDNF to increase dendritic arborization in cell bodies. This process depended on TrkB activity, but not p75 expression. In axons, BDNF/TrkB co-localized with Rab5 endosomes and increased active Rab5. Also, dynein was required for BDNF long-distance signaling, consistent with sorting and transport of signaling endosomes. Using neurons derived from $TrkBF^{616A}$ knock-in mice and the 1NM-PP1 inhibitor, we were able to demonstrate that TrkB receptors activated in the axons by BDNF, were required in the neuronal cell body to increase TrkB activity and phosphorylation of CREB. Also, we were able to visualize endosomes containing activated TrkB. PI3K activity was not required in the axons for dynein dependent BDNF responses. However, dendritic arborization induced by axonal BDNF signaling required both nuclear CREB and PI3K activation in cell bodies. Consistently, axonal BDNF increased protein translation in cell bodies and CREB and PI3K and mTOR activity were required for this process. Altogether, these results show that BDNF/TrkB signaling endosomes generated in axons allows long-distance control of dendritic growth coordinating both transcription and protein translation. Our results suggest a role of BDNF-TrkB signaling endosomes wiring circuits in the CNS.

Disclosures: G. Moya-Alvarado: None. **W.C. Mobley:** None. **C. Wu:** None. **E. Perlson:** None. **F.C. Bronfman:** None.

Digital Abstract Session

P015. Dendritic Growth and Branching

Program #/Poster #: P015.04

Topic: A.05. Axon and Dendrite Development

Support: NIH R01 NS086082

Title: Molecular and cellular functions of the PP2A serine/threonine phosphastase in dendritic diversification

Authors: *S. BHATTACHARJEE[1], E. N. LOTTES[1], S. NANDA[2], G. ASCOLI[2], D. N. COX[1]; [1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]George Mason Univ., Fairfax, VA

Abstract: Uncovering the molecular mechanisms that regulate dendritic diversification is essential to understanding the formation and modulation of functional neural circuitry. However, the molecular mechanisms by which this is achieved remain incompletely understood. Studies in *Drosophila* multidendritic (md) sensory neurons reveal that the Cut homeodomain transcription factor regulates cell-type specific dendritogenesis through cellular pathways that converge on cytoskeletal architecture. Neurogenomic and phenotypic analyses identified the highly conserved PP2A serine/threonine phosphatase complex as a downstream effector of Cut. The PP2A complex is composed of a catalytic subunit encoded by *microtubule star (mts),* a scaffolding subunit encoded by *PP2A-29B* and one of four alternate regulatory subunits encoded by *widerborst (wdb), twins, well-rounded (wrd)* and *CG4733.* Mutant analyses of *mts* and *PP2A-29B* reveal severe reductions in dendritic arborization with *wdb* functioning as the relevant regulatory subunit in Class IV (CIV) md sensory neurons. In contrast, mutations in *mts* and *PP2A-29B* leads to increased dendritic complexity via *de novo* filopodia formation in Class I (CI) md sensory neurons. Mechanistically, while Mts and PP2A-29B are also part of the STRIPAK complex with Cka as the regulatory subunit, our data indicate that the PP2A and STRIPAK complexes function in parallel to regulate dendritic architecture. Live imaging reveals that *mts* mutations leads to microtubule (MT) destabilization. In addition, dynamic EB1::GFP imaging of MTs reveals Mts is required to maintain dendritic MT polarity. In contrast to its effect on MTs, *mts* knockdown leads to F-actin reorganization with a proximal shift towards the soma. Loss of *mts* leads to significant reductions in dendritic localization of organelles including mitochondria and satellite Golgi outposts in CIV neurons, while in CI neurons, *mts* mutants show increased Golgi outpost trafficking along the dendritic arbor. Further, *mts* mutant neurons exhibit defects in neuronal polarity as revealed by Golgi outposts appearing in the proximal axon whereas in controls Golgi outposts are primarily restricted to the soma and at satellite locations on dendrites. Furthermore, in *mts* mutants selective markers of dendritic and axonal compartments are detected in the opposing compartments. At a regulatory level the β-tubulin subunit 85D represents a common PP2A target across these md neuron subtypes. Collectively, these studies provide novel insights into the functional roles of the PP2A phosphatase in promoting dendritic diversity.

Disclosures: S. Bhattacharjee: None. **E.N. Lottes:** None. **S. Nanda:** None. **G. Ascoli:** None. **D.N. Cox:** None.

Digital Abstract Session

P015. Dendritic Growth and Branching

Program #/Poster #: P015.05

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant MH081254

Title: Mir-9 regulates the structure of spiny neurons in Area X of zebra finches

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Abstract: Area X, the avian basal ganglia, plays important roles in song related sensory-motor learning and performance in zebra finches. We have recently shown that the expression of miRNA miR-9 is regulated in Area X during song development and overexpression of miR-9 in Area X of juveniles leads to impairments in vocal learning and vocal performance. We have also shown that miR-9 regulates FOXP2 (a gene associated with speech and language development) and many FOXP2 downstream genes that have been associated with neuronal structural plasticity. These observations prompted us to examine miR-9's role in modulating neuronal structure in Area X. We made a lentiviral vector that expresses miR-9 and a fluorescent marker mCherry, and stereotaxically injected the lentivirus into the Area X at 25-30 days old juveniles. We sacrificed the animals at 60 days and 100 days of age, imaged virally labeled neurons, traced neuronal dendritic structure, and quantitatively analyzed neuronal dendrite and spine structure. We report that miR-9 overexpression in juvenile Area X reduces dendrite length and complexity of spiny neurons. miR-9 overexpression also reduced spine density of these spiny neurons. In addition, miR-9 downregulates DARPP-32, a molecule for dopamine signaling. These impairments are long lasting and persist into adulthood. Together, these results provide strong evidence for miR-9's roles in regulating basal ganglia neuronal structure. As the basal ganglia is key to many neurological and psychiatric disorders, dysregulation of miR-9 may contribute to these disorders.
Key words – basal ganglia, Area X, medium spiny neurons, dendrite, spines, zebra finches

Disclosures: H. Jarrell: None. **X. Li:** None. **Z. Huang:** None. **Z. Shi:** None. **Y. Ding:** None.

Digital Abstract Session

P016. Postnatal Neurogenesis: Temporal and Spatial Patterns

Program #/Poster #: P016.01

Topic: A.02. Postnatal Neurogenesis

Support: CIHR

Title: Delayed development of input plasticity at lateral perforant path synapses onto adult-born dentate granule neurons.

Authors: *N. P. VYLETA[1], J. S. SNYDER[2];
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Abstract: In the mammalian brain, the dentate gyrus of the hippocampus uniquely generates new neurons throughout the lifetime of the animal (adult neurogenesis). Adult-born granule cells undergo a period of maturation during which their membranes are more excitable and inputs from neurons in the medial enthorinal cortex (via medial perforant path) can more easily induce long-term potentiation of synaptic responses. This previously described "critical window" exists between approximately four- and six-weeks of cell age. Whether signaling from the other major cortical input to the dentate gyrus—the lateral enthorinal cortex via the lateral perforant path—undergoes the same timecourse of maturation remains unknown. We measured synaptic responses to stimulation of lateral perforant path axons at adult-born granule cells between three- and thirty-weeks old. Surprisingly, we find that long-term potentiation of synaptic responses matures over an extended timecourse, with a peak at around 15-weeks of cell age. Cells between 4- and 6- weeks of age are more likely to experience long-term depression of lateral perforant path inputs than are older cells. We find that facilitation of transmitter release increases over a similar timecourse as long-term potentiation. Furthermore, the magnitude of long-term potentiation could be predicted by either the starting level of facilitation or by the reduction of facilitation (increase in release probability) after induction of the potentiation. Therefore, these data suggest that an increase in presynaptic release probability at lateral perforant path synapses is the major mechanism of long-term potentiation, and that this plasticity is more prominent at inputs onto older neurons.

Disclosures: N.P. Vyleta: None. **J.S. Snyder:** None.

Digital Abstract Session

P016. Postnatal Neurogenesis: Temporal and Spatial Patterns

Program #/Poster #: P016.02

Topic: A.02. Postnatal Neurogenesis

Title: Excitatory to Inhibitory Transition in GABAergic Currents Guides Circuit Formation of Cortical Interneurons

Authors: *K. ZAVALIN, A. HASSAN, C. FU, E. DELPIRE, A. LAGRANGE;
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Abstract: Rationale: Excitatory GABA is a crucial developmental cue that guides perinatal neuronal migration, synaptogenesis, and circuit formation. However, maturing neurons typically switch to hyperpolarizing responses through onset of KCC2 expression. We investigated how indefinitely-prolonged excitatory GABA responses in cortical interneurons (INs) adversely affect cortical circuit development using an IN-specific KCC2 knockout (KO).
Methods: We created the KO by crossing the IN-specific Dlx5:cre-IRES-eGFP and the $KCC2^{flox}$ lines. We measured seizure susceptibility by latency to onset of flurothyl-induced GTC seizure. We recorded sIPSCs using whole-cell voltage clamp in layer 5 (L5) pyramidal neurons in acute brain slices. We used immunohistochemistry and epifluorescence/confocal imaging to visualize expression of KCC2 and $GABA_A$ receptor subunits α1-5, and IN distribution by Dlx5-driven

reporters and somatostatin (SST)/parvalbumin (PV) antibodies. Experiments focused on P12-14 barrel field.
Results: KO mice exhibit abnormal neurological development, including: spontaneous seizures, a 45% faster onset of flurothyl-induced seizures, reduced body weight, and late postnatal mortality, but normal gross and histological anatomy of organs, including feeding structures and the brain. We observed the earliest cortical KCC2 expression in L5 INs, as early as E16-18 in wild-type mice. We expected these cells to be the most affected by loss of KCC2, and indeed found a 30% increase in density of INs specific to L5. However, we observed that the INs and inhibitory synapses of KO appear normal in many aspects: distribution of immature Dlx5+ INs, frequency of L5 principal cell sIPSCs, and $GABA_A$ receptor subunit expression.
We postulated that the effects of KCC2 loss might be specific to IN subtypes, which in L5 consist mainly of SST and PV INs with distinctly different circuit functions and developmental sequences. We found opposing changes in densities of these subtypes: a 12% increase in L5 density of SST INs in KO, but a decrease in PV IN density in L2-4 and L6.
Conclusions: Cortical INs are vital in regulating neuronal excitability and circuit function, and improper GABAergic excitation of INs underlies genetic and acquired epilepsies. Our data show that loss of KCC2 in developing INs causes failure to thrive and promotes seizures, which may be a result of altered IN distribution. To determine the underlying causes, we are now investigating (1) the early distribution of INs in the KO that precedes the changes in SST/PV distribution, and (2) whether GABAergic responses are anachronously excitatory in maturing INs in KO.

Disclosures: K. Zavalin: None. **A. Hassan:** None. **C. Fu:** None. **E. Delpire:** None. **A. Lagrange:** None.

Digital Abstract Session

P017. Synapse Formation

Program #/Poster #: P017.01

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: R01GM108970
R35GM135160
F31NS113381

Title: Vasp ubiquitination regulates actin dynamics and neuronal morphogenesis

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Abstract: In neurons, filopodia are critical for neuritogenesis, axon guidance, and dendritic spine formation. Defects in these processes can result in improper synaptic connectivity, neurodevelopmental disorders, and psychiatric syndromes. The actin polymerase VASP localizes to the filopodial tip complex and influences actin dynamics. Previously, the Gupton lab showed

VASP transiently co-localizes with the E3 ubiquitin ligase TRIM9 at the filopodial tip. TRIM9 was required for the reversible, non-degradative ubiquitination of VASP and this modification was associated with decreases in growth cone filopodia number and stability. Although the dynamic actin cytoskeleton and VASP are also appreciated to play important roles in the postsynapse, it is not known how VASP activity is regulated in dendritic filopodia and the maturing dendritic spine. Here we show VASP, TRIM9 and ubiquitinated VASP (VASP-Ub) localize to the PSD following differential centrifugation, suggesting a role for VASP-Ub in dendritic spines. Cultured murine cortical neurons overexpressing VASP or VASP-KR (a non-ubiquitinatable construct) exhibit no significant changes in dendritic filopodia number. Although the guidance cue netrin promotes synaptogenesis, neurons overexpressing VASP or VASP KR demonstrated a decrease in dendritic filopodia number following netrin treatment. To understand this puzzling result, we are currently utilizing live cell imaging to quantify the lifetime of these filopodia. Ongoing work is also examining *Trim9* deletion, as well as VASP and VASP-KR overexpression, on dendritic spine number, maturity, and synaptic plasticity. Future work will explore the mechanistic impact of ubiquitination on actin-VASP interactions through in vitro biochemical reconstitution assays.

Disclosures: L.E. McCormick: None. **S.L. Gupton:** None.

Digital Abstract Session

P018. Synapse Maturation and Remodeling

Program #/Poster #: P018.01

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant R00MH110665
The Mall Family Foundation

Title: Kit Ligand Influences Cerebellar Molecular Layer Interneuron Migration and Synapse Formation

Authors: T. ZAMAN[1], Q. ALSABIA[1], A. STAFFORD[1], B. W. LUIKART[2], ***M. R. WILLIAMS**[1];
[1]Pediatrics & Human Develop., Michigan State Univ., Grand Rapids, MI; [2]Mol. and Systems Biol., Geisel Sch. of Med. at Dartmouth Col., Hanover, NH

Abstract: Mutation of the receptor tyrosine kinase Kit is associated with rare cases of intellectual disability and with autism spectrum disorder, suggesting that Kit is important for brain development. Kit Ligand (KL) promotes the development of Kit expressing cells in the body periphery; KL may also act in the brain to influence Kit+ neuron development. In human and rodent brains, KL and Kit are differentially expressed in synaptically coupled neurons. This complementary expression is consistent with a role for KL/Kit in circuit formation, but this model has not been tested. In both human and mouse brains, Kit expression is highest in cerebellar molecular layer interneurons (MLIs), while cerebellar KL is restricted to Purkinje cells

(PCs). In development, MLIs migrate to and synapse upon PCs. This MLI maturation happens largely postnatally in the mouse and is experimentally accessible. We therefore sought to test the hypothesis that target-derived (PC) KL attracts Kit+ cells (MLIs) to migrate and form synapses. This was accomplished through in vivo manipulations of KL, followed by analyses of MLI migration and of MLI:PC synapses. Leveraging a mouse expressing eGFP under the Kit promoter, we could observe the normal migration and morphology of Kit expressing MLIs. By conditional knockout, we found that PC KL was *not necessary* for normal MLI migration. However, by ectopic expression of KL, we found that KL *was sufficient* to attract and arrest MLI migration. We therefore investigated, instead of acting in an all or none fashion, whether KL influenced the local strength or specificity of MLI:PC synapses. By viral transduction, we sparsely overexpressed or knocked out KL, from PCs. By patch clamp electrophysiology, we found that increasing or decreasing PC KL caused a corresponding change in GABAergic input from MLIs. This suggests that PC KL levels may act as a rheostat controlling MLI synapse strength and/or number. This role was not relegated to neurons natively expressing KL; ectopic expression of KL in cerebellar Golgi cells induced MLIs to sprout axon collaterals to invest KL+ Golgi cells. Having established that modulating KL levels in a postsynaptic cell is sufficient to change input from Kit+ MLIs, we are now investigating whether presynaptic Kit is required for normal synapse formation. Our long-term goal for this project is to elucidate the molecular biology through which KL/Kit signaling elicits synapse formation, not only to inform normal development, but to understand neurodevelopmental disorders in which Kit is implicated, and to investigate whether the synaptogenic potential of Kit signaling may be leveraged therapeutically.

Disclosures: M.R. Williams: None. **T. Zaman:** None. **Q. Alsabia:** None. **A. Stafford:** None. **B.W. Luikart:** None.

Digital Abstract Session

P018. Synapse Maturation and Remodeling

Program #/Poster #: P018.02

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant R21NS109750
NIH Grant R41NS108895

Title: Compartmentalized 3D neuronal cultures enhance neuronal maturation and improve access to circuits for measurements and manipulation

Authors: *A. M. TAYLOR, T. NAGENDRAN;
Xona Microfluidics, Inc., Research Triangle Park, NC

Abstract: 3D cultures aim to recapitulate the complex microenvironment found *in vivo* and to bridge the gap between traditional 2D cell culture approaches and *in vivo* animal models. Because of the complexity of the brain, there is a need to develop reliable 3D networks that also provide access to cellular compartments for measurements and manipulations.

Compartmentalized chips made of cyclic olefin copolymer (COC) provide a consistent and reliable platform to compartmentalize neurons and create assessable co-culture model systems. Among different matrices for culturing neurons in 3D, the Matrigel® matrix provides a promising environment for growing neurons, because of its richness in structural proteins found in the brain, such as laminin and collagen. Dissociated neurons from E18 rat combined cortex-hippocampus tissue were grown within the Matrigel® matrix in two compartment COC chips for 12 days *in-vitro* (DIV). Confocal z-stack images of fluorescently labeled neurons within the chamber demonstrate healthy neuron growth and development in 3D pattern. Neurons in 3D cultures that were retrograde labeled with GFP G-deleted rabies virus show increase in dendritic spines compared to 2D controls. In summary, we successfully modeled 3D neuron cultures within compartmentalized chips. These 3D cultures show an increase in dendritic spine formation and suggest a possibility to promote neuronal maturation. Further our results suggest that establishing 3D neuron cultures within these compartmentalized microfluidic devices provides a novel method to visualize the somatodendritic compartments while specifically performing targeted drug manipulations to axons extending into the axonal compartment in 2D. This method helps to develop highly organized *in vitro* assays to mimic and treat brain disorders.

Disclosures: **A.M. Taylor:** A. Employment/Salary (full or part-time):; Xona Microfluidics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Xona Microfluidics, Inc. **T. Nagendran:** A. Employment/Salary (full or part-time):; Xona Microfluidics, Inc..

Digital Abstract Session

P018. Synapse Maturation and Remodeling

Program #/Poster #: P018.03

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Canadian Institutes of Health Research (MOP142215)
Natural Sciences and Engineering Research Council of Canada (RGPIN-2017-03889)
The Scottish Rite Charitable Foundation of Canada (15118)
Michael Smith Foundation for Health Research and British Columbia Schizophrenia Society Foundation Scholar Award (5900)
University of Victoria Fellowship Graduate Award
Natural Sciences and Engineering Research Council of Canada Undergraduate Student Research Award
Canada Foundation for Innovation (29462)

Title: Pannexin 1 regulates cortical neuron dendritic protrusion dynamics and dendritic spine density

Authors: ***J. C. SANCHEZ-ARIAS**, E. VAN DER SLAGT, R. N. CANDLISH, L. SWAYNE; Div. of Med. Sci., Univ. of Victoria, Victoria, BC, Canada

Abstract: Dendritic spines develop from highly dynamic, filopodia-like protrusions arising from the dendritic shaft; here we present recent data from our lab demonstrating that the ATP-release channel protein pannexin 1 (PANX1) regulates mouse cortical neuron dendritic protrusion dynamics and dendritic spine density. Cultured cortical neurons lacking PANX1 exhibited increased dendritic spine density and formed larger cortical neuron networks. PANX1 was enriched in cerebral cortex synapses and its expression levels were downregulated during the critical period for synapse formation in the mouse sensory cortex. Panx1 knockout (KO) mice exhibited increased cortical neuron dendritic spine density in situ (layer 5 sensory cortex pyramidal neurons) and in vitro, establishing, in part, the mechanism underlying PANX1 regulation of neuronal networks. To determine whether changes in stability of developing dendritic protrusions contributed to the increased spine density observed in Panx1 KO cortical neurons, we next developed new methods to measure dendritic protrusion dynamics at 10 days-in-vitro (DIV10). To improve the detection of dendritic protrusions, we transfected neurons with a fluorescent membrane tag (mCherry-CD9-10). This approach resulted in a ~34% increase in the detection of dendritic protrusions when compared to a cytoplasmic fluorescent label (EGFP). Additionally, we confirmed that Panx1 KO led to higher density of dendritic protrusions, while transient expression of PANX1-EGFP in wildtype and Panx1 KO neurons resulted in a significant decrease in dendritic protrusion density. Moreover, using live cell microscopy, we acquired z-stack images of dendritic segments over a period of 10-minutes (1 frame every 5 seconds). We found that dendritic protrusions in Panx1 KO neurons are more stable, while transient expression of PANX1-EGFP significantly increased the overall movement and turnover of dendritic protrusions. Altogether, these new findings confirm and expand our understanding of PANX1 regulation of neuronal development and suggest that PANX1 regulation of dendritic spines is mediated, in part, by modulating dendritic protrusion dynamics in neurons. This work has been published in eNeuro at https://www.eneuro.org/content/6/3/ENEURO.0503-18.2019 and https://doi.org/10.1523/ENEURO.0079-20.2020.

Disclosures: J.C. Sanchez-Arias: None. **E. van der Slagt:** None. **R.N. Candlish:** None. **L. Swayne:** None.

Digital Abstract Session

P018. Synapse Maturation and Remodeling

Program #/Poster #: P018.04

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant R21NS111255
Harry T. Mangurian Jr. Foundation

Title: Sez6 family proteins are novel complement inhibitors expressed by neurons.

Authors: W. QIU[1], S. LUO[1], S. MA[1], P. SAMINATHAN[1], H. LI[1], J. GUNNERSEN[3], H. A. GELBARD[4], ***J. W. HAMMOND**[2];

[2]Neurol., [1]Univ. of Rochester, Rochester, NY; [3]Univ. of Melbourne, Melbourne, Australia; [4]Neurol., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: The Sez6 family consists of Sez6, Sez6L, and Sez6L2. Its members are expressed throughout the brain and have been shown to influence synapse numbers and dendritic morphology. They are also linked to various neurological and psychiatric disorders. All Sez6 family members contain 2-3 CUB domains and 5 complement control protein (CCP) domains, suggesting that they may be involved in complement regulation. We show that all Sez6 family members inhibit C3 deposition by the classical and alterative pathways with varying degrees of efficacy. For the classical pathway, Sez6 is a strong inhibitor, Sez6L2 is a moderate inhibitor, and Sez6L is a weak inhibitor. Using Sez6L2 as the representative family member, we show that it specifically deactivates C3 convertases by accelerating the decay or dissociation of the C3 convertase components. Sez6L2 also deactivates C3 convertases of the alternative pathway by serving as a cofactor for Factor I to facilitate the cleavage of C3b. However, Sez6L2 has no cofactor activity toward C4b. In summary, the Sez6 family are novel complement regulators that inhibit C3 convertases.

Disclosures: W. Qiu: None. **S. Luo:** None. **S. Ma:** None. **P. Saminathan:** None. **H. Li:** None. **J. Gunnersen:** None. **H.A. Gelbard:** None. **J.W. Hammond:** None.

Digital Abstract Session

P019. Neural Circuit Maturation and Remodeling

Program #/Poster #: P019.01

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIDCD R01DC008860

Title: Efferent feedback controls bilateral auditory spontaneous activity

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Abstract: In the developing auditory system, spontaneous activity generated in the cochleae propagates into the central nervous system to promote circuit formation. Effects of peripheral firing patterns on spontaneous activity in the central auditory system are not well understood. Here, we describe wide-spread bilateral coupling of spontaneous activity that coincides with the period of transient efferent modulation of inner hair cells from the brainstem medial olivocochlear system. Knocking out α9/α10 nicotinic acetylcholine receptors, a requisite part of the efferent pathway, abolishes these bilateral correlations. Pharmacological and chemogenetic experiments confirm that the efferent system is necessary and sufficient to produce bilateral coupling. Moreover, auditory sensitivity at hearing onset is reduced in the absence of pre-hearing

efferent modulation. Together, our results demonstrate how afferent and efferent pathways collectively shape spontaneous activity patterns and reveal the essential role of efferents in linking otherwise independent streams of bilateral spontaneous activity during the prehearing period.

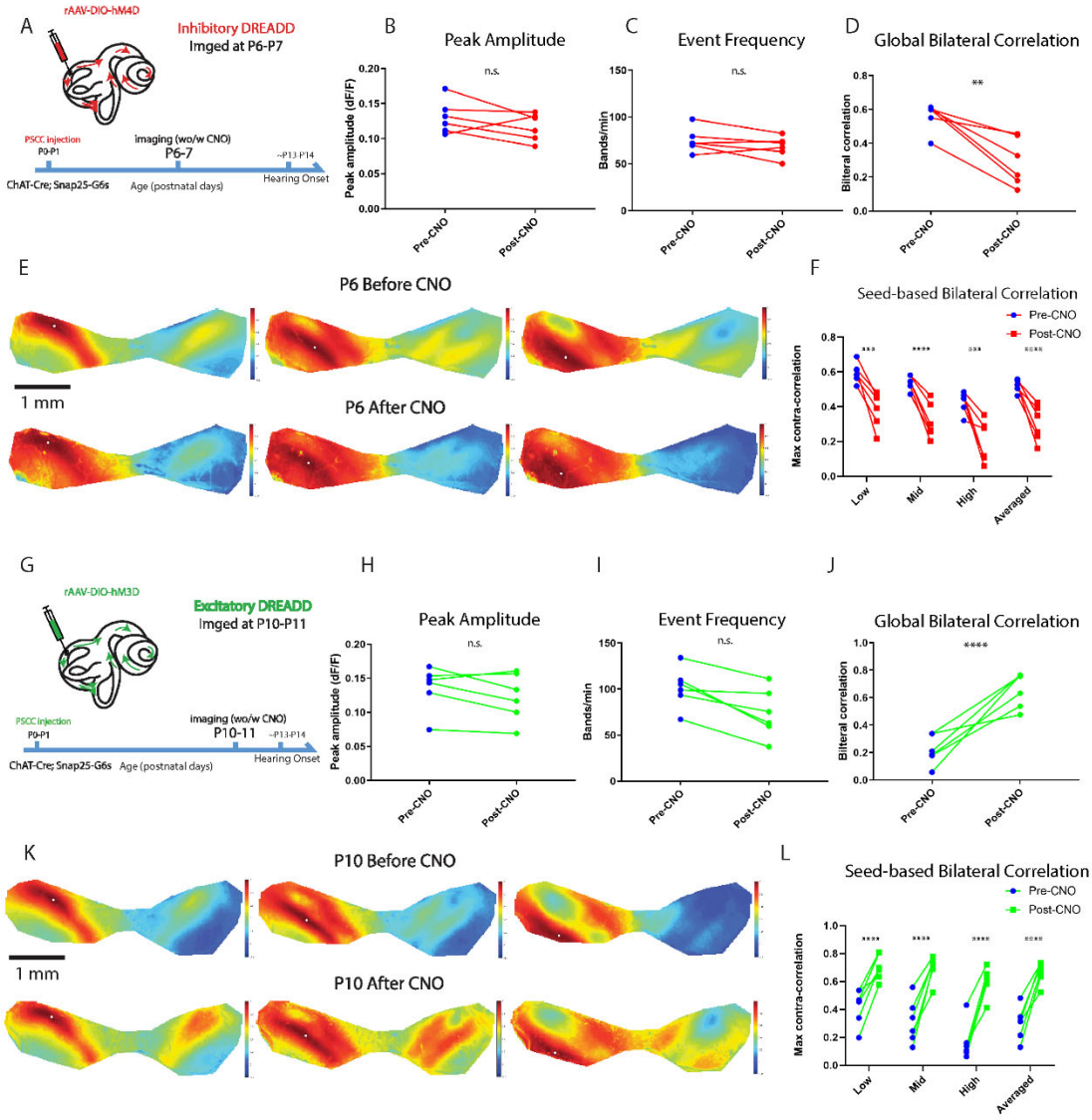

Figure 4. Chemogenetic manipulations can suppress or enhance bilateral coupling in vivo

Disclosures: **Y. Wang:** None. **M. Sanghvi:** None. **A. Gribizis:** None. **Y. Zhang:** None. **L. Song:** None. **B.J. Morley:** None. **D. Barson:** None. **J. Santos-Sacchi:** None. **D. Navaratnam:** None. **M.C. Crair:** None.

Digital Abstract Session

P019. Neural Circuit Maturation and Remodeling

Program #/Poster #: P019.02

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: F32NS100033801
K99NS112604

Title: Folding and lamination of the human neocortex depend on the sodium potassium pump alpha 3 (ATP1A3) subunit

Authors: ***R. S. SMITH**[1], C. A. WALSH[2];
[1]Genet. and Genomics, Boston Childrens Hosp. / Harvard Med. Sch., Boston, MA; [2]Genet. and Genomics, Boston Children's Hosp., Boston, MA

Abstract: ABSTRACT
Osmotic equilibrium and membrane potential in animal cells depend on concentration gradients of sodium (Na^+) and potassium (K^+) ions across the plasma membrane, a function that is catalyzed by the Na,K-ATPase alpha subunit. In vertebrates, four paralogous genes, *ATP1A1-4*, encode distinct alpha subunit isoforms ($a_{1\text{-}}a_4$), three of which (a_1, a_2, a_3) are expressed in the brain, and two (a_1, a_3) predominantly in neurons. The a_3 isoform, encoded by *ATP1A3*, is critical to neuronal physiology, and a growing spectrum of neurological diseases are associated with *ATP1A3* pathogenic variants, with ages of onset ranging from early childhood to adulthood. Here, we describe *ATP1A3* variants encoding dysfunctional a_3 subunits in children affected by polymicrogyria, a developmental malformation of the cerebral cortex characterized by abnormal folding and laminar organization. To gain cell-biological insights into the spatiotemporal dynamics of prenatal *ATP1A3* expression, we established a transcriptional atlas of *ATP1A3* expression during cortical development using mRNA *in situ* hybridization and transcriptomic profiling of ~125,000 individual cells with single-cell RNA sequencing (Drop-Seq) from various areas of the midgestational human neocortex. We find that fetal expression of *ATP1A3* is restricted to a subset of excitatory neurons carrying transcriptional signatures of neuronal activity and maturation characteristic of the developing subplate. Furthermore, by performing Drop-Seq on ~52,000 nuclei from four different areas of an infant human neocortex, we show that *ATP1A3* expression persists throughout early postnatal development, not only within excitatory neurons across all cortical layers, but also and more predominantly in inhibitory neurons, with specific enrichment in fast-spiking basket cells. In addition, we show that *ATP1A3* expression, both in fetal and postnatal neurons, tends to be higher in frontal cortical areas than in occipital areas, in a pattern consistent with the rostro-caudal maturation gradient of the human neocortex. Finally, we discover distinct co-expression patterns linking catalytic α subunit

isoforms (*ATP1A1,2,3*) and auxiliary isoforms (*ATP1B1,2,3*), suggesting the ATPase pump may form non-redundant, cell-type specific α-β combinations. Together, the importance of the developmental phenotypes and dynamic expression patterns of *ATP1A3* point to a key role for *a3* in the development and function of human cortex.

Disclosures: R.S. Smith: None. **C.A. Walsh:** None.

Digital Abstract Session

P019. Neural Circuit Maturation and Remodeling

Program #/Poster #: P019.03

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NSF grant (IOS-1656838)
DARPA grant (HR0011-18-2-0019, TA2)

Title: Early postnatal TrkB activity protects receptive field refinement in superior colliculus of dark reared adults by maintaining GABA levels rather than by maintaining $GABA_A$ receptor, NMDA receptor, gephyrin, PSD-95 or chloride pump protein levels.

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Abstract: Sensory experience during a critical period is typically thought to be crucial for development of neural circuits. However, our previous work on critical period plasticity in the visual pathway showed that Syrian hamsters do not require visual experience and that spontaneous activity is sufficient for visual receptive fields (RFs) in superior colliculus (SC) to refine. Instead, early (P33-P40) visual experience is necessary to stabilize RF size in adulthood via a BDNF-TrkB dependent mechanism. Without this early TrkB activation, RFs of dark-reared (DR) hamsters begin to enlarge around P60, due at least in part to a reduction in lateral inhibition from local GABAergic interneurons. Our pharmacological assays also suggested reduced $GABA_A$ receptor function in visually deprived animals. Visual perception assays revealed that RF re-enlargement significantly reduces the visual processing abilities of DR hamsters, but treatment with a TrkB agonist during the critical period rescued normal visual ability. In this study, we sought to identify the possible postsynaptic changes that could underlie RF re-enlargement in SC of DR adults and explain how reduced TrkB activation affects visual function. Using western blotting, we tested whether light exposure during the early critical period stabilizes adult RF size by affecting the composition or anchoring of $GABA_A$ receptors ($GABA_AR$). We predicted that dark rearing would interfere with the maturation of $GABA_AR$ subunit composition, resulting in a higher α2/α1 (immature/mature) subunit ratio. We also considered that the α5/α1 (extrasynaptic/synaptic) $GABA_A$ receptor subunit ratio in DR hamsters might be higher than normal, resulting in reduced synaptic inhibition. We then studied gephyrin and PSD-95 because reduced synaptic accumulation of these scaffold proteins could reduce the

stability of synaptic $GABA_A$ and glutamate receptors (NMDAR and AMPARs). Alternatively, dark rearing could reintroduce a juvenile chloride pump (lower NKCC1/KCC2 ratio), reducing the overall inhibitory effect of GABA. Lastly, we tested whether dark rearing results in an immature NMDAR composition (higher NR2B/2A ratio), slowing the decay kinetics of NMDARs in previously formed synapses in SC, thus expanding the correlation window. We found no significant differences in levels of these proteins in SC between DR and normal animals. Our results rule out several alternative explanations for the loss of $GABA_A$ receptor function identified by our earlier work, and support reduction in presynaptic GABA rather than postsynaptic alterations as the primary explanation for the loss of RF stability in DR animals.

Disclosures: P.S. Juvale: None. **D.B. Mudd:** None. **N.S. Ramesh:** None. **S.L. Pallas:** None.

Digital Abstract Session

P019. Neural Circuit Maturation and Remodeling

Program #/Poster #: P019.04

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant R01EY027003

Title: Developmental roles of Pcdh1a in neural circuit assembly

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Abstract: Vertebrate neural development is a dynamic process that is shaped by complex spatial and temporal interactions between cells. These interactions influence the trajectory of development to determine the final structure of the brain, which in turn underlies its functional capacity. Protocadherins (pcdhs) are homophilic cell adhesion molecules that are critical effectors during development. Additionally, pcdhs are both causative and risk genes for various neurodevelopmental disorders including autism spectrum disorder, schizophrenia, and epilepsy. In this project, we investigated the effect of the loss of Pcdh1a on neural development and brain organization at the local circuit level. To accomplish this, we performed whole brain *in vivo* calcium imaging of spontaneous neuronal activity in wild type and *pcdh1a* mutant zebrafish larvae over the course of the first developmental week. This time window represents a critical period of circuit assembly and structural maturation. We analyzed these data with graph theory to evaluate the network development using the principle that neurons that fire together are physically connected. This approach allowed us to quantitatively compare wild type and mutant networks over time. We observed that *pcdh1a* mutants display increases in several complex network measures like clustering, transitivity, and assortativity, and decreases in modularity. These specific alterations suggest that *pcdh1a* mutants have an altered developmental trajectory and pattern of connectivity than wild type controls, although they do not show any overt morphological defects. This could be the result of increased neurogenesis as well as dysregulation of synaptogenesis at the local circuit level. We are currently investigating the

cellular roles of Pcdh1a that may contribute to the altered network phenotypes we observed. Ultimately, we theorize that pcdhs influence brain architecture by locally regulating and coordinating developmental processes. Our long-term goals are to determine the molecular and cellular mechanisms of pcdhs in order to better understand how they guide brain development and how their loss of function leads to disease.

Disclosures: S. Light: None. **J. Jontes:** None.

Digital Abstract Session

P019. Neural Circuit Maturation and Remodeling

Program #/Poster #: P019.05

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: Enhancing adult neuroplasticity by epigenetic regulation of PV cells

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Abstract: A traumatic event can form an indelible fear memory trace and behavioral therapy for post-traumatic stress disorder (PTSD) is limited by the restricted adult brain plasticity level. Parvalbumin-expressing GABAergic interneurons (PV) are characterized by their highly complex axon, contacting hundreds of neurons. In adults, their somas are surrounded by a matrix-like structure, the perineuronal nets (PNN). Decreasing PV inhibition or digesting the PNN are sufficient to foster adult brain plasticity. However, the molecular mechanisms that regulate PNN are not well understood. To enhance adult brain plasticity and increase fear extinction retention, we studied PNN formation around PV somata. Activity-regulated and synaptic plasticity-associated genes are controlled by the chromatin acetylation level. We generated conditional *PV_Cre;Hdac2$^{lox/lox}$* mouse line (cKO) and found that adult mice show enhanced fear memory extinction retention, along with reduced PNN around PV cells in prefrontal cortex (PFC) and basolateral amygdala, but not in somatosensory cortex. Drop-Seq analysis, followed by RNAScope *in situ* hybridization, in the PFC yielded the *acan* gene, which encodes for the aggrecan protein, as a PNN component specifically expressed by PV cells. We confirmed the reduced aggrecan agglomeration around PV cells in the cKO PFC which was then rescued by Cre-dependent viral overexpression of *Hdac2*. Further, we show that a single injection of a novel Hdac2 inhibitor, before extinction training, is sufficient to increase fear extinction retention in WT adult mice, and concomitantly reduce *acan* expression and destabilize PNN in PFC. Altogether, our work supports a model in which PV cells and PNN play a pivotal role in brain plasticity and suggests that modulation of either Hdac2, or the *acan* gene, along with therapy could improve PTSD treatment.

Disclosures: **M. Lavertu Jolin:** None. **B. Chattopadhyaya:** None. **A. Vlad Varlan:** None. **K. Gauvin:** None. **D. Robertson:** None. **H. Affia:** None. **G. Andelfinger:** None. **G. Pineyro:** None. **G. Di Cristo:** None.

Digital Abstract Session

P019. Neural Circuit Maturation and Remodeling

Program #/Poster #: P019.06

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: The Canadian Institutes of Health Research (CIHR)

Title: Role of p75 neurotrophin receptor in cortical parvalbumin-positive GABAergic interneurons and cognitive flexibility

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Abstract: Parvalbumin (PV)-positive GABAergic interneurons constitute the majority of inter-neurons in the cortex and play a key role in the function and synchronization of cortical networks. Alterations in PV-interneuron connectivity, especially in the medial prefrontal cortex (mPFC), have been found in different psychiatric disorders. We have previously shown that the expression of the p75 neurotrophin receptor (p75NTR) regulates the time course of PV cell synapse maturation in a cell-autonomous fashion. Here, we show that p75NTR removal in postnatal PV cells affects the connectivity and physiology of PV interneurons and mPFC function in PvCre dependent conditional knockout (cKO) mice. We first analyze the effect of p75NTR removal on the efferent and afferent connectivity of PV cells. We quantified putative perisomatic synapses formed by PV cells, identified by the juxtaposition of PV and gephyrin, a post synaptic scaffolding protein of GABAergic synapses. Both the density of PV+gephyrin+ puncta and the percentage of perisomatic PV puncta showing juxtaposed gephyrin were significantly increased in mPFC but not in visual cortex of cKO mice. Analysis of putative glutamatergic synapse density on PV cell somata is ongoing. An important indication of PV cell maturation is the appearance of specialized extracellular matrix structures called perineural nets (PNNs) around the soma and primary dendrites of mature cortical PV cells. p75 cKO mouse show a significant increase in the number of PV cells encircled by PNN, as revealed by WFA staining, in mPFC but not visual cortex. These mice also show deficits in attentional set-shifting task, a measure of attention and cognitive flexibility in mPFC, and in extinction of fear memories, both of which rely on mPFC function. Moreover, extracellular recordings in the cKOs show that p75 removal induces an increase in ɣ activity, an oscillation dependent on PV cells, in the mPFC of anesthetized mice. Taken together, our data suggests that p75NTR expression in postnatal PV cells play a role in their connectivity specifically in mPFC function.

Disclosures: **P. Chehrazi:** None. **B. chattopadhyaya:** None. **M. Carreño-Muñoz:** None. **M. Lavertu Jolin:** None. **G. Di Cristo:** None.

Digital Abstract Session

P019. Neural Circuit Maturation and Remodeling

Program #/Poster #: P019.07

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: European Union's FP7/2007-2013/ programme under REA grant agreement No 327409
Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 705452
International Brain Research Organization Return Home Fellowship
L'ORÉAL Baltic "For Women In Science" fellowship with the support of the Lithuanian National Commission for the United Nations Educational, Scientific and Cultural Organization (UNESCO) and the Lithuanian Academy of Sciences

Title: Phospholipid scramblase Xkr8 is required for developmental axon pruning

Authors: *U. NENISKYTE[1,2], A. VADISIUTE[1], K. JEVDOKIMENKO[1], L. COLETTA[3], D. DABKEVICIENE[1], E. PERLAS[2], U. KULIESIUTE[1], D. RAGOZZINO[4], A. GOZZI[3], C. GROSS[2];
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Abstract: The mature brain connectome emerges during development via the extension and pruning of connections. Glial cells have been identified as key players in the phagocytic elimination of neuronal synapses. However, our understanding of pruning has been hampered by a lack of knowledge about the neuronal *eat-me* signals and associated transduction systems involved. Here we identified Xk-related protein 8 (Xkr8), a phospholipid scramblase, as a key factor for the pruning of axons in the developing mammalian brain. We found that phosphatidylserine scrambling is upregulated during synaptic pruning and preferentially found exposed on synaptic structures to promote microglia-synapse interactions. Mice lacking Xkr8 showed excess excitatory synapses, increased corticospinal projections and global brain hyperconnectivity. These data identify phospholipid scrambling by Xkr8 as a central process in the labelling and discrimination of developing neuronal projections for pruning in the mammalian brain.

Disclosures: **U. Neniskyte:** None. **A. Vadisiute:** None. **K. Jevdokimenko:** None. **L. Coletta:** None. **D. Dabkeviciene:** None. **E. Perlas:** None. **U. Kuliesiute:** None. **D. Ragozzino:** None. **A. Gozzi:** None. **C. Gross:** None.

Digital Abstract Session

P019. Neural Circuit Maturation and Remodeling

Program #/Poster #: P019.08

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH NS106244

Title: Maturation of cFOS expression during development follows information flow through the hippocampal formation circuitry

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Abstract: The hippocampal formation codes for spatial memory and navigation. In mammals, sensory information from cortical areas reaches the layer 2/3 of entorhinal cortex (Ent L2/3) and then flows to the dentate gyrus granule cell layer (DGC), Ammon's horn pyramidal cell layer (CA3 and CA1) before returning to Ent L5 which distributes information to cortical areas. In development, these functions seem to start around eye opening, when environment exploration starts (Speed and Dobrunz 2009), but the specific order of maturation is unclear. Here we used cFOS immunofluorescence (a marker of activation) to assess whether the mature hippocampal cFOS expression pattern is reached simultaneously among regions, or whether maturation occurs gradually following the direction of activity through the circuitry or other principles (for example, following the "rhinal to dentate" neurogenetic gradient). We compared cFos expression in mouse pups at postnatal day (P) 4, 9, 13 and 17 with adults (n=4 for all ages). All animals were kept awake for 1-2 hours, then immediately anesthetized and perfused with fixative. The brains were frozen and cut at a cryostat in 40μm thick sections. Adult mice showed cFOS in Ent (all layers), DGC, CA1 and CA3. We observed initial cFOS expression in Ent L2 and DGC on P4, and Ent L3, CA3 and CA1 expression on P9. In DGC, the initial staining was present only at the border with the molecular layer, where cells mature earlier than in DGC proper (as measured by decreasing doublecortin expression; Allen Institute for Brain Science 2008). The staining became distributed throughout the DGC only after P17. An adult-like level of staining was present in DGC on P9, in CA3 at P13, and CA1 after P17. Initial cFOS was seen in Ent L5 on P9-P13 and it was not adult-like even at P17. Thus cFOS shows a "maturation" sequence in the hippocampal formation that roughly follows the activation sequence through the circuitry. This may indicate a general principle of sequential activity maturation.

Disclosures: M. Pompeiano: None. **M.T. Colonnese:** None.

Digital Abstract Session

P019. Neural Circuit Maturation and Remodeling

Program #/Poster #: P019.09

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NSF OMA-0835976

Title: Voltage based spike-timing dependent myelin plasticity

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Abstract: There is an increasing body of evidence from both in vivo studies (Varela et al., 2001; Wang, 2010; Siegel, Donner, & Engel, 2010) and simulated networks (Izhikevich & Edelman, 2008; Noori et al. 2020) that the brain's processing requires synchronization of spike arrival times. One of the most promising mechanisms suggested to meet this requirement is compensating myelination, where adaptive levels of myelin insulation reduce longer conduction delays to synchronize with shorter ones (Salami et al., 2003; Vicente et al., 2008; Seidl, 2014). This compensation is produced through homeostatic regulation of myelin levels, converging onto a particular level of myelin for a given phase-locked pattern of stimulation (Domingues et al. 2016, Noori et al. 2020).
We introduce a plausible biological mechanism for the regulation of activity dependent myelin plasticity, and a computational model of this mechanism which ultimately produces stable, homeostatic dynamics analogous to voltage-based STDP models. Our model synchronizes coherently out-of-phase inputs via changes in conduction velocity, and makes predictions about the proteins, neurotransmitters, and ions involved in adaptive CNS myelin plasticity.

Disclosures: S. Berteau: None. **A. Yazdanbakhsh:** None.

Digital Abstract Session

P020. Animal Models of Autism: From Molecular Mechanisms To Behavior

Program #/Poster #: P020.01

Topic: A.07. Developmental Disorders

Support: NIMH K99MH115143
SFARI pilot award #401457
CIHR fellowship to KYC

Title: Oxytocin normalizes altered social circuit connectivity in the Cntnap2 Knockout mouse

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Abstract: Aberrant functional connectivity (FC) is frequently found in autism spectrum disorders (ASD), notably correlating with the degree of social impairment (Supekar et al., 2013). We previously reported that administration of oxytocin (OXT) improves social deficits in mice lacking an ASD risk gene, *Cntnap2* (Penagarikano et al., 2015). Given the ability of OXT to increase circuit signal-to-noise (Owen et al., 2013), we hypothesized that OXT might exert its prosocial effects via stimulating socially-relevant brain regions and rescuing potentially present FC alterations in *Cntnap2* KO mice. To test this, we used high field (7T) fMRI to measure brain-wide BOLD responses and changes to resting-state FC after administering OXT to wild-type (WT) and *Cntnap2* KO mice (n=15/group). In KO mice, we observed significantly lowered mean FC between regions with established roles in social behavior (e.g. PVN, nucleus accumbens (NAc), medial prefrontal cortex), and higher mean FC between these and other regions not typically involved in social functions (e.g. sensory cortices, thalamus; $p<0.001$ vs WT). Strikingly, OXT robustly increased the BOLD signal in various social regions, and reversed both FC phenotypes in these mice ($p<0.001$), including circuits involving the NAc. To validate these results, we assessed the brain-wide patterns of neuronal activity with lightsheet imaging of iDISCO+ cleared brains. In parallel with fMRI results, OXT strongly increased the number of c-Fos+ cells in several social brain regions in KO mice (e.g. NAc, BNST, insula), but not WT ($p<0.005$; n=5/group). Further investigation revealed a lower excitatory synaptic drive in the NAc of KO mice, latter of which was rescued by local agonist activation of OXT receptors (n=6-7 cells/group). As OXT axons directly project to the NAc, we next tested whether stimulating the terminal release of OXT onto the NAc *in vivo* is sufficient for rescuing the social impairment of adult KO mice. To do this, we selectively expressed the excitatory opsin ChETA in PVN OXT neurons (OXT-ChETA), and stimulated the NAc with blue light (457 nm at 30 Hz, 5 ms pulse width, ~10-15 mW) through bilaterally implanted optic fibers. Our results revealed that adult KO mice with OXT-ChETA spent a significantly longer time interacting with an unfamiliar juvenile WT mouse, compared to OXT-eYFP control KO mice ($p=0.03$; n=5/group), confirming that increasing endogenous OXT in the NAc is sufficient for reversing the social deficits of these mice. Collectively, these results suggest that the prosocial effects of OXT in *Cntnap2* KO mice may involve an enhancement of concerted activity across social brain areas, and identify the NAc as a key area in this process.

Disclosures: K.Y. Choe: None. **D. Geschwind:** None. **L. DeNardo:** None. **P. Golshani:** None. **R. Bethlehem:** None. **M. Safrin:** None. **H. Dong:** None. **E. Salman:** None. **Y. Li:** None. **N. Harris:** None. **O. Penagarikano:** None. **V. Grinevich:** None.

Digital Abstract Session

P020. Animal Models of Autism: From Molecular Mechanisms To Behavior

Program #/Poster #: P020.02

Topic: A.07. Developmental Disorders

Support: Swedish Research Council

Title: Behavioural and neurochemical responses of oxytocin-receptor-mutant zebrafish to group and individual social stimulation: support for zebrafish autism model

Authors: *S. SHAMS, L. WESTBERG;
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Abstract: Autism is a family of neurodevelopmental disorders characterized by social deficits and abnormalities in brain areas associated with social and emotional behaviour. Autism affects roughly 1% globally and no effective pharmacological treatments are currently available. Recently, intranasal oxytocin has shown promise as a potential treatment for social impairments seen in autism. Oxytocin is a neuropeptide that regulates a wide range of mammalian behaviours relevant to autism, such as bonding, social recognition, and anxiety. As the mechanisms underlying oxytocin's role in improving social function in autistic patients are not well-understood, animal models with capacity for sophisticated genetic manipulation are necessary. Zebrafish (*Danio rerio*) is a highly social vertebrate with phylogenetic conservation in oxytocin and key neurotransmitter systems and we exploit these features to develop an animal model of autism. Our aim was to investigate social interaction and neurochemistry in zebrafish mutants with genetically manipulated oxytocin system. Using adult male and female CRISPR-Cas9-mutants lacking either of the two zebrafish oxytocin receptor genes, *oxtr* and *oxtrl*, we measured socialization between four fish (same-genotype, $n \geq 20$ for each group) in a large open-field (40x body length). We measured shoaling, group excursions, general locomotion, and specific motor patterns of mutant and wild-type fish in the large pool. Following stimulation with automated visual-only social stimulus individually, we also measured the levels of neurotransmitters and amino acids (dopamine, serotonin, norepinephrine, glutamate, GABA, and glycine) in multiple brain regions of these mutants using high precision liquid chromatography and compared them with wild-type control fish. Our data show that zebrafish lacking oxytocin receptors display social deficits and changes in anxiety-related behaviour, in a receptor-specific and context-dependent manner. We also found significant differences in neurotransmitter levels in the measured brain regions (olfactory bulbs and telencephalon, mesencephalon, diencephalon, cerebellum & hindbrain), indicating a role of oxytocin in regulation of neurotransmitters associated with social and emotional behaviour. These results suggest that the two oxytocin receptor play important but distinct roles in zebrafish social behaviour. These findings advance our understanding of neural mechanisms underlying oxytocin-regulated social interaction in zebrafish and highlight the potential of future investigation of zebrafish oxytocin system towards generating better therapeutic treatments for autism.

Disclosures: S. Shams: None. **L. Westberg:** None.

Digital Abstract Session

P020. Animal Models of Autism: From Molecular Mechanisms To Behavior

Program #/Poster #: P020.03

Topic: A.07. Developmental Disorders

Support: NIH Grant R01MH105610
Scripps SURF program

Title: Altered connectivity of dopaminergic projections from the ventral tegmentum to prefrontal cortex in a mouse model of autism/macrocephaly syndrome

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Abstract: Dysregulation of the dopamine system has been hypothesized as a factor contributing to behavioral and cognitive deficits in autism spectrum disorder (ASD) based on the role of dopamine in processing natural and conditional rewards and social behavior. Previous findings of social behavioral deficits in a mouse model of conditional deletion of the autism risk gene *Pten* in dopaminergic neurons further implicates the dopamine system in ASD-relevant behavioral phenotypes. *PTEN* is mutated in approximately 15% of individuals with autism spectrum disorder and macrocephaly and is a negative regulator of the PI3K-Akt-mTOR pathway. Signaling through mTOR is a critical effector of neuronal growth and connectivity; however, the role of dysregulated mTOR signaling in the development of dopaminergic circuitry underlying social behavior is unknown. We hypothesized dopaminergic neurons in the ventral tegmental area (VTA) and their projections to the mPFC (an area previously found to be hyperexcitable in *Pten* mutant mice) will be increased in germline $Pten^{+/-}$ mice, which are a model of macrocephaly/autism syndrome, due to dysregulated mTOR signaling. To test this hypothesis, we immunostained VTA and mPFC sections of wildtype and *Pten* mutant mice with anti-tyrosine hydroxylase and phospho-S6, a marker for mTOR activity. Measurements of soma size and axon fiber density were collected and analyzed blind to genotype. Our findings demonstrate that the soma of dopaminergic neurons residing in the VTA are enlarged in $Pten^{+/-}$ mice compared to WT controls. Dopaminergic neurons in the VTA also display greater mTOR activity compared to non-dopaminergic cells in $Pten^{+/-}$ mice. Analysis of VTA to mPFC axonal projections reveals that the distribution of dopaminergic axons in the mPFC is altered in $Pten^{+/-}$ mice, with deeper layers receiving greater axon innervation compared to upper layers. Increased axon fiber density in deeper layers may drive increased activity in the mPFC in response to social stimuli, leading to social behavioral deficits. To test this hypothesis, we attempted a rescue by reducing VTA to mPFC neuronal activity using chemogenetics (DREADDs). Upon reducing VTA to mPFC neuronal activity, social interaction increased in $Pten^{+/-}$ mice. Our findings suggest *Pten* mutations drive aberrant dopaminergic projections to the mPFC and decrease social interactions due to dysregulated mTOR signaling, pointing to a novel candidate pathophysiological mechanism in macrocephaly/autism syndrome for further investigation.

Disclosures: **A. Lish:** None. **A. Zucca:** None. **A. Clipperton-Allen:** None. **J. Sejourne:** None. **H. Swick:** None. **D. Page:** None.

Digital Abstract Session

P020. Animal Models of Autism: From Molecular Mechanisms To Behavior

Program #/Poster #: P020.04

Topic: A.07. Developmental Disorders

Support: SFARI

Title: Altered interval timing in the Nrxn+/-mouse model of Autism spectrum disorder

Authors: K. M. RODDICK[1], E. B. HABIB[1], F. BALCı[2], *R. E. BROWN[1];
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Abstract: Autism spectrum disorder (ASD) is characterized by impaired social interaction and communication and increased repetitive and stereotypical behavior. Neuroimaging shows functional abnormalities in brain areas involved in temporal processing of ASD patients (Allman & Meck, 2012, Brain, 135:656-77), and individuals with ASD show deficits in interval timing (Allman & Falter, 2015, In: Time Distortions in Mind:37-56). Neurexin (Nrxn) genes have been identified in a wide variety of neuropsychiatric disorders, including ASD, and *Nrxn1+/-* mice possess a mutation that disrupts both alpha and beta isoforms of Nrxn1, a gene that is involved in the structure of synapses (Flaherty et al., 2019, Nat Genet, 51:1679-90). We investigated the interval timing abilities of the *Nrxn1*+/- mouse model of ASD in the peak interval procedure with a 15s target interval. Two-month-old male *Nrxn1*+/- and C57BL/6J littermate control mice were trained to obtain sucrose liquid rewards 15s after the onset of a discriminative stimulus (discrete fixed-interval training) and their timing responses were tested in probe trials that lasted much longer and reinforcement was omitted. We found that the *Nrxn1+/-* mice responded earlier than the control mice on the probe trials, resulting in a leftward shift in their average response curve. This shift in response is typically seen in animals with hippocampal lesions (Balcı et al., 2009, In: Animal models of human cognitive aging:161-201), and is similar to the shift in timing responses of the 5xFAD mouse model of Alzheimer's disease on the same task (Gür et al., 2019, J Neurosci Res. 97:817-27). No differences were found in measures of temporal differentiation, response rate, or response amplitude. While a prior study of timing behaviour in individuals with ASD found a similar leftward response shift (Allman et al., 2011, Am J Intellect Dev Disabil, 116:165-78), another study found no such shift (Doenyas et al., 2019, Autism Res, 12:239-48). Leftward shifts were also found in a study of a different mouse model of ASD (Acosta et al., 2018, Eur J Neurosci, 47:19-630). In conclusion, these findings are suggestive of hippocampal dysfunction in the *Nrxn1+/-* mouse model of ASD, and add to the growing knowledge of timing behaviour in ASD.

Disclosures: K.M. Roddick: None. **E.B. Habib:** None. **F. Balcı:** None. **R.E. Brown:** None.

Digital Abstract Session

P020. Animal Models of Autism: From Molecular Mechanisms To Behavior

Program #/Poster #: P020.05

Topic: A.07. Developmental Disorders

Support: NIH R01MH16553

Title: Examining the impact of neuroimmune dysregulation on social play behavior of male and female juvenile rats

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Abstract: Recent literature indicates that along with genetic risk factors, there also are perinatal environmental factors that contribute to the risk of developing Autism Spectrum Disorder (ASD). These environmental factors are known to activate the immune system, such that dysregulation of the immune system early in life may have negative consequences on neural function and the development of appropriate social behavior. Our experiment is designed with the intent of modeling the multitude of social behavior deficits seen in many neurodevelopmental disorders, including autism and schizophrenia. We hope to be able to eliminate some of the questions still present in these disorders. Microglia, which are the immune cells of the brain when activated, release inflammatory cytokines throughout which in effect “primes” these cells. The exaggerated cytokine release from the microglia, as well as a second “hit” later in life, alters neuronal function and leads to behavioral disorders. Our model introduces an immune challenge to rats on day 4 of life (P4) and then again on day 25 (P25). This result stems from a technique called a “two-hit model of neuroinflammation," the model that we currently use in our experiments. Play behaviors such as pouncing, pinning, chasing, and social exploration were observed and analyzed in juvenile male and female rats. Our previous work shows these play behaviors for a juvenile dyad consisting of one control animal and one experimental animal (treated with two-hit model). New data consisting of the same play behaviors and dyad were scored by looking at behaviors for the experimental animal alone. With this data we can observe the differences in the participation vs initiation in a play bout with our experimental animals compared to controls. Our goal is to further understand the impact of the two-hit model of neuroinflammation on the development and expression of social play behaviors in male and female juvenile rats. Additionally by looking at this data we can further investigate the differences present between the sexes. Funding: NIH R01MH16553 to JMS.

Disclosures: E. McAuley: None. **J. Schwarz:** None. **A. Turano:** None.

Digital Abstract Session

P020. Animal Models of Autism: From Molecular Mechanisms To Behavior

Program #/Poster #: P020.06

Topic: A.07. Developmental Disorders

Support: NIH grant R01MH106553

Title: Examining the impact of neuroimmune dysregulation on locomotive activity in male and female juvenile rats

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Abstract: Evidence suggests that microglia, the immune cells of the brain, have an important role in the development of brain circuits. As a result, activation of the immune system early in life significantly increases the risk of various neurodevelopmental disorders, including AutismSpectrum Disorder. Perinatal exposure to infectious pathogens is suggested to be an environmental risk factor that has a role in activating the developing immune system, and the subsequent onset of neurodevelopmental diseases. Furthermore, the "two-hit hypothesis of immune activation" suggests that not just one, but multiple exposure to immune activation further increases the risk of neurodevelopmental disorders. That is immune activation during a"sensitive period" of neurodevelopment may result in an exaggerated immune response to subsequent immune challenges and the onset of behavioral disorders during the juvenile period.Microglia number and activation profiles differ between males and females during early development. Thus, males might be more susceptible to the consequences of immune activation as a result of the two-hit model, thereby leading to a greater prevalence of males diagnosed with neurodevelopmental disorders. In order to better understand the two-hit model of neuroinflammation and its effect on the expression of social behaviors in males and females, we tested the impact of neonatal bacterial infection (E.coli) and a subsequent juvenile immune challenge (lipopolysaccharide, LPS) on two well established tests of rodent social behavior - social interaction and social recognition. Using an open-source ImageJ plugin, MouBeAt, locomotive behavior; such as total distance traveled, total freezing time, average velocity and more were examined during these behavioral tests. Preliminary analysis of the data thus far indicates that the timing (neonatal or juvenile) and nature (E.coli or lipopolysaccharide) of the neuroimmune activation have a different effect on the expression of locomotive activity in male and female juvenile rats during a social behavior test.

Disclosures: M. Muench: None. **A. Turano:** None. **J.M. Schwarz:** None.

Digital Abstract Session

P020. Animal Models of Autism: From Molecular Mechanisms To Behavior

Program #/Poster #: P020.07

Topic: G.05. Mood Disorders

Title: Impact of Clostridium celatum on behavior in a murine model of Autism Spectrum Disorder

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Abstract: Autism Spectrum Disorder (ASD) has become increasingly prevalent in recent years, withthe CDC estimating that it now affects 1 in 59 children. Recent studies have linkedneuropsychiatric phenotypes of ASD to both genetic and environmental factors,and amongthem have established a promising link between the gut microbiome and autisms. Mousestudies for example have highlighted the growing importance of the role of the gutmicrobiota in ASD, and neurotypical behaviors were rescued in a mouse model of autismusing the commensal bacteria *Bacteroides fragilis,* establishing a direct association betweenthe gut microbiome and ASD. In human subjects, studies comparing the microbiomes ofASD-affected and neurotypical children found that the former were depleted of*Bifidobacterium* spp. and *Prevotella* spp. and enriched in *Clostridium* spp, particularly*Clostridium celatum,* a non-pathogenic bacterial taxa. In order to establish whether *C.celatum* can effectively modulate the ASD phenotype, we investigate the mechanismsunderlying these effects by using behavioral assays with and without the bacteria in anautism-like mouse Maternal Immune Activation model. Here we assess anxiogenic behavior,general activity levels, restrictive and repetitive behavior, and social cognition, and socialnovelty through the elevated plus maze, open field habituation, marble burying, andthree-chamber sociability test. In addition, gastrointestinal content has been sampled fromthe duodenum, jejunum, ileum, and caecum of selected subjects and used to assess thestructure and diversity of microbial communities of the gut using 16S analysis. Our studyfinds potential colonization of *Clostridium* in the mice and evidence for modulation of theanxiogenic phenotype between mice treated with and without the bacteria.

Disclosures: G.E. Deitzler: None. **A. Phillips:** None. **P. Singla:** None. **M.M. David:** None. **A. Martin-Varnum:** None.

Digital Abstract Session

P021. Autism: Genetic Models

Program #/Poster #: P021.01

Topic: A.07. Developmental Disorders

Support: 1R01NS104428-01A1
SC INBRE NIGMS P20GM103499
COBRE NIGMS P20GM103641
SC EPSCoR/IDeA 18-SR04

Title: Ash1l regulates the structural development of human neurons by modulating bdnf/trkb signaling

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Abstract: Autism spectrum disorder (ASD) is highly heritable and associated with alterations in neuronal connectivity. Genetic studies suggest that there is an overrepresentation of chromatin regulatory genes associated with ASD. ASH1 Like Histone Lysine Methyltransferase (ASH1L) is a major genetic risk factor for ASD. ASH1L methylates Histone H3 on Lysine 36 this histone modification is proposed to be primarily involved in transcriptional activation. However, how mutations in ASH1L lead to deficits in neuronal connectivity associated with ASD pathogenesis is largely understudied. Using stem cell-derived human neurons, we found that ASH1L regulates neuronal morphogenesis by counteracting the Polycomb Repressive complex 2 group (PRC2) catalytic activity. Analysis of changes in chromatin landscape and gene expression by ATAC seq suggest that ASH1L regulates pathways associated with neurite projection development, neuronal morphogenesis, and synaptic function. In particular, we find a strong correlation of our neuronal morphogenesis phenotypes with decreased expression of the neurodevelopmentally important neurotrophin receptor TrkB. Therefore, we present here a novel mechanism regulating neuronal morphogenesis that implicates an epigenetic modifier-ASH1L as a key regulator of the BDNF-TrkB signaling pathway in humans and that might underlie ASD pathogenesis.

Disclosures: J. Vacharasin: None. **S. Cheon:** None. **A. Culver:** None. **A. Bagnell:** None. **F. Ritchie:** None. **M. McCord:** None. **C. Papendorp:** None. **E. Chukwurah:** None. **A. Smith:** None. **M. Cowen:** None. **P. Ghate:** None. **S. Davis:** None. **J. Liu:** None. **S. Lizarraga:** None.

Digital Abstract Session

P021. Autism: Genetic Models

Program #/Poster #: P021.02

Topic: A.07. Developmental Disorders

Support: Partnership for Pediatric Epilepsy Research grant, Autism Speaks
Norton Children's Hospital grant

Title: Neuroinflammation and platelet-endothelial injury with disruption of the blood brain barrier in a genetic model of autism with GABAergic neuron-specific deletion of Semaphorin 3F

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Abstract: Background: Semaphorin impacts neurogenesis and vasculogenesis and regulates cell and neurite motility of neurons and endothelial cells. Autism spectrum disorder (ASD) is

associated with neuroinflammation but vascular and blood brain barrier (BBB) involvement is unclear. We previously reported neuroinflammation, oxidative stress, epileptogenesis and ASD behavior in GABAergic interneuron-specific DLX5/6Cre+ Sema 3F KO mice. We aimed to determine whether neuroinflammation and oxidative stress involve platelet-endothelial injury and BBB dysfunction in this genetic model of ASD.
Methods: We examined the expression and distribution of platelets (CD61), platelet activation (p-selectin and serotonin) markers, fibrinogen and albumin leakage as an indicator of blood brain barrier disruption and endothelial injury, in DLX5/6Cre+FF Sema 3F KO mice. Furthermore, we examined superoxide levels (DHE staining) in the heterozygous DL5/6Cre+F/WT mouse and whether the excitatory neuron-specific EMX1-Cre+ FF mouse that shows no epileptogenesis, exhibits similar increases in oxidative markers. We also compared brain weight and electrophysiological (EEG) changes of both DLX5/6 Cre+FF and EMX1-Cre+FF Sema 3F KO mice.
Results: Sema 3F deletion resulted in 10% brain weight loss and decreased the alpha and beta frequency EEG power in the DLX5/6Cre+FF but not in EMX1-Cre+ FF mice. Increased superoxide was also detected in heterozygous DLX5/6 Cre + F/WT but not in EMX1-Cre + FF mice. Platelet deposition (CD61) and activation (p-selectin, serotonin, fibrinogen) occurred in multiple brain regions important in ASD. Finally, along with platelet activation and fibrinogen accumulation, albumin leakage and uptake suggest BBB deficits.
Conclusions: Electrophysiological changes may underlie increased epileptogenesis and ASD behavior in DLX5/6Cre+ mice. Additionally, platelet activation, with increased serotonin release that has previously been associated with the severity of ASD behavioral deficits, as well as protein leakage suggest that Sema 3F deletion in inhibitory interneurons causes platelet-endothelial injury and BBB breach, further enhancing neuroinflammation. These observations may provide some insights into new therapeutic strategies targeting platelet-endothelial dysfunction and neuroinflammatory pathways to improve symptoms and behavioral deficits associated with ASD.

Disclosures: **R. Jagadapillai:** None. **Z. Li:** None. **E. Gozal:** None. **G.N. Barnes:** None.

Digital Abstract Session

P021. Autism: Genetic Models

Program #/Poster #: P021.03

Topic: A.07. Developmental Disorders

Support: Grant # UL1 TR001866 from the National Center for Advancing Translational Sciences (NCATS, National Institutes of Health (NIH) Clinical and Translational Science Award (CTSA) program

Title: Novel Astn2 mutant mouse shows aberrant cerebellar synaptic structure and physiology and exhibits autism-like behaviors

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Abstract: The cerebellum has recently been recognized as an important contributor to cognition and neurodevelopmental disorders such as autism spectrum disorder (ASD). We examine the role of the predominantly cerebellar gene *ASTN2* in cerebellar circuit function and ASD-related behaviors. Copy number variations in *ASTN2* have been identified as a significant risk factor for ASD, suggesting that *ASTN2* mutations lead to altered cerebellar synaptic function. In addition, we recently reported a family with a paternally inherited intragenic *ASTN2* duplication that manifested a range of neurodevelopmental disorders, including ASD, learning difficulties, and speech and language delay (Behesti et al, 2018). Our previously published cellular and molecular studies on mouse cerebellum show that ASTN2 binds to and regulates the trafficking of multiple synaptic proteins, including Neuroligins, which have been genetically linked to ASDs, and modulates cerebellar Purkinje cell (PC) synaptic activity (Behesti et al, 2018). To provide a genetic model to study cerebellar circuit function and the role of the cerebellum in neurodevelopmental disorders, we generated a novel global *Astn2* knockout mouse line. Our electrophysiology experiments indicate that P30 mice lacking *Astn2* have a decrease in evoked excitation relative to inhibition in PCs. We found a reduced PC dendritic spine density using Golgi staining at P21, and a reduction of vGlut1 excitatory synapses between granule cells parallel fibers and PCs via immunohistochemistry at P21 and 6 months old. These results suggest a specific cerebellar circuit defect in the *Astn2* mutants. In addition, we observe motor deficits and defects in ASD-related behaviors in *Astn2* mutants. When compared to wild type littermates, *Astn2*$^{-/-}$ animals show a marked decrease in the number of calls in separation induced pup ultrasonic vocalizations (male and female P2-P14 pups), hyperactivity, anxiety, and repetitive behavior phenotypes in the open field test (male and female 8-12 week old animals), and reduced sociability and social novelty seeking in the three-chamber test (male and female 8-12 week old animals). As other findings do not indicate major defects in cerebellar development, we hypothesize that the behavioral defects we observed relate to defects in the cerebellar circuitry with underlying changes in synaptic receptor trafficking.

Disclosures: **M. Hanzel:** None. **S. Maloney:** None. **Z. Horn:** None. **H. Behesti:** None. **S. Gong:** None. **J.D. Dougherty:** None. **C. Hull:** None. **M.E. Hatten:** None.

Digital Abstract Session

P021. Autism: Genetic Models

Program #/Poster #: P021.04

Topic: A.07. Developmental Disorders

Support: University of Michigan
University of Michigan, Department of Human Genetics

Title: Characterizing the role of the histone methyltransferase, Ash1I, in mouse brain development

Authors: *K. P. TOOLAN, M. L. BRINKMEIER, S. BIELAS, S. A. CAMPER;
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Abstract: Over 100 genes have been implicated in the genetic etiology of autism spectrum disorder (ASD). Many high confidence ASD genes are involved in chromatin remodeling, histone modifications and DNA methylation. Among these is *ASH1L* (Absent, Small, Homeotic-Like), which catalyzes histone H3K36 methylation and has a role in activating transcription by counteracting polycomb repression. ASD patients have been identified with heterozygous *ASH1L* variants that are predicted to cause loss of function, and they include premature termination, frame shifts, and missense variants throughout the protein. While ASH1L is expressed in a number of regions of the developing and adult brain, its role has yet to be defined. We hypothesize that gene expression changes caused by *Ash1l* deficiency will alter cortical development and cause molecular abnormalities in cortical layers. To investigate the role of *Ash1l* in mammalian neural development and function, we established novel mouse models of *Ash1l* deficiency with cre-loxP technology starting with the $Ash1l^{tm2a(EUCOMM)Hmgu}$ mouse line. Using FlpO, we generated an allele with loxP sites flanking exon 4, $Ash1l^{fl}$. *Cre*-mediated excision of exon 4 results in a premature stop codon in exon 5, p.V1693Afs*2, a presumptive null allele. Using an *EIIA*-cre mouse line we generated *Ash1l* heterozygous null mice ($Ash1l^{+/-}$) and confirmed that $Ash1l^{-/-}$ mice are underrepresented at postnatal day 14 ($p = 0.021$). Preliminary results revealed defects in corpus callosum development at embryonic day 18.5-P0 in $Ash1l^{-/-}$ mice relative to littermate controls. We are in the process of histological analysis of brain development in $Ash1l^{-/-}$ embryos, including assessments of cell proliferation and death, and cortical layer organization. Additionally, we used $Emx1\text{-}Cre^{ERT2}$ to induce cortical-specific knockouts of *Ash1l* and we are in the process of determining gene expression changes via single cell RNA sequencing on $Emx1\text{-}Cre^{ERT2}; Ash1l^{fl/fl}$ mouse embryos. By better understanding the role of *Ash1l* in the mouse cortex, we aim to provide insight into the developmental and molecular pathogenesis of ASD in patients with *ASH1L* loss-of-function variants. Well-characterized *Ash1l* knockout mouse strains will be a valuable for future assessment of therapeutic treatments.

Disclosures: **K.P. Toolan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Editas Medicine. **M.L. Brinkmeier:** None. **S. Bielas:** None. **S.A. Camper:** None.

Digital Abstract Session

P021. Autism: Genetic Models

Program #/Poster #: P021.05

Topic: A.07. Developmental Disorders

Support: MINECO RTI2018-101427-B-I00

Title: Structural abnormalities in Purkinje neurons in the Cntnap2 mouse model of autism

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Abstract: The cerebellum has traditionally been associated with motor function, however, there is increasing evidence of its role in modulating non-motor behaviors, including social cognition, which has wide implications for disorders such as autism. Indeed, several cerebellar abnormalities have been found in postmortem brain tissue of individuals with autism, including reduced number and size of Purkinje cells (PCs). Animal models provide a major avenue to test the implication of a certain neuropathology in the disorder. In humans, mutations in the CNTNAP2 gene are associated with cerebellar abnormalities in individuals with autism. To investigate its potential role in the disorder, in this study, we characterized the neuroanatomical structure of PCs in a mouse model of autism, knockout for the Cntnap2 gene, which has been shown to display cerebellar functional abnormalities.We performed biocytin injection upon patch clamp recordings of PCs in the Crus I region of the cerebellum (an area shown to be involved in social behavior) in male mice. We then reconstructed the dendritic tree of PCs and performed Sholl analysis with ImageJ to study neuronal arborizations. Furthermore, the relative spine density was also estimated semi-automatically using ImageJ.We found a reduction in PC length and number of ramifications in knockout mice, indicating a less complexity of neuronal arborization. This result could be related to an underdevelopment of this region and it could trigger alterations in the functionality of those cells. Conversely, the density of dendritic spines appears to be increased in knockout mice, perhaps to counteract that underdevelopment.Further studies are needed to identify potential functional abnormalities associated with the structural alterations observed, which will give new insights into the pathophysiology of autism.

Disclosures: T. Sierra-Arregui: None. **M. Fernández:** None. **S. Knafo:** None. **O. Peñagarikano:** None. **J. Llorente:** None.

Digital Abstract Session

P021. Autism: Genetic Models

Program #/Poster #: P021.06

Topic: A.07. Developmental Disorders

Support: NIH RO1 Grant ES026896

Title: Dysregulation of autism associated genes foxg1, shank3, and homer1 following postnatal exposure to bisphenol a

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Abstract: Autism Spectrum Disorder (ASD) is a heterozygous disorder marked by social impairments and varying levels of genetic dysregulation. Some commonly dysregulated genes including RORA and SHANK3 are modulated by actions at gonadal hormone receptors, making these genes vulnerable to the effects of endocrine disrupting chemicals. Bisphenol A is a known estrogenic compound though it is unclear what impact BPA could have on the expression of hormonally regulated genes due to BPA having distinct mechanisms at nuclear estrogen receptors. Increased postnatal levels of BPA have been associated with an increased risk of developing ASD, though it is unclear what role BPA may have in the pathology of the disorder. Decreased SHANK3 expression has been seen in children with ASD and has been linked with social deficits in mice. FOXG1 is implicated in inhibitory neuron differentiation, with increase expression of FOXG1 resulting in increased inhibitory neurons. The present study aims to assess whether postnatal exposure to BPA results in dysregulation of these hormonally regulated genes. Male and female Long Evans hooded rats were dosed from postnatal day 6 to 8 via oral gavage with either 0, 40, or 400 mg/kg BPA suspended in corn oil. A rt-qPCR was run to determine relative expression of commonly ASD associated genes RORA, FOXG1, HOMER1, and SHANK3. Males exposed to BPA had increased HOMER1 expression, which was most notable in the 400mg/kg group. SHANK3 expression was increased in males exposed to 40mg/kg BPA but decreased in males exposed to 400mg/kg BPA. FOXG1 expression was increased in males exposed to 400 mg/kg BPA. These findings suggest a dose and sex specific response to BPA, where males exposed to 400mg/kg BPA showed the highest level of dysregulation. Males exposed to 400 mg/kg BPA exhibited dysregulation of FOXG1 and SHANK3, in a similar pattern as seen in children with ASD.

Disclosures: **A.S. Brinks:** None. **C.M. Drzewiecki:** None. **J.M. Juraska:** None.

Digital Abstract Session

P021. Autism: Genetic Models

Program #/Poster #: P021.07

Topic: A.07. Developmental Disorders

Support: National Institutes of Mental Health (NIMH)
Simons Foundation, Binational Science Foundation
Kavli Foundation, Spector Fund
National Genetics Fund and Swebilius Foundation

Title: Functional analysis of ASD risk genes in zebrafish

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Abstract: Autism spectrum disorders (ASDs) are a group of complex neurodevelopmental disorders that affect social behavior and communication, and are characterized by the presence of repetitive, restrictive behaviors. There are still no pharmacological interventions available that target the core deficits in ASD as much is unknown about the neurobiology of the disorder. Whole-exome sequencing has recently revealed “high confidence” risk genes associated with ASD (hcASD), enabling a series of more in depth studies, however our knowledge about the mechanisms by which hcASD gene disruption alters brain development, remains elusive. The zebrafish has become an optimal vertebrate model for genetic studies due to its fast development, transparency of its embryos, availability of efficient methods for generating mutant lines and tractability for high-throughput analyses. Using CRISPR/Cas9 technology, we have generated zebrafish mutant lines of ten hcASD genes. To investigate how gene disruption alters basic sensory processing and sleep-waking activity, we have established a novel behavioral high-throughput in vivo system. This system is used to assay visual-startle and rest-wake behavior across the different mutants and in parallel, for the screening of over 750 FDA-approved drugs to identify their effects on these behaviors. By comparing the effect of these drugs in wild-type fish to the mutant behavioral profiles, we aim to identify dysregulated pathways in mutants and potential suppressors of hcASD mutant behavioral phenotypes. Preliminary analyses have identified a range of behavioral phenotypes across hcASD mutants.. Using these profiles, we are beginning to identify pharmacological compounds that target these phenotypes and investigate the mechanisms by which certain phenotypes could be reverted with these anti-correlating drugs. The identification of phenotypes in the behavioral profile of hcASD zebrafish mutants and the discovery of new pharmacological suppressors of these phenotypes has the potential for identifying drug candidates for further investigation in ASDs. RNA-seq analysis of zebrafish brains during the same developmental stages of behavior profiling, are also being carried for all mutant lines. The exploration of disrupted pathways, gene expression and upstream regulators related to these hcASD genes, may elucidate other common molecular players of this complex neuro disorder.

Disclosures: **H. Weinschutz Mendes:** None. **T. Chen:** None. **W. Wu:** None. **J. Elibott:** None. **B. Vander Wyk:** None. **A. Wang:** None. **E. Hoffman:** None.

Digital Abstract Session

P021. Autism: Genetic Models

Program #/Poster #: P021.08

Topic: A.07. Developmental Disorders

Support: NIH Grant
Simons Foundation

Title: Whole-brain activity mapping in zebrafish ASD risk gene models

Authors: ***T. CHEN**[1], H. WEINSCHUTZ MENDES[1], D. S. JIN[2], B. M. ROONEY[1], X. PAPADEMETRIS[3,4], E. J. HOFFMAN[5,6];

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Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that affects approximately 1 in 54 children in the U.S. Whole-exome sequencing has led to the identification of at least 100 high confidence risk genes that are strongly associated with ASD. However, the mechanisms by which the disruption of risk genes alters basic neural signaling during vertebrate brain development is not well understood. To investigate this, we generated zebrafish mutants in 10 high confidence ASD risk genes in zebrafish using CRISPR/Cas9 and developed a novel pipeline for the analysis of whole-brain activity in mutants.. Because we found that zebrafish ASD risk gene mutants lack major structural abnormalities, but display behavioral deficits, we hypothesized that loss of ASD risk gene function might lead to changes in baseline brain activity. To analyze this, zebrafish mutant and sibling- or cousin-matched wild-type larvae were fixed at 6 days post fertilization and stained for phospho-ERK (pERK) and total-ERK (tERK), followed by confocal imaging. ERK is a downstream reporter of calcium signaling, such that pERK stains only active neurons, while tERK antibodies stains all neurons. Using our pipeline, confocal images were registered to a standard zebrafish reference brain and mapped onto a zebrafish brain atlas. This allows us to quantify regional differences in brain size and activity. Our findings show that zebrafish ASD risk gene mutants display alterations in baseline brain activity affecting several brain regions, though these patterns were non-overlapping in different mutants. For example, zebrafish *scn1lab* mutants show significant deficits in baseline activity throughout the brain, most prominently in the telencephalon, while *dyrk1a* mutants show deficits in the mesencephalon. These findings suggest that ASD risk gene disruption might differentially affect baseline brain activity. In future studies, we will perform in vivo functional imaging in zebrafish ASD risk gene mutants to identify changes in brain activity patterns during behavioral tasks.

Disclosures: T. Chen: None. **H. Weinschutz mendes:** None. **D.S. Jin:** None. **B.M. Rooney:** None. **X. Papademetris:** None. **E.J. Hoffman:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.01

Topic: A.09. Adolescent Development

Support: NIH Grant GM122657

Title: Alterations in the Perineuronal Nets, Microglial Cells, and Open-Field Activity of Prenatally Restricted Mice

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Abstract: Inadequate food consumption during pregnancy can negatively impact fetal brain development, which is mediated in part by perineuronal nets and microglial cells. Perineuronal nets are a neuronal specific form of the extracellular matrix which envelop the cell bodies and proximal processes of predominantly GABAergic interneurons. As specialized macrophages, microglia clear dead neurons and other cellular debris to maintain CNS health. To study how low food consumption impacts offspring, adult female CD-1 mice were placed on a calorically restricted diet before and during pregnancy. Pups produced from this group were compared to pups born from ad libitum-fed mothers. Perineuronal nets and microglia in pups were visualized using histochemistry to better understand how maternal caloric restriction impacts offspring brain development. An open-field paradigm was also conducted in which mice were allowed to freely explore an arena while locomotor activity was measured. Mice that were calorically restricted in utero had significantly fewer perineuronal nets compared to non-restricted mice. There was also a sex difference with female offspring being more affected by maternal caloric restriction than male offspring. The microglia of prenatally restricted mice were more rammified compared to non-restricted mice. Female prenatally restricted pups also expressed less willingness to explore in the behavioral paradigm compared to the control pups. These results suggest that food shortage may have a degenerative effect on the brain plasticity and anxiety levels of prenatal offspring. From studying the impact of food restriction in mice, this data can be used to better understand the effects of inadequate caloric intake on children born to mothers during times of food shortage.

Disclosures: K.E. Berta: None. **S. Lutchman:** None. **J.C. Brumberg:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.02

Topic: A.09. Adolescent Development

Support: NSF IOS 1735934
NSF DBI 1906523
NIH R01 MH102456
Ford Foundation Postdoctoral Research Fellowship

Title: Sex-specific effects of development on social recognition in rats.

Authors: *K. E. YOEST[1], M. G. HENRY[1], H. A. VELISEK[1], R. PEGUERO[2], A. H. VEENEMA[1];
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Abstract: The ability to recognize previously encountered conspecifics is crucial for normal social interaction. This social recognition ability is well characterized in adults of both sexes but remains largely unexplored in juveniles. We first determined whether juvenile male and female

rats show similar temporal patterns of social recognition to adults, by testing their ability to recognize a previously encountered same-sex stimulus rat 30, 60, or 90 min following initial investigation. Similar to adults, juvenile males showed social recognition 30 and 60 min, but not 120 min, following the initial encounter. Juvenile females, however, did not show social recognition at any time point tested. We then determined at what age during development social recognition ability is established in female rats, by testing females as juveniles, adolescents, young adults, and adults. We found that only young adult and adult females showed social recognition. This developmental difference in social recognition ability was driven by a decrease in the amount of time females investigated the previously encountered (familiar) stimulus. Based on these findings, we hypothesized that social recognition is impaired in juvenile and adolescent females due to a lack of circulating estradiol. To test this, we administered estradiol benzoate (EB) 48 hours prior to testing for social recognition in juvenile female rats. EB treatment induced a preference to investigate the familiar over a novel social stimulus, the opposite behavioral pattern observed in typical rodent social recognition. Also, EB treatment increased investigation of the social stimulus during the initial investigation period. Together, this suggests that additional factors contribute to the lack of social recognition ability in juvenile females. This may include sex and age differences in activation of the bed nucleus of the stria terminalis, a brain area implicated in adult social recognition which shows social stimulus-induced activation in juvenile females, but not males. These findings provide the first evidence of a development-specific sex difference in social recognition ability. Ongoing work seeks to tease apart the underlying neurobiological mechanisms by investigating the phenotype of BNST cells activated following social investigation, as well as the role of ovarian hormones during development on social recognition ability. Research supported by NSF IOS 1735934 to KEY, NSF DBI 1906523 and NIH R01 MH102456 to AHV.

Disclosures: K.E. Yoest: None. **M.G. Henry:** None. **H.A. Velisek:** None. **R. Peguero:** None. **A.H. Veenema:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.03

Topic: A.09. Adolescent Development

Support: NSERC Discovery Grant RG203596-13

Title: The sex-specific effects of adolescent omega-3 supplementation and exposure to environmental enrichment on adulthood anxiety and avoidance behaviour in Wistar rats

Authors: *A. MORIN, M. POITRAS, J. RAYMOND, H. PLAMONDON;
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Abstract: The adolescent period is known as a critical stage for brain development and for its vulnerability to various external influences. In fact, dietary supplementation of omega-3 (n-3)

and exposure to environmental enrichment (EE), defined as housing rodents in larger, more stimulating cages equipped with toys, running wheels, and more cagemates, have both separately shown beneficial effects on synaptic plasticity, glucocorticoid receptor (GR) expression, and anxiety when exposed during the developmental period. However, as of now, no study has assessed the long-term behavioural and molecular effects of their combined time-limited exposure. This study aimed at investigating putative synergistic and sex-specific actions of n-3 and EE during adolescence on anxiety-like behaviour and fear avoidance in adulthood. Wistar rats (n=32 males; n=32 females) aged 23 days old arrived at facility and were assigned to one of four conditions based on dietary supplementation [fish oil (FO; n-3 (20-31%); eicosapentaenoic acid (10-15%); docosahexaenoic acid (8-15%)) or control soybean oil (CSO)] and housing [EE or regular cages (RC)] (n=8/group). Supplementation regimen (0.3 ml/100 g body weight) took place from postnatal day (PND) 28 to 47 and EE was provided between PND 28-58, after which all rats returned in RC. In adulthood (PND 90), a modified version of the forced swim test was used to trigger stress response. The following days, anxiety-like behaviours were assessed using the Open Field Test (OFT) and Elevated-Plus Maze (EPM - PND 92) and measures of aversive conditioning memory were obtained with the Y-Maze Passive Avoidance Task (YMPAT) on PND 94. Brain tissue was collected on PND 97 and immunohistochemistry was used to quantify GR expression in the CA1 and CA3 hippocampus sublayers. In the OFT, exposure to EE during adolescence reduced adult anxiety-like behaviours in males having received CSO, in part due to elevated global activity. Overall, females displayed more anxiety-like behaviours than males. No significant differences were observed in the YMPAT. In the CA3, females showed elevated GR expression compared to males, as did CSO- compared to FO-treated groups. These results did not support adolescent FO exposure to have beneficial effects on delayed anxiety-like behaviours. Concordant with other studies, females showed heightened emotional/neuronal response to anxiogenic environments. No treatments affected fear-associated learning. Taken together, these results provide a better representation of complex, sex-specific, and long-term effects of adolescent diet-environment interactions on adulthood behaviour and neuronal function.

Disclosures: **A. Morin:** None. **M. Poitras:** None. **H. Plamondon:** None. **J. Raymond:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.04

Topic: A.09. Adolescent Development

Support: CNPq Scholarship 141700/2017-3

Title: Maternal hyperglycemia and snack intake effects on offspring sucrose preference and behavior in adolescence

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Abstract: The effects of maternal diabetes and diet manipulation have been thoroughly studied separately. Previous studies have shown that snack intake during pregnancy and lactation further impair glucose tolerance on hyperglycemic female rats. Nonetheless, there is no evidence of how diet manipulations may aggravate behavioral and metabolic impairments in the offspring of hyperglycemic dams. Since maternal metabolism and nutritional status are key factors for a healthy offspring intrauterine and postnatal development, the aim of the present study was to investigate the impact of mild hyperglycemia associated with snack intake on offspring behavior in adolescence. Newborn female Wistar rats were divided into Control (citrate buffer, s.c.) or STZ (streptozotocin, 100 mg/kg, s.c.) groups. On postnatal (PND) 90, rats from both groups were mated and subdivided into four groups: females fed with standard chow (Control, n = 22; STZ, n = 23); and females fed with standard chow plus potato chips and 1.5% sucrose solution from pregnancy day 0 to lactation day 14 (Control-snack, n = 23; STZ-snack, n = 23). Dams gave birth naturally and on PND 1, litters were culled to 6 pups (3 males, 3 females). On PND 30, male and female offspring were evaluated in the open field arena and the elevated plus maze, followed by a glucose tolerance test and a sucrose preference test. All experimental procedures were approved by the local ethics committee (Protocol number 919). As expected, female offspring explored more the open field arena, but neither maternal metabolism nor snack intake impaired exploratory behavior. Maternal snack intake increased open arms entries in the elevated plus maze in both sexes, as well as the time female offspring spent in open arms. Regarding sucrose preference, the association between maternal metabolism and snack intake had opposing effects in male and female, leading to a decreased preference in the former and an increased one in the latter. Finally, offspring from snack-fed dams, regardless of maternal metabolism, showed lower glycemic levels during the oral glucose tolerance test. In conclusion, snack intake during pregnancy and lactation reduced anxiety-like behavior in adolescent offspring and changed sucrose preference in a sex-dependent way. Further studies will analyze if offspring behavior is affected by this impaired maternal metabolism in adulthood.

Disclosures: **M.G. Martins:** None. **A.C.I. Kiss:** None. **J.A.C. Horta:** None. **B. Woodside:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.05

Topic: A.09. Adolescent Development

Support: Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Open-field behaviors in adolescent rats: Influence of sex and immune activation on variable reliabilities and intercorrelations

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Abstract: Introduction. This study examined the reliabilities of open-field (OF) locomotor and anxiety-like behavioral measures in adolescent rats. The OF is an apparatus frequently used in rodent behavioral studies. Some previous examinations of reliabilities of OF variables exist, but we expanded the range of variables, examined sex differences and the effects of an immune stressor (lipopolysaccharide [LPS]) during early adolescence on OF variable reliabilities and cross-correlations in later adolescence. We predicted that score aggregation would increase reliabilities across test days, exposure to LPS would decrease reliabilities and/or cross-correlations and that the effects would be more pronounced in females than in males.
Methods. Long-Evans male (n = 32) and female (n = 32) rats were injected with LPS (200 μg/kg) or vehicle control on postnatal day [PND] 30 and 32. Locomotor and anxiety-like behaviors were measured in an OF on PND 38-40. The locomotor variables included total horizontal distance moved (TD), number of horizontal movements (NHM), distance per horizontal movement (D/HM), vertical time (VT), and number of vertical movements (NVM). The anxiety-like variables included central duration (CD), central nosepokes (CNP), number of peripheral to central OF transitions (NT), time per vertical movement (T/VM), and thigmotaxis ratio (THIG).
Results. High positive correlations were found for both locomotor and anxiety-like behavioral measures from PND 38 - 40 (range = .401 - .842). These correlations increased substantially when the data was aggregated across the 3 test days (range = .723 - .945). When cross-correlations among the aggregated OF variables were examined, the male rats had significantly reduced correlations for the LPS relative to the vehicle control group for locomotor variables correlated with NHM. Significant differences were also found for correlations between NHM and CNP and NT, and D/HM correlated with CD and NT, in the males. In the females there were significant differences between the vehicle control and the LPS group in THIG correlated with NVM and CNP as well as TD and NVM.
Discussion. In line with our predictions, aggregation increased the reliability of both locomotor and anxiety-like behavioral measures. Exposure to LPS decreased the cross-correlations for some locomotor measures. Contrary to our predictions, LPS related changes were more pronounced in males than in females. These results suggest that the OF locomotor and anxiety-like behavioral measures are reliable. However, when rats are previously exposed to an immunogen, such as LPS, the cross-correlations, especially of locomotor measures in male rats, are reduced.

Disclosures: I. Bishnoi: None. **M. Kavaliers:** None. **K. Ossenkopp:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.06

Topic: A.09. Adolescent Development

Support: NIEHS T32 ES007326
NIH R21 ES026896
NIH P01 ES002848-Project 3

Title: The role of puberty in Esr2 expression in the rat medial prefrontal cortex, motor cortex, and striatum: An RNAscope analysis

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Abstract: Adolescence, often characterized as the period encompassing puberty, is a unique time of neurodevelopment within the medial prefrontal cortex (mPFC). Our laboratory has previously demonstrated extensive neuroanatomical changes occurring during adolescence in the mPFC. For example, a significant reduction in neuron and synapse number occurs during adolescence, and these changes tend to coincide with pubertal onset, suggesting a role for gonadal hormones in these processes. Recently, we have examined the number of perineuronal nets (PNNs), components of the extracellular matrix that preferentially surround parvalbumin (PV) expressing interneurons, during adolescence and have shown that they are significantly downregulated 24 hours after pubertal onset in females (Drzewiecki et al, 2020). Estrogen receptor beta (ERβ) is the prominent ER present in the adult mPFC, and it is highly colocalized with PV, providing a candidate mechanism for the neural reorganization occurring during adolescence. Several studies have shown that ERβ expression is altered in the presence of estrogens, though the direction of these effects is region specific (Osterlund et al., 1998; Patisaul et al., 1999). We therefore examined the expression of *Esr2*, the gene encoding ERβ, during puberty in female rats. Subjects were observed daily for pubertal onset, marked by vaginal opening. When a female reached puberty, the rat and an age- and sex-matched, pre-pubertal littermate were sacrificed 24 hours later. The brains of pre-pubertal and post-pubertal female pairs were processed using RNAScope to label *Esr2* in the mPFC. The motor cortex and striatum were also analyzed to better understand whether observed changes are unique to the mPFC. In the mPFC, there was a significant reduction in *Esr2* expression, as evidenced by decreased mean fluorescent intensity ($p = 0.04$) and fewer fluorescent puncta ($p = 0.006$) in post-pubertal subjects compared to their pre-pubertal siblings. Analyses of the motor cortex and striatum are in progress. These results indicate that ERβ is present in the mPFC at puberty and estrogen feeds back to decrease its expression. Estrogen acting at ERβ may be involved in many of the neuroanatomical changes at puberty.

Disclosures: E.P. Sellinger: None. **C.M. Drzewiecki:** None. **J.M. Juraska:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.07

Topic: A.09. Adolescent Development

Support: CIHR 275228
CIHR 106445

Title: The impact of vicarious stressor exposure on fear expression, social behaviours and acoustic startle in juvenile male and female rats

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Abstract: Vicarious stressors can be experienced by witnessing trauma occurring to others and rodent studies have shown that affective states are shared between a witness and a demonstrator (the subject directly experiencing the stress) such as fear expression, anxiety-like behaviours, and HPA-axis activity. In addition, there are opposing findings that suggest that prior stressor experience in witnesses is required for vicarious stress to occur in rats and mice. In addition, most studies utilize adult animals however less is known about the effects of vicarious stress during the juvenile period. The objective of this study was to explore if anxiogenic behaviours can be induced in juvenile rats that experience vicarious stress and if prior stressor exposure enhances the effects of vicarious stressor exposure. Juvenile male and female Sprague-Dawley rats were divided into four witness groups: Control (no experience and no witnessing of footshocks. n=8-10/sex), Context (experienced, no witnessing to footshocks, n=10/sex), Naive (no experience and witnessed footshocks, n=10/sex) and Experienced (experienced and witnessed footshocks, n=10/sex). On post-natal day (PD) 27, Experienced and Context groups were subjected to ten 1.0 mA footshocks over 12 minutes. On PD 28, both Naive and Experienced witnesses watched their cagemate endure the same footshock protocol the day prior from a perforated transparent barrier. Control and Context groups did not watch their cagemate experience any footshocks. Short-term (24hrs) and long-term (7 days) effects on fear expression were observed. Social interaction with a novel rat and acoustic startle response was recorded in early adolescence. Vicarious stress increased fear expression in juvenile male and female rats that had prior stressor exposure, with no significant sex differences however, the time spent freezing diminished significantly by the seventh day. In addition, sniffing and following behaviours were reduced in Experienced witnesses in females and only playfighting was significantly greater in males. The acoustic startle response at 90db, 105db, and 120db did not result in any group or sex differences. The significance of this study provides insight as to how vicarious stress alters fear expression and social interaction and that prior stressor experience is required in juvenile rats for this to occur.

Disclosures: E. Ali: None. **M. Anam:** None. **M. Gebre:** None. **J. James:** None. **C. Cayer:** None. **A. Abizaid:** None. **Z. Merali:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.08

Topic: A.09. Adolescent Development

Support: SC2GM109811
SC3GM130467

Title: Adolescent fluoxetine exposure induces persistent gene expression changes in the hippocampus of adult male C57BL/6 mice

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Abstract: Fluoxetine (FLX), a selective serotonin reuptake inhibitor, is the first line of pharmacological intervention in pediatric patients suffering from affect-related illnesses, such as anxiety and depression. Although the use of this antidepressant treatment has been deemed efficacious in the juvenile population, the enduring neurobiological consequences of adolescent FLX exposure are not well understood. Thus, we explored for persistent molecular adaptations in the adult hippocampus, as a function of adolescent FLX pretreatment. To do this, we administered daily peritoneal injections of FLX (20 mg/kg/day) to male C57BL/6 mice during adolescence (postnatal day [PD] 35-49). After a 21-day washout period (PD70), whole hippocampal tissue was dissected, and subsequent qPCR analysis was ran to assess changes in the expression of genes associated with neuronal survival. Specifically, we evaluated major intracellular signal transduction pathways, including the Ras-mitogen-activated protein kinase (MAPK) and phosphatidylinositide-3-kinase (PI3K)/AKT pathways, as well as several transcription factors. Our results indicate that adolescent FLX treatment results in a long-term upregulation of mRNA levels across numerous genes from the ERK, and PI3K/AKT pathways, along with increases of the transcription factors CREB, ΔFosB, and zif268. Additionally, adolescent FLX treatment resulted in persistent increases of transcripts associated with cytoskeletal integrity (β-actin) and caspase activation (DIABLO), while decreasing genes associated with metabolism (fucose kinase) and overall neuronal activation (c-Fos). Collectively, these data indicate that adolescent FLX exposure mediates persistent alterations in hippocampal gene expression in adulthood, thus, questioning the safety of early-life exposure to this antidepressant medication.

Disclosures: A. Themann: None. **F.J. Flores-Ramirez:** None. **O. Lira:** None. **S.D. Iñiguez:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.09

Topic: A.09. Adolescent Development

Support: Oklahoma State University Start-up Finds
SFN Tulsa Chapter Grant- Dana Foundation 2019

Title: Oxycodone Conditioned Place Preference effects on Brain Derived Neurotropic Factor in the Prefrontal Cortex, Nucleus Accumbens and Cerebellum of Adult male rats.

Authors: *Z. D. SIMSEK[1], A. TORRES[2], K. S. CURTIS[3], D. B. VAZQUEZ SANROMAN[4]; [1]Dept. Pharmacol. & Physiol. - Anat. and Cell Biol., [2]Anat. and Cell Biol., Oklahoma State Univ. Col. For Hlth. Sci., Tulsa, OK; [3]Dept. Pharmacol & Physiol, Oklahoma State Univ. Ctr. for Hlth. Sci., Tulsa, OK; [4]Anat. and Cell Biol., Oklahoma State Univ., Tulsa, OK

Abstract: Introduction: Oxycodone drug-seeking behavior involve alterations in brain-derived neurotrophic factor (BDNF) levels in the brain. Nonetheless, little information is available about whether oxycodone preferentially affects the precursor (pro-BDNF) or mature form (mature-BDNF) of BDNF. **Aim:** Here, we first assessed drug/reward-seeking behavior after oxycodone using conditioned place preference (CPP) and then evaluated mature-BDNF and pro-BDNF levels in the (PFC), nucleus accumbens (NAcc) and cerebellar vermis (Vm) of adult male rats. **Methods:** During the CPP, rats were placed and confined to the non-preferred side of the chamber and given injections of either oxycodone (3mg/kg. SC) or saline (0.9 ml/kg. S.C.) on alternating days for ten consecutive days; control groups always received saline injections. For the postconditioning test, rats have access to all CPP chambers for 15 minutes. The time spent in each comportment was recorded, and an oxycodone-CPP was derived by dividing the total duration in drug-paired compartment by the total duration in both the drug and vehicle compartments. Note that a score of 0.5 denoted no preference and a score above 0.5 denoted a preference for oxycodone. Forty-five minutes after the post conditioning test, rats were terminated, pro- and mature-BDNF levels assessed. **Results:** In drug-treated animals, oxycodone induced CPP and controls exhibit less than 0.5 score preference [$F(1, 21) = 5.31$; $p < 0.05$)]. Further analysis demonstrated that oxycodone decreased pro-BDNF levels in the NAcc [$F(1) = 14.46$, $p<0.001$] and in the PFC [$F(1,21) = 34.97$, $p<0.001$], but increases pro-BDNF levels in the Vm [$F(1,21) = 42.36$, $p<0.001$]. **Conclusion:** Our results demonstrate that pro-BDNF, in PFC , NAcc and Vm, is associated with oxycodone drug seeeking properties.

Disclosures: Z.D. Simsek: None. **A. Torres:** None. **K.S. Curtis:** None. **D.B. Vazquez Sanroman:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.10

Topic: A.09. Adolescent Development

Support: NSERC Discovery Grant 203596-13

Title: Effects of prepubertal stress and AM251 on social behaviors and memory in pubescent male rats

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Abstract: Stress exposure during sensitive periods of development before adulthood has been associated with increased risks of developing neuropsychiatric disorders. Rodents stress models' studies have shown that juvenile and adolescent stress exposure has a negative impact on pro-social behaviours in adulthood and on cognitive functions. Previous research also showed effects of CB1 receptor antagonism on memory and stress, possibly mitigating pro-social behaviours and memory in rats exposed to stress immediately prior or during adolescence. This study aimed to investigate the effects of an heterotypic stress paradigm on prepubescent rats and the short-term consequences on pro-social behaviours and memory and learning. Male Wistar rats (aged postnatal day [PND] 30) were randomly assigned to a 10-day heterotypic stress condition or no stress condition (n = 16 per group). The heterotypic stress consisted of a 10-day paradigm alternating between two types of stressors from PND30 to PND39. On even numbered days, animals were stressed using a restrain procedure of 30 min. On odd numbered days, animals were stressed using the forced swim for 15 min. Following this period, rats were tested in the social interaction and preference test (SIT and SP) and in the Y-Maze passive avoidance test (YMPAT). Forty-five minutes prior to each test, half of the stress and no stress groups received an injection of AM251 (1mg/kg; i.p.) and the other half received only the vehicle (n = 8 per group). A rest period of 48 hours occurred between the SIT and SP, and the YMPAT. AM251-treated rats showed reduced direct exploration ratio in the SIT than vehicle-treated rats. There was also an effect of the stress exposure on the direct interaction ratio, animals that were stressed spending less time proportionally with the stranger rat than the non-stressed animals. Effects on memory of both stress and AM251 are mitigated.

Disclosures: E.L. Person: None. **A. Morin:** None. **H. Plamondon:** None. **J. Côté:** None. **V. Boisvert:** None. **P. Paradis:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.11

Topic: A.09. Adolescent Development

Title: Adolescent fluoxetine exposure mediates a long-term anxiogenic phenotype and decreases ERK signaling in the hippocampus and prefrontal cortex of female C57Bl?6 mice

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[1]Psychology, The Univ. of Texas At El Paso, El Paso, TX; [2]Scripps Res. Inst., San Diego, CA; [3]Psychology, THE UNIVERSITY OF TEXAS AT EL PASO, El Paso, TX

Abstract: Adolescent fluoxetine exposure mediates a long-term anxiogenic phenotype and decreases ERK signaling in the hippocampus and prefrontal cortex of female C57BL/6 mice

Minerva Rodriguez, Francisco J. Flores-Ramirez, and Sergio D. Iñiguez
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The objective of this study was to evaluate whether juvenile fluoxetine (FLX) exposure induces long-term changes in baseline responses to anxiety-inducing environments, and if so, whether its re-exposure in adulthood would ameliorate this anxiogenic-like phenotype. An additional goal was to assess the impact of adolescent FLX pre-treatment, and its re-exposure in adulthood, on serotonin transporters (5-HTT) and brain-derived-neurotrophic-factor (BDNF)-related signaling markers (TrkB-ERK1/2-CREB-proBDNF-mBDNF) within the hippocampus and prefrontal cortex. To do this, we exposed adolescent female C57BL/6 mice to FLX in their drinking water (250 mg/l) from postnatal day [PD]-35 to PD49. After a 21-day washout period (PD70), mice were either euthanized (for tissue collection) or assessed in adulthood on responsivity to the elevated plus-maze (EPM) or the light-dark box (LDB) tests – behavioral paradigms commonly used to assess anxiety-like responses in rodents. To evaluate whether FLX re-exposure would reverse the antidepressant-induced molecular and anxiety-related alterations observed in adulthood, we reinstated FLX treatment in separate groups of mice (PD70-84). Twenty-four hours later (PD85) mice were either euthanized (tissue collection) or evaluated on the EPM or LDB tests. Our results indicate that juvenile FLX history induced a persistent anxiogenic-like profile, along with decreases in BDNF-signaling markers, but not 5-HTTs or TrkB receptors, within both brain regions. Interestingly, FLX re-exposure in adulthood reversed the enduring FLX-induced anxiety-related responses across all behavioral tasks, while restoring ERK2-CREB-proBDNF markers to control levels and increasing mBDNF within the prefrontal cortex, but not the hippocampus. Collectively, the results indicate that adolescent FLX history mediates neurobehavioral adaptations that endure into adulthood, which are indicative of a generalized anxiogenic-like phenotype, and that this persistent effect is ameliorated by later-life FLX re-exposure, in a prefrontal cortex-specific manner.

Disclosures: M. Rodriguez: None. **F.J. Flores-Ramirez:** None. **S.D. Iniguez:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.12

Topic: A.09. Adolescent Development

Support: NIGMS-1SC3GM130467

Title: Lasting effects of juvenile ketamine exposure for reward-related stimuli in C57BL/6 mice

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Abstract: Ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, induces rapid antidepressant efficacy in adolescent treatment-resistant MDD patients. However, the long-term effects of ketamine exposure during early development are unknown. To address this gap in the literature, we examined whether adolescent exposure to ketamine and/or vicarious defeat social stress (VDS; where a rodent witnesses the physical defeat of a conspecific), results in long-lasting changes in sensitivity to the rewarding properties of cocaine and sucrose in adulthood using male C57BL/6 mice. Specifically, mice received ketamine (0 or 20 mg/kg) after each 10-minute VDS exposure for 10 consecutive days during adolescence (Postnatal Day [PD] 35-44). Once mice reached adulthood (PD70), we assessed their behavioral responsivity to sucrose (1%) on a 2-bottle choice test, or cocaine (0, 5, 10 mg/kg) using the conditioned place preference test. Our results show that adult mice pre-exposed to ketamine or VDS alone, displayed an enhanced preference for sucrose and environments paired with cocaine. However, no long-term differences in these behavioral measures were evident between the groups when adolescent mice underwent concomitant social stress (VDS) and ketamine treatment. Collectively, we demonstrate that in the absence of social stress history, adolescent ketamine exposure increases the rewarding valence of sucrose and cocaine in adulthood.

Disclosures: I. Garcia-Carachure: None. **J. Preciado-Piña:** None. **S.D. Iñiguez:** None. **A.R. Zavala:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.13

Topic: A.09. Adolescent Development

Support: CIHR Grant
NSERC Grant
FRQS Grant

Title: Examining Litter Specific Variability in Mice and its Impact on Neurodevelopmental Studies.

Authors: *V. VALIQUETTE[1,4], E. GUMA[1,4], R. PATEL[2,4], E. PLITMAN[3,4], D. GALLINO[4], G. A. DEVENYI[4,3], M. CHAKRAVARTY[3,4,2,1];
[1]Integrated Program in Neurosci., [2]Dept. of Biol. and Biomed. Engin., [3]Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada; [4]Computional Brain Anat. Laboratory, Cerebral Imaging Centre, Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada

Abstract: Our current understanding of litter variability in developmental studies using mouse models limits the translation and replicability of findings. Higher variance of measures across litters than within may be attributable to maternal care, intrauterine, and postnatal environment. Here, we seek to characterize variability across litters through development.
Behavioral assessments (marble burying, prepulse inhibition, and open field tests) were

performed at postnatal day (PND) 38 and 90. T1-weighted magnetic resonance images (MRI; 100 um3 isotropic voxels) were previously acquired on 42 C57bl/6 mice (9 litters: 20F, 22M; litter 6-7 one sex only) at PND 21, 38, 60, and 90 using a 7T Bruker small animal MRI scanner. Volumes of 72 brain structures were obtained using the MAGeT brain pipeline. Levene's test was used to evaluate within- and between- litter variance per timepoint. Subsequently, principal components analysis (PCA) was performed across all brain measures to find patterns explained by the litter-effect. Finally, a partial least squares (PLS) analysis was used to evaluate patterns of covariance between behavior and anatomy. K-means clustering was used to evaluate grouping of PCA and PLS results by litters.

Levene's test revealed significant variability, after multiple comparisons correction in the thalamus ($q < 0.02$) at PND38 and for specific prepulse inhibition results ($q < 0.04$) at PND38 and 90. The first latent variable derived from PLS (var. expl.: 38%, $p = 0.01$) at PND38 revealed relationships between specific covariant brain regions (Principal component (PC) 4, var. expl.: 6%) and exploratory behaviors (Figure 1). Clusters corresponding to the number of litters were found in PC4 and LV1 (n=7; silhouette score: 0.58-0.60).

Factors specific to a litter modulate mouse development. Our results show greater variability between- than within- litter, mainly in the adolescence period (PND38). Improved analysis decisions, such as including litter as a random effect in statistical models, should be considered to better account for this litter-effect.

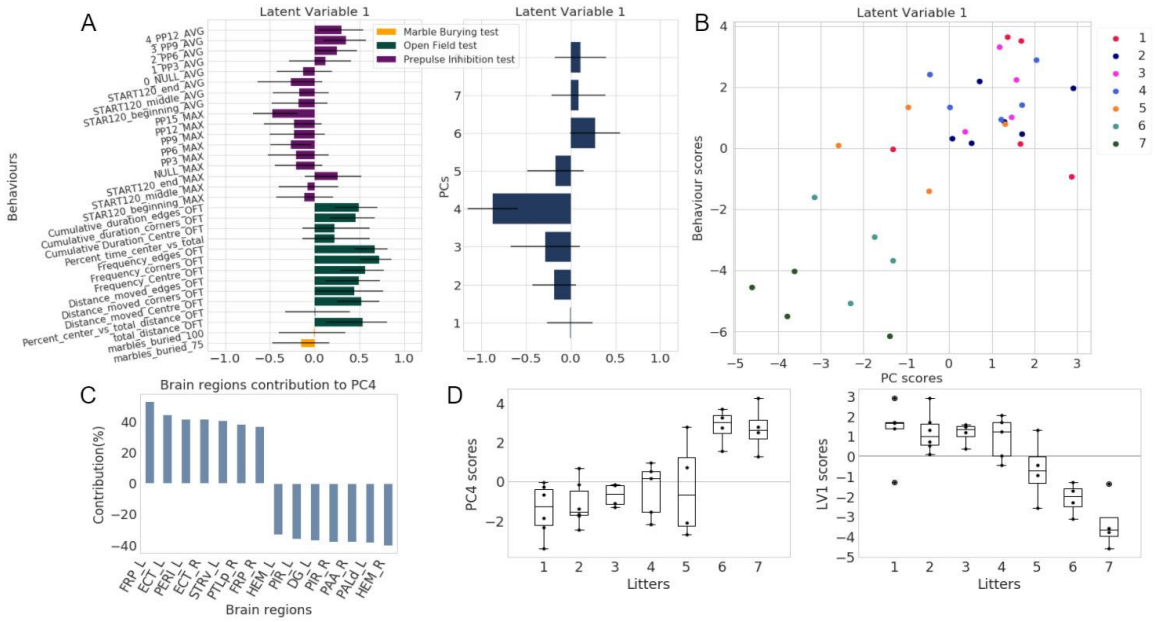

Figure 1: Results from PCA and PLS analysis at PND38 A) Behavior and PCs weights/correlation for each variable to LV1, B) Correlation of behaviour and PC scores for LV1, C) Percentage of contribution (%) of each regions to PC4, D) Distributions of PC4 and LV1 scores by litters. FRP: Frontal pole cerebral cortex, ECT: Ectorhinal area, PERI: Perirhinal area, STRv: Striatum ventral, PTLp: Posterior parietal association areas, HEM: Hemispheric regions, PIR: Piriform area, DG: Dentate Gyrus, PAA: Piriform-amygdalar area, PALd: Pallidum dorsal region.

Disclosures: V. Valiquette: None. **E. Guma:** None. **R. Patel:** None. **E. Plitman:** None. **D. Gallino:** None. **G. A. Devenyi:** None. **M. Chakravarty:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.14

Topic: A.09. Adolescent Development

Title: Characterizing the Dopaminergic-Circadian Rhythm Network Across Time and Age

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Abstract: The circadian rhythm is strongly implicated in many neuropsychiatric and neurodegenerative disorders, all of which are associated with altered dopamine (DA) neurotransmission in the substantia nigra and ventral tegmental area. Progress has been made in elucidating the circadian rhythm-dopaminergic network and its role in the onset of neuropsychiatric and neurodegenerative disorders. Previous research suggests that circadian rhythm transcription factors are responsible for directly regulating the DA phenotype; however, it is currently unknown what the relationship between the circadian rhythm and dopaminergic regulators looks like with respect to age and time of day. Using a transgenic mouse model with Cre recombinase expression under control of dopamine transporter (DAT^{Cre}) and yellow fluorescent protein (YFP) Cre reporter and immunohistochemistry techniques, we are able to differentiate between 'former' and 'current' DA neurons in the ventral midbrain. Current DA neurons are identified using tyrosine hydroxylase (TH), the rate limiting enzyme in DA synthesis; whereas former DA neurons are YFP+/TH- demonstrating that they expressed DAT at some point in their lifecycle but are not currently synthesizing DA. Previous research in our lab has revealed that the DA neuron phenotype is regulated by the circadian rhythm, as demonstrated by significant differences in the number of DA neurons throughout a 24-hour circadian cycle. In this study, mice transgenic for DAT^{Cre}/YFP were analyzed at postnatal day 0 (P0), P21, P35 and adulthood (>P70). Each time point included mice taken at subjective dawn (circadian time 0) and subjective dusk (CT12), excluding P0 mice. Results revealed that between P21 and P35, there was a significant loss of the dopamine neuron phenotype at CT12, as compared to CT0. There was no statistical difference between P35 and adults at CT0 or CT12. This suggests that between P21 and P35, dopamine neurons begin to transition to a 'former' phenotype throughout the circadian rhythm. Additionally, qRT-PCR data revealed abnormal circadian rhythm gene mRNA levels at P21. Elucidating the molecular characteristics of these dopamine neurons is crucial to understanding the biological mechanisms behind the dopaminergic-circadian rhythm network, which will have future implications in understanding neuropsychiatric and neurodegenerative disorders.

Disclosures: S. Barker: None. **H. Partington:** None. **J. Nutter:** None. **J.B. Eells:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.15

Topic: A.09. Adolescent Development

Support: Internal SURF Grant

Title: Effects of early maternal separation on behavior in rats

Authors: S. ROBERTS, L. D. GRIFFITH, A. L. BUCKLER, C. PANIAGUA-UGARTE, Z. HANNABASS, H. A. DAVIS, *P. A. JACKSON;
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Abstract: Extended maternal separation in mammals in early development has been shown to have lasting negative effects on maturation and sociability, causing long term disruptive effects on brain structure and individual behavior (Haller et al., 2014; Lundberg et al., 2017). Maternal contact in the early postnatal period is vital for development. Our goal was to assess if contact with siblings or proximity to the dam (mother) during repeated separations decreased the lasting negative effects. Litters were randomly assigned to a separation period of either 180 minutes (experimental group) or 15 minutes (control group) during postnatal days 1-14 (PND). Within each litter, rat pups were randomly assigned to either isolation in a cage with a dividing wall between the pups and the dam, or in the same barrier-type cage without the dam present. Pups were then assigned to be either alone in isolation or with siblings. Preliminary results showed that pups separated from the dam for a significantly longer period had lower body weights and increased signs of discomfort. There were significant gender differences. Females were more strongly affected by the 180-minute isolation and males were more strongly affected by no dam present. Behavioral data collection is ongoing. One male and one female rat from each group (per litter) was tested during adolescence for anxiety-like behavior and sociability. The first behavioral task (on PND 50) was the elevated-plus-maze (EPM). On PND 55, after a 4-day break, we conducted open-field testing (OF). On PND 56 rats were placed in the same OF apparatus with walls inserted to create a three-chamber test of sociability. Experimental rats were placed in the center chamber and the chamber to either side contained a cage, one empty and the other with a same-sex, unfamiliar conspecific in it. Social discrimination was assessed by measuring interactions with the familiar rat and a new stranger rat 24-hours later. The implications of this study can be applied to scenarios where young children are developing in an environment where contact with the maternal figure is limited, such as premature infants in the Neonatal Intensive Care Unit (NICU). If negative long-term effects of maternal separation can be lessened by having siblings present, it could have major implications for children in foster care (Turney & Wildeman, 2016). Having the dam present but not accessible could be compared to parents that use the TV as a babysitter as well as ‘latchkey kids’. This study adds to existing literature and presents a novel test of sibling separation and dam proximity.

Disclosures: **S. Roberts:** None. **P.A. Jackson:** None. **L.D. Griffith:** None. **A.L. Buckler:** None. **C. Paniagua-Ugarte:** None. **Z. Hannabass:** None. **H.A. Davis:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.16

Topic: A.09. Adolescent Development

Support: F31MH123041
R01MH101729
R01 MH049698
T32 DK059803

Title: Altered excitability of infralimbic glutamatergic pyramidal neurons as a potential mechanism behind adolescent stress induced resilience

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Abstract: Adolescence is a critical period of brain development in humans as well as in other mammalian species. The match-mismatch hypothesis of brain development postulates that certain forms of early life adversity can promote an adaptive phenotype later in life, allowing individuals to cope with adverse situations in adulthood and contributing to resilience. Preliminary data from the lab indicate that subjecting adolescent rats to chronic variable stress (CVS) prevents traumatic single prolonged stress (SPS)-induced enhancement of fear responses in adulthood, suggesting that early life stress may promote stress resilience under certain circumstances. SPS causes infralimbic (IL) prefrontal cortex hypoactivity which is thought to underlie exaggerated fear responses and abnormal fear extinction. Therefore, we tested the hypothesis that adolescent stress leads to greater prefrontal drive in adulthood, helping to protect against SPS induced plasticity in the IL cortex. Patch clamp electrophysiology was used to test intrinsic excitability and firing frequency of IL pyramidal neurons. Our results show SPS-induced decreases in excitability were prevented by adolescent CVS. Our results suggest that adolescent CVS increases the excitability of IL pyramidal neurons, leading to greater prefrontal drive and top-down control when exposed to SPS in adulthood, resulting in resilience to SPS impairment in extinction of fear conditioning. Our findings hint at potential mechanism of altered excitability of IL glutamatergic pyramidal neurons towards developmental stress induced resilience.

Disclosures: **N. Nawreen:** None. **E. Cotella:** None. **M.L. Baccei:** None. **J.P. Herman:** None. **K. Oshima:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.17

Topic: A.09. Adolescent Development

Support: NIH Grant R01MH106553

Title: Effects of juvenile immune challenge with lipopolysaccharide (LPS) on learning in context fear-conditioning paradigms

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Abstract: Early-life immune activation has been linked to learning deficits commonly associated with neurodevelopmental disorders such as Autism, ADHD, and Schizophrenia. Microglia, the immune cells of the brain, are thought to be important for the formation of circuits during development which underlie learning. The Context Pre-exposure Facilitation Effect (CPFE) is a fear-conditioning paradigm in which context learning, acquisition of a context-shock association, and expression of contextual fear are separated across three days. Whereas, in standard contextual fear conditioning (sCFC), context learning and acquisition of the context-shock association occur on the same day. We have found that immune activation with lipopolysaccharide (LPS), on P21 in rats, produces learning deficits in the CPFE paradigm from P24-P26. Thus, we sought to elucidate the involvement of hippocampus (HP) and medial prefrontal cortex (mPFC) in the observed LPS-induced learning deficits. Male and female Sprague-Dawley rats were treated with LPS (100 ug/ml; i.p.) on P21. Rats were then tested in sCFC from P24-P25 and in a variation of CPFE from P24-P26 that included a measure of freezing immediately after the shock on Day 2. In CPFE, there was a main effect of treatment such that LPS-treated rats showed deficits in freezing both post-shock on Day 2 (P25) and during testing on Day 3 (P26). This suggests that P21 LPS prevents rats from learning the context on Day 1 (implicating PFC or HP processing) and/or expressing a post-shock fear response on Day 2 (implicating HP processing). Moreover, in sCFC, LPS-treated rats showed no deficits in learning during testing on Day 2 (P25), as compared to controls. This suggests that P21 LPS either does not disrupt HP functioning or that neocortical systems are compensating for LPS-induced HP dysfunction in sCFC. Overall, these findings suggest that context fear-conditioning learning deficits, induced by LPS immune activation, are likely not driven exclusively by changes in mPFC functioning, but likely involve alterations in HP functioning.

Disclosures: M.B. Bielicki: None. **J.M. Schwarz:** None.

Digital Abstract Session

P022. Behavioral Outcomes: Animal Models

Program #/Poster #: P022.18

Topic: A.09. Adolescent Development

Support: NIH-R01DA03791
CIHR-MOP-74709

Title: Enduring effects of cannabinoids in adolescence on inhibitory control

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Abstract: Adolescence is a period of heightened vulnerability to psychopathology, including drug abuse. Yet, there is a gap in knowledge regarding the cellular and molecular processes underlying adolescent brain development and how drugs of abuse influence them. Dopamine axons continue to grow to the prefrontal cortex across adolescence, remaining very vulnerable to environmental changes. This gradual maturation of the dopamine input to the prefrontal cortex occurs in parallel to the development of cognitive control. In this study, we assessed the effects of cannabinoids exposure in early adolescence on the organization of dopamine connectivity in the adult prefrontal cortex and on behavioral inhibition. Male 57BL/6 mice were treated with four doses of the cannabinoid receptor - 1 (CB1-R) agonist WIN-55,212-2 (0, 0.5, 2, 4 mg/kg i.p.) every-other-day from postnatal day (PND) 21 to PND 31. In adulthood, mice were assessed for behavioral inhibition using the Go/No-Go task. Adult mice that received WIN during early adolescence showed dose-dependent improvement in performance in the Go/No-Go task when contrasted against controls. Mice that received WIN 2 and 4 mg/kg began to inhibit their behavior during earlier trials than vehicle-treated groups and had significantly fewer commission errors. There were no differences in the number of correct responses in Go trials (HITS). In a separate group of adult mice administered WIN (2mg/kg i.p.) or vehicle in early adolescence, we quantified dopamine connectivity in the pregenual prefrontal cortex using stereology. Mice that received WIN 2 mg/kg in adolescence showed a significant increase in the total number and density of dopamine varicosities in both prelimbic and infralimbic subregions compared to vehicle-treated mice. Our results contrast with previous research, carried out by our group using recreational-like doses of d-amphetamine and suggest that different drugs may affect behavioral inhibition and dopamine varicosities in opposite ways. The observed increase in dopamine varicosities might produce an increase of dopamine transmission in the medial prefrontal cortex. This increase might facilitate inhibitory control and behavioral flexibility.

Disclosures: G.A. Hernandez: None. **A. Fallahi:** None. **D. Nuel:** None. **B. Livingston:** None. **N. Zlebnik:** None. **J.F. Cheer:** None. **C. Flores:** None.

Digital Abstract Session

P023. Fetal Alcohol Exposure Models

Program #/Poster #: P023.01

Topic: D.01. Sensory Disorders

Support: NIH grant AA026421

Title: Prenatal ethanol exposure impairs visual processing in male and female rats, effects normalized by environmental enrichment

Authors: *R. WANG[1], C. D. MARTIN[1], A. L. LEI[1], K. A. HAUSKNECHT[1], M. TURK[1], V. MICOV[1,2], F. KWARTENG[1,2], K. ISHIWARI[1], S. OUBRAIM[1], J. B. RICHARDS[1], S. HAJ-DAHMANE[1], R.-Y. SHEN[1];
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Abstract: Background. Individuals with fetal alcohol spectrum disorders (FASD) often show sensory processing deficits. Using an operant sensation seeking model, we tested if prenatal ethanol exposure (PE) could alter responding and habituation to contingent visual stimuli in rats. We also explored if postnatal environment enrichment could ameliorate the effects of PE. **Materials and Methods.** Pregnant Sprague Dawley rats were gavaged twice/day with 0 or 3 g/kg/treatment ethanol (15% w/v) during gestational days 8 – 20, mimicking second-trimester heavy PE in humans. The offspring were reared in the standard housing condition or underwent an environmental enrichment procedure. An operant light reinforcement experiment was conducted in adult rats, which comprised a pre-exposure phase, during which no light was presented, and a light-onset phase, during which contingent light-onset (turning on light) served as a sensory reinforcer. Both short- and long-access procedures were used. A dishabituation test was conducted to further characterize the habituation process. An additional non-operant open field test was also performed to investigate PE effects on sensory processing. **Results.** The results showed reinforcer effectiveness of the contingent light-onset in all groups. Prenatal ethanol exposure increased responding in both sexes reared in the standard housing condition. Such an effect was not observed in rats reared in the enriched condition, which led to an overall decrease in responding. Greater dishabituation and between-session recovery of responding were also observed in PE rats. Furthermore, the long-access procedure reduced responding and facilitated between-session habituation in both control and PE rats. Lastly, in the open field test, augmented locomotor responding was observed in PE rats due to blunted responses to the increase in illumination level. **Conclusions.** Prenatal ethanol exposure leads to deficits in processing of visual stimuli, including increased responding and impaired habituation in rats reared in the standard housing condition. Such effects may underlie enhanced sensation seeking/impaired habituation in individuals with FASD. The PE-induced effects could be ameliorated by postnatal environmental enrichment as well as longer exposure to the testing environment. The observation might inform a suitable animal model to study possible intervention strategies for FASD.

Disclosures: R. Wang: None. **C.D. Martin:** None. **A.L. Lei:** None. **K.A. Hausknecht:** None. **M. Turk:** None. **V. Micov:** None. **F. Kwarteng:** None. **K. Ishiwari:** None. **S. Oubraim:** None. **J.B. Richards:** None. **S. Haj-Dahmane:** None. **R. Shen:** None.

Digital Abstract Session

P023. Fetal Alcohol Exposure Models

Program #/Poster #: P023.02

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant GM112696

Title: Brain Organoids as a Models for Alcohol-induced Developmental Neurotoxicity

Authors: *T. ARZUA[1], Y. YAN[1], C. JIANG[1], S. LOGAN[1], R. ALLISON[1], C. WELLS[1], S. KUMAR[1], R. SCHÄFER[2], X. BAI[1];
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Abstract: Maternal alcohol exposure during pregnancy can substantially impact the development of the fetus, causing a range of symptoms, known as Fetal Alcohol Spectrum Disorders (FASDs), with the pathophysiology and mechanisms largely unknown. Recently developed cerebral organoids from human induced pluripotent stem cells are more similar to fetal brains in the aspects of development and structure being a more relevant in vitro system to study FASDs. Using human cerebral organoids, we sought to quantify the downstream toxic effects of alcohol (ethanol) on neural pathology phenotypes and signaling pathways. The results revealed that alcohol induced apoptosis, as well as ultrastructural changes, especially affecting the mitochondria. The apoptotic effects of alcohol on the organoids depended on the alcohol concentration and varied between cell types. Specifically, neurons were more vulnerable to alcohol-induced apoptosis than astrocytes. Alcohol exposure also resulted in mitochondrial dysfunction and metabolic stress in the organoids as evidenced by decreased mitochondrial respiration and an increase of non-mitochondrial respiration. Furthermore, we found that alcohol treatment affected the expression of 199 genes out of 17,195 genes analyzed. Bioinformatic analyses showed the association of these dysregulated genes with 37 pathways related to clinically relevant pathologies. This study extends for the first time animal models of binge drinking-related FASDs to a human model, allowing in-depth analyses at the tissue, cellular, subcellular, and gene levels. Hereby, we provide novel insights into alcohol-induced pathologic phenotypes, cell type-specific vulnerability, and affected signaling pathways and molecular networks, that can contribute to a better understanding of the neurotoxic effects of binge drinking during pregnancy.

Disclosures: T. Arzua: None. **Y. Yan:** None. **C. Jiang:** None. **S. Logan:** None. **R. Allison:** None. **C. Wells:** None. **S. Kumar:** None. **R. Schäfer:** None. **X. Bai:** None.

Digital Abstract Session

P024. Neurodevelopmental Disorders: Animal Models

Program #/Poster #: P024.01

Topic: A.07. Developmental Disorders

Support: NIH Grant R21AA025751-01A1
Nebraska Center for Substance Abuse Research

Title: Behavioral alterations in a mouse model of Fetal Alcohol Spectrum Disorders

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Abstract: Fetal alcohol spectrum disorders (FASD) is one of the leading causes of developmental abnormalities worldwide. Maternal consumption of alcohol during pregnancy leads to a diverse range of cognitive and neurobehavioral deficits. Here, we use a model of maternal voluntary alcohol consumption throughout gestation to investigate the effects of prenatal alcohol exposure on behavioral phenotypes in mice. Metabolic studies were performed to study the effects of prenatal alcohol exposure on body composition and energy expenditure. Baseline behaviors including feeding, drinking, movement and their circadian rhythm were examined by performing home cage monitoring in adult mice. We also evaluated a range of behaviors that examined motor function, motor skill learning, anxiety-related behavior and sensorimotor gating to study the long-lasting behavioral alterations. Our data show that prenatal alcohol exposure alters offspring growth and body weight and affects body composition. Results from a battery of behavioral tests show long-lasting behavioral impairments in male and female offspring. To understand whether prenatal alcohol exposure results in persistent alterations in the synaptic proteome, we performed label-free proteomic analysis of cortical synaptosomes. We found that prenatal alcohol exposure altered proteins associated with synaptic function. Our results show that prenatal alcohol exposure (PAE) results in long-lasting behavioral impairments and alterations in the synaptic proteome.

Disclosures: T. Chaudoin: None. **S.J. Bonasera:** None. **A. Dunaevsky:** None. **P. Ragunathan:** None.

Digital Abstract Session

P024. Neurodevelopmental Disorders: Animal Models

Program #/Poster #: P024.02

Topic: A.07. Developmental Disorders

Support: NIH Grant R21HD097561
Friedman Brain Institute Research Award by the Fascitelli Family
Beatrice and Samuel A. Seaver Foundation

Title: Deciphering DDX3X syndrome: cellular and molecular insights

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Abstract: Mutations in the X-linked gene DDX3X account for ~2% of intellectual disability in females, often in co-morbidity with behavioral problems, motor deficits, and brain malformations. DDX3X encodes an RNA helicase with emerging functions in corticogenesis and synaptogenesis. Here, we present the first characterization of a Ddx3x haploinsufficient mouse (Ddx3x+/-) with construct validity for DDX3X loss-of-function mutations. Ddx3x+/- mice show physical, sensory, and motor delays that evolve into behavioral anomalies in adulthood, including hyperactivity, anxiety-like behaviors, cognitive impairments, and motor deficits. Motor function further declines with age. These behavioral changes are preceded by reduction in brain volume postnatally, with some regions (e.g., cortex and amygdala) disproportionally affected. Cortical thinning is accompanied by defective cortical lamination, indicating that Ddx3x regulates the balance of glutamatergic neurons in the developing cortex. While supporting face validity for a novel pre-clinical mouse model, these data shed new light on the developmental mechanisms driving DDX3X syndrome.

Disclosures: M. Garcia-Forn: None. **A. Boitnott:** None. **D. Ung:** None. **K. Niblo:** None. **D. Mendonca:** None. **M. Flores:** None. **S. Maxwell:** None. **Y. Park:** None. **J. Ellegood:** None. **D.E. Grice:** None. **J. Lerch:** None. **M. Rasin:** None. **J.D. Buxbaum:** None. **E. Drapeau:** None. **S. De Rubeis:** None.

Digital Abstract Session

P024. Neurodevelopmental Disorders: Animal Models

Program #/Poster #: P024.03

Topic: A.07. Developmental Disorders

Support: GRK 2318, DFG Research Training Group "TJ-Train"

Title: Lrp2 receptor integrates signaling and scaffolding function to facilitate neural tube formation

Authors: *I. KOWALCZYK[1], N. MECKLENBURG[1], J. GÖRNE[1], K. FEISTEL[2], A. HAMMES[1];
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Abstract: Early brain development is a complex morphogenetic process integrating various molecular and cellular mechanisms. One of the key signaling molecules at the onset of neurulation is morphogen SHH, required for ventral midline induction. In LRP2-deficient mice, SHH signaling in the ventral forebrain is disturbed, leading to holoprosencephaly (HPE; Christ et al., 2012). In addition, *Lrp2* mutants show a phenotype independent of the SHH pathway, affecting the dorsolateral neural folds. Our recent work identified that the penetrance of HPE strongly depends on the genetic background of the inbred mouse strain, suggesting that strain dependent modifiers affect the SHH pathway. However, fully penetrant, disturbed dorsal neural tube morphology indicates a new, conserved role of LRP2.
We used mouse genetics to understand the variability of HPE penetrance, comparing the severely affected *Lrp2* mutant C57BL/6N mice and FVB/N mice with a full rescue of the ventral midline defects. Transcriptome analysis identified candidate genes that trigger the SHH machinery independent of LRP2 receptor, thus rendering FVB/N mice less susceptible to SHH pathway disturbances in the ventral midline. Functional assessment of genes revealed PTTG1 as a novel component of SHH signaling, a positive regulator triggering target genes, and importantly a new component of primary cilium.
Having clarified the strain-dependent penetrance of HPE in *Lrp2* mutants, we next tried to understand the mechanisms underlying the strain independent role of LRP2. Neural plate bending and closure is a dynamic process involving cytoskeletal remodeling and apical constriction. Analysis of LRP2-deficient neural fold morphology using electron and confocal microscopy revealed impaired neuroepithelial integrity affecting the entire neuroepithelial sheet. Altered dorsolateral hinge point formation and upfolding of the neural folds was caused by impaired apical constriction in mutants, placing LRP2 in context of cytoskeletal and apical membrane remodeling. Our results strongly suggest that intracellular adaptor proteins containing PDZ domains serve as a bridge linking the LRP2 to the intracellular scaffold. Endocytic activity of the receptor facilitates apical membrane remodeling during apical constriction and simultaneously contributes to the apicobasal distribution of VANGL2, indicating a close interface between cell shape control and maintenance of planar cell polarity.
We conclude that crosstalk between signaling pathways and cytoskeletal remodeling allows LRP2 to integrate different morphogenetic processes during neurulation, thereby ensuring proper neural tube formation.

Disclosures: I. Kowalczyk: None. **N. Mecklenburg:** None. **J. Görne:** None. **K. Feistel:** None. **A. Hammes:** None.

Digital Abstract Session

P024. Neurodevelopmental Disorders: Animal Models

Program #/Poster #: P024.04

Topic: A.07. Developmental Disorders

Support: Canadian Institutes of Health Research (CIHR)
Heart & Stroke Foundation of Canada

Réseau pour transformer les soins en autisme (RTSA) - Transforming Autism Care Consortium (TACC) - Quebec Autism Research Training (QART) Program Fellowship
Foundation CHU Sainte-Justine Bourse D'Excellence doctorale
Bourses De Recrutement FESP Département De Neurosciences

Title: Effects of neonatal hypoxia on the development of serotonergic innervation and cognitive functions

Authors: *K. K. Y. LEE[1,2], B. CHATTOPADHYAYA[1,2], A. S. FERNANDES DO NASCIMENTO[2], G. DI CRISTO[1,2];
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Abstract: Perinatal hypoxia is caused by prolonged oxygen deprivation to newborn infants during birth. Although the specific cellular and network changes caused by mild perinatal hypoxia (MPH) are still not well understood, this is a crucial issue since recent studies suggest that children who experienced MPH show long lasting subtle cognitive and behavioral deficits. In particular, population-based case-control studies suggested that children who experience perinatal hypoxia have a higher probability to be diagnosed with autism spectrum disorders than the general population.
Serotonin (5-HT) is essential for cognitive and social functions. Few studies have shown that severe hypoxia-ischemia lead to reduced 5-HT neurons and innervation, however whether 5-HT dysregulations contribute to MPH-induced cognitive problems is unclear.
We have recently established a mouse model of MPH, which shows long-term deficits in social interaction, attention, cognitive flexibility and memory. To investigate whether MPH affects 5-HT system development, we characterized 5-HT expression levels and innervation in the auditory and prefrontal cortex of female and male MPH mice. Preliminary data suggest both 5-HT expression and innervation complexity are reduced in adult MPH mice especially in the prefrontal cortex. We are currently investigating whether pharmacological 5-HT modulation will rescue MPH-induced cognitive impairment in adult mice. Identifying MPH effects on serotonergic system development will lead to a better understanding of how social, attention, cognitive flexibility and memory dysfunctions associated with MPH occur and may pave a path towards the development of pharmacological strategies for treating children exposed to MPH.

Disclosures: K.K.Y. Lee: None. **B. Chattopadhyaya:** None. **A.S. Fernandes do Nascimento:** None. **G. Di Cristo:** None.

Digital Abstract Session

P024. Neurodevelopmental Disorders: Animal Models

Program #/Poster #: P024.05

Topic: A.07. Developmental Disorders

Support: NIH R01 NS093704
NIH R61 NS111074
Swebilius Foundation
American Epilepsy Society postdoctoral fellowship

Title: Filamin A inhibition reduces seizure activity in a mouse model of focal cortical malformations

Authors: *L. ZHANG[1], T. HUANG[1], S. TEAW[1], L. NGUYEN[1], L. HSIEH[1], X. GONG[1], L. BURNS[2], A. BORDEY[1];
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Abstract: Epilepsy treatments for patients with mechanistic target of rapamycin (mTOR) disorders, such as tuberous sclerosis complex (TSC) or focal cortical dysplasia type II (FCDII), are urgently needed. In these patients, the presence of focal cortical malformations is associated with the occurrence of lifelong epilepsy, leading to severe neurological comorbidities. Here, we show that the expression of the actin cross-linking protein filamin A (FLNA) is increased in resected cortical tissue that is responsible for seizures in patients with FCDII and in mice modeling TSC and FCDII with mutations in phosphoinositide 3-kinase (PI3K)-ras homolog enriched in brain (Rheb) pathway genes. Normalizing FLNA expression in these mice through genetic knockdown limited cell misplacement and neuronal dysmorphogenesis, two hallmarks of focal cortical malformations. In addition, Flna knockdown reduced seizure frequency independently of mTOR signaling. Treating mice with a small molecule targeting FLNA, PTI-125, before the onset of seizures alleviated neuronal abnormalities and reduced seizure frequency compared to vehicle-treated mice. In addition, the treatment was also effective when injected after seizure onset in juvenile and adult mice. These data suggest that targeting FLNA with either short hairpin RNAs or the small molecule PTI-125 might be effective in reducing seizures in patients with TSC and FCDII bearing mutations in PI3K-Rheb pathway genes.

Disclosures: L. Zhang: None. **T. Huang:** None. **S. Teaw:** None. **L. Nguyen:** None. **L. Hsieh:** None. **X. Gong:** None. **L. Burns:** None. **A. Bordey:** None.

Digital Abstract Session

P024. Neurodevelopmental Disorders: Animal Models

Program #/Poster #: P024.06

Topic: A.07. Developmental Disorders

Support: LouLou Foundation Grant CDKL5-19-104-02

Title: Dysregulation of central and peripheral microtubule proteins in a transgenic mouse model of Cdkl5 deficiency disorder

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Abstract: Background: CDKL5 Deficiency Disorder (CDD) is a rare brain disorder characterized by severe early-onset seizures, intellectual disability, motor and social impairments. CDD is an X-linked disorder caused by heterogenous mutations in the *Cdkl5* gene. Microtubule (MT) dynamics are fundamental for brain development and are involved in the pathogenesis of CDD. Here, we examined brain and plasma α-tubulin post translational modifications (PTMs) associated with MT dynamics in *Cdkl5*-KO (exon 4) male mice compared to age-matched wild-type (WT) mice at postnatal day (P) 20, 60 and 180. **Method:** Infrared western blot measured α-tubulin PTMs including Acetylated-Tubulin (Acet-Tub; stable MT), Tyrosinated-Tubulin (Tyr-Tub; dynamic MTs), Detyrosinated-Tubulin (Glu-Tub; stable MTs) and Delta2-Tubulin (Δ2; neuron-specific) in the cortex, hippocampus and plasma of WT and *Cdkl5*-KO mice. Tyr-Tub and Glu-Tub were analysed as a ratio, while expression of Acet-Tub and Δ2-Tub was normalized to Total-Tubulin (Tot-Tub). **Results:** Age-related changes were evident in α-tubulin PTMs including a significant increase in Acet-Tub/Tot-Tub and Δ2/Tot-Tub ratios and a decrease in Tyr-Tub/Glu-Tub ratio in the hippocampus and cortex at P60 compared to P20 in WT and *Cdkl5*-KO mice. However, Acet-Tub/Tot-Tub and Δ2/Tot-Tub are over-expressed in *Cdkl5*-KO mice at both P20 and P60 in the hippocampus and cortex, respectively compared to WT. Similarly, Tyr-Tub/Glu-Tub ratio is further downregulated in *Cdkl5*-KO mice at P20 in the cortex and P60 in the hippocampus. Intriguingly, Acet-Tub/Tot-Tub is increased is in the plasma of *Cdkl5*-KO mice compared to WT at both P60 and P180, while Tyr-Tub/Glu-Tub was not changed. **Conclusion:** These data confirm earlier findings showing age-related changes in brain α-tubulin PTMs consistent with physiological neurodevelopment. However, *Cdkl5*-KO mice exhibit alterations in α-tubulin PTMs indicating dysregulated MT dynamics during neurodevelopment; which may serve as a novel therapeutic target for CDD. Moreover, plasma Acet-Tub may represent a potential biomarker of disease progression in CDD.

Disclosures: A.M. Thornton: None. **C.K. Callaghan:** None. **N. McDyer:** None. **A. Taki:** None. **O. Mamad:** None. **I. Barbiero:** None. **C. Kilstrup-Nielsen:** None. **M. Bianchi:** None.

Digital Abstract Session

P024. Neurodevelopmental Disorders: Animal Models

Program #/Poster #: P024.07

Topic: A.07. Developmental Disorders

Support: NIH Grant R15S088776

Title: Vitamin D fortified diet increases percent survival, decreases microglia, but does not correct bone abnormalities in the NS-Pten mouse

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Abstract: Individuals who experience recurrent spontaneous seizures are at a high risk for bone fractures (independent of seizure-related falls), as well as an increased likelihood of a comorbid diagnosis of Autism spectrum disorder (ASD). There is also evidence that individuals with epilepsy have vitamin D deficiencies. Previously, this deficit was hypothesized to be related to anti-seizure medications, however, there is a lack of consensus between studies. The neural subset-specific (NS) *Pten* knockout (KO) mouse has been well characterized to show autistic-like deficits in addition to having a lower bone mineral density. *PTEN* is an important component of the mTOR (mammalian target of rapamycin) pathway, which is important for cell proliferation and survival. As a result of the mutation, the NS-*Pten* knockout (KO) mouse exhibits hyperactive mTOR signaling pathway and has been linked in epilepsy. This study investigated whether a vitamin D enriched diet could be a potential dietary intervention to a mouse model of epilepsy, specifically the deletion of *Pten.* This study focuses on the potential of a dietary therapy where treatment mice received a vitamin D diet for a total of 5 weeks. Neuronal tissue was collected to look at downstream markers of the mTOR pathway and inflammatory markers, in addition to the scan and analysis of the mouse femurs. Mice on the vitamin D enriched diet showed a decreased level of microglia marker IBA-1 and mTOR targets like S6 and AKT. We did not find an effect of vitamin D on the bone abnormalities previously found in this mouse model, however we found that many differences between WT and KO mice including a significant reduction in bone mineral density (BMD) and an increase in bone volume and trabecular cortical expansion. Additionally, we found that females KO mice had a more significant reduction in percent bone volume over trabecular volume (BV/TV) compared to female WT mice, and this finding was sex specific as we did not see this in the male mice. Most significantly, the vitamin D fortified diet increased percent survival in male and female KO animals. Overall, these findings suggest that a vitamin D enriched diet had a significant impact on the survivability and molecular pathology of NS-*Pten* KO mice, suggesting that dietary manipulations like vitamin D supplementation could be a potential therapeutic option in addition to current epilepsy treatment, as well as an alternative for those with treatment resistant epilepsy.

Disclosures: P.D. Womble: None. **S.L. Hodges:** None. **S.O. Nolan:** None. **M. Binder:** None. **A. Holley:** None. **R. Herrera:** None. **S. Senger:** None. **E. Kwok:** None. **D. Narviaz:** None. **A. Faust:** None. **C.J. Hernandez-Zegada:** None. **D. Santana-Coelho:** None. **K.J. Blandin:** None. **J.N. Lugo:** None.

Digital Abstract Session

P024. Neurodevelopmental Disorders: Animal Models

Program #/Poster #: P024.08

Topic: A.07. Developmental Disorders

Support: NIH Grant R01DE024217

Title: Disrupted trigeminal ganglion development in a mouse model of Familial Dysautonomia

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Abstract: Neurons in the trigeminal ganglion (TG) relay somatosensory information from the face and oral cavity. TG neurons arise from both neural crest cell (NCC) and ectodermal placode cell precursors that ultimately produce neurons that are specific for one sensory modality. TG neuron identity, established during embryogenesis, is determined by particular molecular markers, including mutually exclusive expression of the neurotrophin receptors TrkA, TrkB, or TrkC, which are required for long-term neuronal function and survival. While little is known about mechanisms that establish and maintain distinct neuronal subpopulations in the TG, disease studies offer some insight. Familial Dysautonomia (FD) is a hereditary neuropathy caused by mutation in *Elongator complex protein 1* (*ELP1*), which leads to reduced ELP1 levels in sensory and autonomic neurons. FD patients have clinical deficits pointing to abnormal TG function, including impaired sensation of facial pain and temperature and smaller trigeminal nerves, but no obvious signs of degeneration, indicating a role for ELP1 in TG neuron development. However, the function of ELP1 in TG neurons and the mechanisms underlying facial sensory deficits in FD are still unclear. Our preliminary data reveal Elp1 depletion from Wnt1-positive NCCs in mouse leads to abnormal TG morphology and fewer trigeminal nerve branches as early as embryonic day (E)11. Moreover, TrkA-expressing neurons, which typically function in nociception, are vulnerable to loss of Elp1 in NCCs. By E13, we observe decreased TrkA expression and fewer TrkA-positive neurons in the TG. Interestingly, TUNEL staining to assess cell death indicates progenitor cells, rather than neurons themselves, may undergo aberrant apoptosis in Elp1 conditional knockout TG. These findings confirm a role for Elp1 in the development of TG neurons, and may partially explain the loss of facial sensation experienced in FD.

Disclosures: C. Leonard: None. **L. Taneyhill:** None.

Digital Abstract Session

P024. Neurodevelopmental Disorders: Animal Models

Program #/Poster #: P024.09

Topic: A.07. Developmental Disorders

Support: fondation Pierre Lavoie/RDDM
Fondation Savoy

Title: A novel mouse model of FASN-associated neurodevelopmental disorders

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Abstract: Developmental epileptic encephalopathies are clinically and genetically heterogeneous disorders. Recent studies revealed mutations in the *FASN* gene in two unrelated children with early-onset epilepsy. We have now identified a cohort of 11 children carrying recessive or *de novo FASN* mutations and presenting a spectrum of neurodevelopmental disorders, ranging from global developmental delay and intellectual deficiency to severe developmental epileptic encephalopathy.
The *FASN* gene encodes Fatty acid synthase, a multifunctional protein responsible for *de novo* lipogenesis from acetyl-Coa and malonyl-CoA in the presence of NADPH. *FASN* is ubiquitously expressed in the embryo and its loss results in prenatal lethality. However, its roles in brain development are unclear.
To investigate the mechanisms by which *FASN* mutations disrupt neurodevelopmental processes, we generated a novel mouse model carrying a patient-derived mutation using CRISPR/Cas9 gene-editing. The homozygous $Fasn^{S154N}$ knock-in mice are not viable. However, heterozygous $Fasn^{S154N}$ mice display a clinical phenotype reminiscent of the patients' phenotypes, with anxiety-like behavior, altered spatial learning, spontaneous interictal spikes on electroencephalograms (EEG), and a tendency to a reduced PTZ-induced seizure threshold.
Our work thus reveals the phenotypic spectrum of *FASN*-associated neurodevelopmental disorders, while providing a unique animal model to advance mechanistic studies that will ultimately drive therapeutic innovation for this disorder.

Disclosures: S. Tene Tadoum: None. **P. Kumar Raju:** None. **S. Raghuram:** None. **M. Lachance:** None. **P. Campeau:** None. **E. Rossignol:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.01

Topic: A.07. Developmental Disorders

Support: NIH Grant MH112237
Nancy Lurie Marks Family Foundation

Title: A standardized social preference protocol for measuring social deficits in mouse models of autism

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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social communication deficits and other behavioral abnormalities. The three-chamber social preference test is often used to assess social deficits in mouse models of ASD. However, varying and often contradicting phenotypic descriptions of ASD mouse models can be found in the

scientific literature, and the substantial variability in the methods used by researchers to assess social deficits in mice could be a contributing factor. Here we describe a standardized three-chamber social preference protocol, which is sensitive and reliable at detecting social preference deficits in several mouse models of ASD. This protocol comprises three phases that can all be completed within 1 d. The test mouse is first habituated to the apparatus containing two empty cups in the side chambers, followed by the pre-test phase in which the mouse can interact with two identical inanimate objects placed in the cups. During the test phase, the mouse is allowed to interact with a social stimulus (an unfamiliar wild-type (WT) mouse) contained in one cup and a novel non-social stimulus contained in the other cup. The protocol is thus designed to assess preference between social and non-social stimuli under conditions of equal salience. The broad implementation of the three-chamber social preference protocol presented here should improve the accuracy and consistency of assessments for social preference deficits associated with ASD and other psychiatric disorders.

Disclosures: B. Rein: None. **K. Ma:** None. **Z. Yan:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.02

Topic: A.07. Developmental Disorders

Title: A test for impaired behavioral flexibility in a mouse model of autism spectrum disorder

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Abstract: We hypothesized that impaired behavioral flexibility might underlie an array of symptoms associated with autism spectrum disorder (ASD), especially repetitive and restricted behavior. To explore this hypothesis, we tested Shank2 knockout (Shank2-KO) mice in a probabilistic reversal learning task. Two different odor cues were presented to a head-fixed mouse and paired with a reward (water) or a punishment (air-puff) each with 75% probability. Even though both Shank2-KO and wild-type mice showed higher anticipatory licking responses to the reward-predicting than punishment-predicting cues during the initial training, it took significantly longer for Shank2-KO than wild-type mice to show higher anticipatory licking responses to the new reward-predicting cue upon the reversal of cue-outcome contingency. Shank2-KO mice also showed enhanced fear responses, as measured by pupil diameter, than wild-type mice in the task, raising the possibility that enhanced fear responses might contribute to impaired reversal learning. When we trained the animals using a mild air-puff that induced similar fear responses between Shank2-KO and wild-type mice, no significant difference was found in reversal learning between the two animal groups. Moreover, when we trained the animals with only appetitive outcomes (75 versus 25% reward probabilities), no significant difference was found in reversal learning, either, between the two animal groups. These results

suggest that impaired reversal learning of Shank2-KO mice in the original appetitive-aversive conditioning is likely because of hypersensitivity to punishment rather than general inflexibility of Shank2-KO mice. Collectively, our results provide evidence against the general behavioral inflexibility hypothesis for ASD.

Disclosures: M. Yun: None. **J. Shin:** None. **M.W. Jung:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.03

Topic: A.07. Developmental Disorders

Support: NIH Grant 105000

Title: Digging Behavior Discrimination Test to Study Burrowing and Exploratory Digging in Laboratory Mice

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Abstract: Digging is an instinctual behavior in mice across strains, pervasive through lab-bred generations, and often used to study mouse models of neurodevelopmental and psychiatric disorders. One of the most common behavioral digging tests used to assay repetitive behaviors like those seen in obsessive-compulsive disorder (OCD) and autism spectrum disorder (ASD) is the marble burying test which measures the number of marbles buried into deep bedding during digging. However, the literature suggests digging is a naturalistic behavior with a variety of motivations; i.e. foraging for food, burrowing for shelter, removing noxious stimuli, or even for recreation as shown for dogs and ferrets. Previous digging assays such as free digging and marble burying are unable to elucidate motivation complicating the interpretation of results. Here we present a goal-directed paradigm, the Digging Behavior Discrimination (DBD) test that uses coordinated measures of free digging and burrowing behavior adapted from the Deacon (Nature Protocols, 2006) to rapidly assess mouse digging and burrowing behavior and help to distinguish why mice dig. To distinguish between exploratory digging and burrowing, we set up a clear cage with deep corncob substrate allowing the mice to choose between a transparent tube filled with soft paper bedding in one corner and a free digging area. By collecting a variety of measures of activity, burrowing efficiency, and free digging we found that the test provides stable results in multiple cohorts. To determine whether digging preference could be shifted, cohorts of male and female mice underwent food restriction leading to 10-15% weight loss, followed by recovery with *ad libitum* feeding. Both male and female mice switched to spending more time outside the burrowing area during food restriction but only males showed a reduction in burrowing efficiency and a significant increase in free digging during that time. The DBD test was then

conducted in a mouse model for ASD and intellectual disability (ID), conditional knock-out (cKO) mice for the ASD/ID gene CC2D1A that had previously shown both hyperactivity and reduced digging in the marble burying. Male Cc2d1a cKO mice burrowed significantly less than wild type male mice and shifted towards exploratory digging, a genotype difference that was not present between female groups. These data suggest that the DBD test may be a more sensitive measure of digging also providing additional information on digging motivation. We found that digging motivation may differ between males and females and special attention must be given when discussing digging behavior, particularly in females.

Disclosures: **A.T. Heller:** None. **B.M. Gural:** None. **M. Manzini:** None. **H.L. Pond:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.04

Topic: A.07. Developmental Disorders

Support: ANU Future scheme
INSERM
CNRS
Bordeaux University

Title: Autism spectrum disorder is a risk factor for PTSD-like memory formation

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Abstract: Recent studies have suggested co-occurrence of post-traumatic stress disorder (PTSD) and ASD in humans. Behaviourally, ASD and PTSD display similar characteristics, including impaired emotional regulation, cognitive rigidity, and fragmented autobiographical memory. Yet, despite several shared neurological alterations, the overlap between the two disorders has remained poorly explored. To assess the susceptibility of developing traumatic memory in ASD, we tested the Contactin-associated protein 2 knock out (*Cntnap2* KO) mouse in an unpaired tone-shock fear conditioning paradigm, modified from a previously used model of PTSD-like memory, and combined with a 30min-restraint stress. Unlike control mice which displayed strong fear of the conditioning context (i.e. normal memory), stressed *Cntnap2* KO mice exhibited strong fear to the tone and little fear to the context. Such a memory profile is characteristic of a PTSD-like memory: hypermnesia for an irrelevant but salient cue, combined with partial amnesia for the context surrounding the traumatic event. Furthermore, this pathological memory was associated with a broad dysfunction of the prefrontal-hippocampo-amygdalar network. Among these structures, we established the pivotal role of the prefrontal cortex (PFC) in the development of PTSD under mild stress conditions, through the

demonstration that PFC optogenetic inhibition successfully prevented pathological memory formation in stressed *Cntnap2* KO mice. Finally, we employed a behaviour-based rehabilitation strategy to rescue traumatic memory, through re-exposure to the tone in the conditioning context. Recontextualization of the trauma after long-term retention restored normal memory. Together, this study provides the first direct demonstration that ASD is a risk factor for developing PTSD-like memory. Indeed, a stressful situation below the threshold for pathological memory in control population gives rise to PTSD in ASD. It urges for autism-specific trauma assessments to improve detection measures and specific treatment of PTSD in vulnerable population with ASD.

Disclosures: A.S. Al Abed: None. **A. Sellami:** None. **A. Marighetto:** None. **A. Desmedt:** None. **N. Dehorter:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.05

Topic: A.07. Developmental Disorders

Support: NIH Grant K01NS107723

Title: Role of Cortical and Hippocampal Integrin β3 in Mediating Social and Repetitive Mouse Behaviors

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Abstract: Many neurological diseases, such as autism spectrum disorder, Alzheimer's, and epilepsy, are thought to be associated with disorders of the synapse. Autism spectrum disorder (ASD) is characterized by repetitive behaviors, deficits in communication, and overall impaired social interaction. Integrins are heterodimeric cell adhesion molecules that bind to the extracellular matrix and regulate cell motility. These molecules are required for normal structural plasticity of dendrites and synapses, as well for construction of cortical and hippocampal circuits within the brain, but the role that Itgb3 plays in the function of social and repetitive behaviors, specifically those related to ASD, is still not well defined. We were able to test for mouse behaviors typically associated with ASD by conditional knockout (cKO) of *Itgb3* in cortical and hippocampal excitatory neurons and glia, which are known to be important for social and repetitive behaviors. We determined whether our cKO mice showed differences in anxiety-like behaviors, social behaviors, and/or repetitive behaviors, the latter two being hallmarks of ASD. Our findings indicated that there were differences in both social and repetitive behaviors between cKO and wild type (WT) mice. We found that cKO mice spent less time grooming in a novel environment and displayed a social preference for novel mice while WT showed increased time spent grooming in the novel environment compared to home cage and showed no social preference for either familiar or novel mice. These findings are markedly different from previous

results of global *Itgb3* knockout mice, suggesting that Itgb3 may be playing a unique role in the cortex and hippocampus.

Disclosures: G.S. Vidal: None. **A. Lopuch:** None. **B. Swinehart:** None. **E. Widener:** None. **Z.L. Holley:** None. **K. Bland:** None. **C. Handwerk:** None. **H. Cook:** None. **A. Kalinowski:** None. **H. Rodriguez:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.06

Topic: A.07. Developmental Disorders

Support: Autism Science Foundation (19-001)
F31 MH120918
R01 HD055741
R01 MH093510
UL1 TR002345

Title: Testing candidate cerebellar presymptomatic biomarkers for autism spectrum disorder

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Abstract: Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder diagnosed on the basis of social impairment and restricted, repetitive behaviors (RRBs). Results from animal and adult neuroimaging studies have generated interest in cerebellar contributions to ASD pathophysiology. Specifically, commentaries have posited that impaired cerebellar-mediated error signaling may account for the emergence of ASD behaviors, implicating cerebellar connectivity as a promising presymptomatic biomarker of ASD. However, supportive evidence from tests of cerebellar function in human infants is lacking. **Objective:** This project tested infant cerebellar connectivity (fcMRI) as a presymptomatic biomarker of ASD in a rigorous manner using multiple analytic approaches. **Methods:** We utilized data from the Infant Brain Imaging Study, a prospective study of infants at high and low familial risk for ASD (*n* = 94, 68 male). Brain-behavior associations were analyzed for 6-month cerebellar connections in relation to 12 and 24-month ASD-associated behaviors (joint attention, motor coordination,

repetitive behaviors) and 24-month ASD outcomes (positive/negative) using univariate, multivariate, and enrichment approaches. In hypothesis-driven tests, we focused on cerebellar-frontoparietal (the FPN has been implicated in error signaling) and cerebellar-default mode (the DMN has been implicated in prior studies of ASD) connections. **Results:** Univariate tests of cerebellar-FPN and cerebellar-DMN connections failed to implicate the cerebellum in ASD in a convincing manner (only .02% of tested connections survived multiple comparisons correction), despite > 80% power to detect medium effects. Multivariate predictive tests in high risk infants using cerebellar-FPN and cerebellar-DMN connections similarly failed to achieve above chance classification accuracy for ASD outcomes, despite utilizing procedures that achieved > 80% positive predictive value in brain-wide work. Whole-brain enrichment identified three 6-month network pairs that were strongly associated with later RRBs. However, the cerebellum was implicated at chance in these networks. **Conclusions:** Contrary to hypotheses, we failed to observe strong associations between 6-month cerebellar fcMRI and 12-24-month ASD behaviors and outcomes, casting doubt on cerebellar theories of ASD etiology and indicating that cerebellar effects, if present, are likely small. Instead, we identified brain-behavior associations between multiple 6-month network pairs and later RRBs, suggesting network-level correlates for emerging ASD behaviors that warrant future testing in independent samples.

Disclosures: Z. Hawks: None. **A. Todorov:** None. **T. Nishino:** None. **M. Talovic:** None. **N. Marrus:** None. **M. Nebel:** None. **S. Davis:** None. **S. Marek:** None. **B. Seitzman:** None. **A. Eggebrecht:** None. **J. Elison:** None. **S. Dager:** None. **M. Mosconi:** None. **L. Tychsen:** None. **A. Snyder:** None. **K. Botteron:** None. **A. Estes:** None. **A. Evans:** None. **G. Gerig:** None. **H. Hazlett:** None. **R. McKinstry:** F. Consulting Fees (e.g., advisory boards); NOUS Imaging. Other; Siemens (meals & travel), Philips Healthcare (meals & travel). **J. Pandey:** None. **R. Schultz:** None. **M. Styner:** None. **J. Wolff:** None. **L. Zwaigenbaum:** None. **J. Piven:** None. **J. Pruett:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.07

Topic: A.07. Developmental Disorders

Support: UK National Institute for Health Research PB-PG-0816-20019

Title: Skills profiles in minimally-speaking autistic children: Functional assessments and touchscreen interactions associate fine motor ability with expressive language

Authors: E. J. L. WEISBLATT[1], A. MCKINNEY[2], K. L. HOTSON[3], Z. B. AHMED[3], C. DÍAS[4], D. BEN SHALOM[5], J. FOSTER[6], S. S. VILLAR[1], S. MURPHY[7], A. JATKAR[8], S. LANGHORNE[9], *M. K. BELMONTE[10];
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Negev, Beer Sheva, Israel; [6]King's Col. London, London, United Kingdom; [7]Univ. of Bedfordshire, Luton, United Kingdom; [8]Univ. of North Carolina, Chapel Hill, NC; [9]Univ. of Manchester, Manchester, United Kingdom; [10]The Com DEALL Trust, Bangalore, India

Abstract: Background: Point OutWords is a caregiver-delivered, iPad-assisted intervention targeting motor and cognitive skills prerequisite to communication, designed specifically for those minimally verbal autistic children whose severe deficits in expressive language are coincident with impaired motor skills.
Objectives: Assess 1) Relationships amongst verbal and motor functioning using parent-report and direct observational measures 2) Motor functioning measured by interactions with the iPad touchscreen, within the intervention group 3) Any change with treatment.
Methods: Our parallel-groups randomised controlled design recruited 30 autistic children aged 3-15 years, speaking fewer than 100 words functionally. The intervention group used Point OutWords 5 times a week for 8 weeks; controls used other iPad apps. Communication (Vineland, Mullen), motor, oromotor (VMPAC) and daily living skills were tested at baseline and post-intervention. Data were analysed with mixed ANOVA for behavioural and questionnaire measures. Children's touchscreen interactions were separated from their caregivers' by Gaussian mixture modelling; each movement was assessed for median jerk (3rd derivative of position), mean direction error, and time to completion. These derived measures were regressed against time in treatment.
Results: Motor and especially language and social skills were impaired for age (all $p<0.001$). Gross motor skills exceeded fine ($p=0.03$). Only 2 children had stronger expressive than receptive language. All had severe impairments of oromotor control. There were no consistent effects of treatment though Mullen motor and language scores improved overall ($p=0.027$). 8 of 12 children significantly lessened the time to complete movements (each $p<0.02$), and every child showed significant increase in jerk ($t(11)=5.81$, $p=0.000118$).
Conclusion: This study recruited a highly impaired group of children with minimal spoken language. A majority demonstrated better gross than fine motor, better motor than verbal, and better receptive than expressive verbal skills. Increased jerk and faster performance suggest a change to more frequent, finer-grained movement corrections. Future work will investigate predictors of such changes and implications for augmentative and alternative communication.

Disclosures: E.J.L. Weisblatt: None. **A. McKinney:** None. **K.L. Hotson:** None. **Z.B. Ahmed:** None. **C. Días:** None. **D. Ben Shalom:** None. **J. Foster:** None. **S.S. Villar:** None. **S. Murphy:** None. **A. Jatkar:** None. **S. Langhorne:** None. **M.K. Belmonte:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.08

Topic: A.07. Developmental Disorders

Title: N170 latency delays are observed in ASD independent of visual attention to specific facial features

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Abstract: Autism spectrum disorder (ASD) is associated with N170 (an event-related potential reflecting temporal face processing) latency delays, indicating slower processing. We aim to better characterize N170 in ASD by cueing attention to specific facial features and analyzing the relationship between feature-specific N170s and autistic traits. We obtained electroencephalography (EEG) data on ASD (n=8) and typically developing (TD) (n=17) subjects. Each trial consisted of a crosshair (600-650 milliseconds (ms)), a neutral face or a house (1000 ms), and an intertrial period (500-650 ms). Crosshairs were presented in four locations, cueing attention to the left eye, right eye, nose, or mouth on the face and corresponding regions on the house. Fixations were monitored with co-registered eye tracking. ASDs had longer N170 latencies for each feature than TDs (Fig. 1). Within diagnostic groups there were no differences in feature-specific latencies [ASD: $F(3,5)=1.68$, $p=.29$; TD: $F(3,14)=1.05$, $p=.40$]. Across groups left eye latency correlated most strongly with autistic traits measured by the Autism Spectrum Quotient, the Broad Autism Phenotype Questionnaire, and the Social Responsiveness Scale (Table 1). These data show that N170 latency delays in ASD are evident irrespective of visual attention to specific facial features. Across groups left eye latency exhibited largest correlations with autistic traits. These data suggest the importance of considering visual attention in EEG studies of face perception.

Figure 1: N170 latencies elicited by specific facial features in ASD and TD participants.

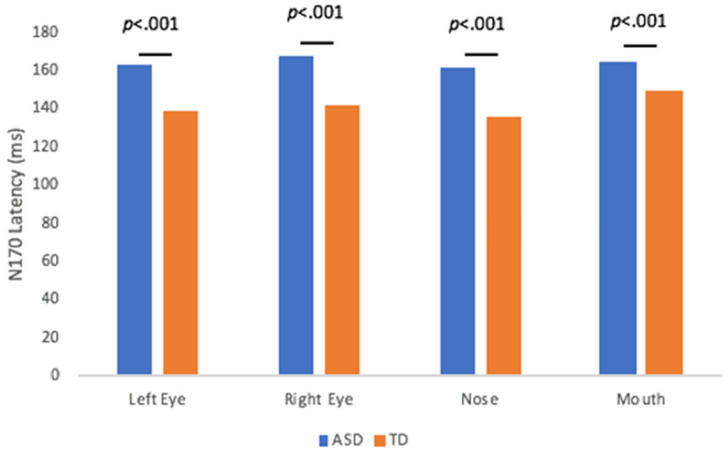

Table 1: Summary of correlations between N170 latency and ASD symptomatology according to the AQ, BAPQ, and SRS across diagnostic groups.

	Autism Spectrum Quotient Total Score		Broad Autism Phenotype Questionnaire Total Score		Social Responsiveness Scale Total Raw Score	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value
Left eye N170 latency	0.441	0.027*	0.532	0.006**	0.563	0.003**
Right eye N170 latency	0.409	0.043*	0.458	0.021*	0.524	0.007**
Mouth N170 latency	0.385	0.058	0.464	0.019*	0.553	0.004**
Nose N170 latency	0.373	0.066	0.426	0.034*	0.488	0.013*

Footnote: Left eye N170 latency correlated most strongly with autistic traits measured by the Autism Spectrum Quotient (r=.441, p=.027), the Broad Autism Phenotype Questionnaire (r=.532, p=.006), and the Social Responsiveness Scale (r=.563, p=.003)

Disclosures: **S. Kala:** None. **A. Bagdasarov:** None. **C. Carlos:** None. **E. Cummings:** None. **A. Naples:** None. **T. McAllister:** None. **J. McPartland:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Janssen Research & Development. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Guilford Press, Lambert, and Springer. F. Consulting Fees (e.g., advisory boards); BlackThorn Therapeutics.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.09

Topic: A.07. Developmental Disorders

Support: NIH Grant 1R15MH118627-01

Title: Sex-specific effects on sociability following reduced midbrain Drd2 expression in mice

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Abstract: Dopamine (DA) modulates locomotor activity and social behavior, the dysfunction of which is a core symptom of Fragile X syndrome (FXS) and autism. We've previously reported in a mouse model of FXS, the *Fmr1*-null mouse, that both wildtype (WT) and knock-out (KO) offspring of *Fmr1* deficient dams exhibit attenuated locomotor-reducing effects of dopamine autoreceptor 2 (D2aR) activation, suggesting that maternal *Fmr1* deficiency may program altered sociability and hyperactivity by downregulating this negative feedback mechanism. Indeed, male offspring of *Fmr1* deficient dams show reduced VTA *Drd2* expression irrespective of their own *Fmr1* genotype. Here we asked if constitutive or adult-only reduction in midbrain *Drd2*

expression is sufficient to induce hypersociability. We generated *Drd2*$^{fl/+}$ DAT-Cre^{+} C57xFVB F1 hybrids with constitutive reduction of *Drd2* expression in DAT+ cells (*Drd2* knock-down (KD)). Additionally, we generated *Drd2*$^{fl/+}$ F1 hybrids and induced adult *Drd2* KD by injecting AAV-TH-Cre into the VTA. Constitutive *Drd2* KD increased sociability, reduced avoidance, and attenuated quinpirole sensitivity in female, but not male mice. This suggests that life-long *Drd2* deficiency leads to sex-specific effects on dopamine neurotransmission. Interestingly, we found opposite sex-dependent effects of adult *Drd2* KD, with male but not female mice showing increased sociability after AAV-mediated midbrain *Drd2* KD. This suggests that D2aR-dependent negative feedback of midbrain DAergic signaling has a direct modulatory role on sociability in male, but not female mice. Furthermore, our data show that reduced midbrain *Drd2* expression is sufficient to increase sociability in adult mice, phenocopying hypersociability of male mice reared by *Fmr1* deficient dams.

Disclosures: A.K. Osterman: None. **A. Khasnavis:** None. **B.A. Sulaman:** None. **B. Zupan:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.10

Topic: A.07. Developmental Disorders

Title: A Minecraft mod to improve theory of other minds and empathy in autistic spectrum disorder children

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Abstract: Autistic spectrum disorder (ASD) affects more than 1% of children. Major genetic determinants of ASD have yet to be found, and molecular, biochemical or neuronal cell biology based treatment is far off, if ever possible. Importantly, these may not be the correct level of reduction to treat ASD. The core deficit in ASD is a lack of appreciation of a theory of mind (ToM) of others/lack of empathy. Some years ago we suggested (Altschuler, 2008) that having an ASD child take care of a virtual pet, could force, in a fun way, the ASD child to think about the world through the eyes and needs of the pet. However, care of a virtual pet may not hold the attention of an ASD (or any) child. Conversely, ASD children greatly enjoy playing Minecraft. Given this, we have built and here describe and demonstrate a Minecraft mod to increase theory of other minds and empathy in ASD children. The mod includes a helper that can fish, farm, mine, and build a shelter inside the game world for the ASD player. The ASD player can ask the helper to perform these tasks using a graphical user interface (GUI). The helper requires tools to perform these tasks which the ASD player can provide by dropping them on the ground and

letting the helper pick them up which is standard in Minecraft. Furthermore, the ASD player can provide positive feedback through the GUI. Positive feedback allows the helper to gain new skills and use more advanced tools, and thus perform more challenging tasks. The helper tries to stay near the ASD player and gives prompts about the current situation and needs, such as danger, hunger, or boredom. The Minecraft mod is designed in such a way that to fully benefit from the helper the ASD player needs to invest in interaction and understanding the needs of the helper. We hope this will lead to the promotion of empathy and theory of mind, as well as developing the confidence of the player in engaging in social interactions. We plan to assess the mod in a small study and then after subsequent refinements in an RCT.

Disclosures: E.L. Altschuler: None. **R. Kangasmäki:** None. **C. Colon:** None. **C. Parkes:** None. **J. pajarinen:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.11

Topic: A.07. Developmental Disorders

Title: Early life sleep disruption and genetic vulnerability interact to drive life-long behavioral deficits in a Shank3 autism model.

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Abstract: Sleep is essential for lifelong brain health and cognitive function. Sleep disruption is a major comorbidity in Autism Spectrum Disorder (ASD), occurring in over 80% of patients. We hypothesize that sleep disruption early in life negatively impacts brain development and drives lasting changes in brain function and behavior seen in ASD. Shank3 is a high-confidence ASD risk gene, and heterozygous mutation of Shank3 is known to cause Phelan McDermid Syndrome, a severe developmental disorder characterized by intellectual disability, autism, and disrupted sleep. Here we report findings of sleep and behavioral analysis of ASD model mice bearing C-terminal truncation (ΔC) of Shank3. Juvenile and adolescent Shank3$^{\Delta C/\Delta C}$ male and female mice showed reduced and fragmented sleep compared to wildtype littermates. In comparison, clinically relevant Shank3$^{+/\Delta C}$ mice showed mild sleep disruption phenotypes. We suspected that Shank3$^{+/\Delta C}$ mice may be especially vulnerable to the effects of developmental sleep loss. Therefore, we tested the lasting effects of early life sleep disruption (ELSD) on wild type and Shank3$^{+/\Delta C}$ mice. ELSD was induced by subjecting mice to intermittent mild mechanical disturbance from postnatal days 14-21. Following ELSD, all animals were allowed to mature undisturbed to adulthood for behavioral testing. Compared to undisturbed controls, adult male Shank3$^{+/\Delta C}$ exposed to ELSD demonstrated a marked deficit in sociability in the 3-chambered social test, reduced acoustic startle reflex, and impaired pre-pulse inhibition. Adult female Shank3$^{+/\Delta C}$ exposed to ELSD showed a reduction in anxiety-like behavior in the elevated plus

maze. These results establish a novel gene x sex x environment interaction model of ASD susceptibility and support early life sleep disruption as a significant vulnerability in the development of ASD.

Disclosures: J.S. Lord: None. **S.M. Gay:** None. **K.M. Harper:** None. **V.D. Nikolova:** None. **S.S. Moy:** None. **G.H. Diering:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.12

Topic: A.07. Developmental Disorders

Support: NIH grant NIMH R21 ES026896

Title: Short term, perinatal BPA exposure decreases juvenile social behaviors in male rats: Implications for autism spectrum disorder

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Abstract: Bisphenol A (BPA) is an endocrine disruptor found in polycarbonate plastics. BPA exposure in humans is nearly ubiquitous and has also been linked to autism spectrum disorders (ASD), a developmental disorder characterized by abnormal social interaction. While the etiology of ASD is undoubtedly multifactorial, our laboratory has previously shown that perinatal BPA exposure disrupts social behaviors in adolescent rats (Wise et al., 2018). To further explore the relationship between BPA and symptoms of ASD, we orally fed male and female rat pups to a 0 (control), 40, or 400 µg/kg/day BPA solution between postnatal day (P)6 and P8. Subjects were left undisturbed until they underwent a social preference task at P15 and P28. This task measured the subjects' preference for a novel, age- and sex-matched conspecific vs a novel object. All subjects, regardless of sex or age, showed significant preference for the novel conspecific over the novel object. We found that at P15, only the males who received the 40 µg/kg/day BPA dose showed decreased social preference for the conspecific when compared to controls. At P28, the males who received the 400 µg/kg/day BPA dose showed decreased social preference compared to controls. BPA did not influence social preference in females at either age. Thus, short-term BPA exposure in early life can induce disruptions to social behaviors in a dose-specific pattern. These results support a growing body of literature that suggests BPA may exacerbate symptoms of ASD, particularly in young males.

Disclosures: C. Drzewiecki: None. **A.D. Doshi:** None. **J.Y. Koh:** None. **J.M. Juraska:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.13

Topic: A.07. Developmental Disorders

Title: The effects of maternal immune activation on the behaviour of mice lacking vesicular zinc

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Abstract: Zinc is important in neural and synaptic development and neuronal transmission. Within the brain, zinc transporter 3 (ZnT3) is essential for zinc uptake into vesicles. Loss of vesicular zinc has been shown to produce neurodevelopmental disorder-like behaviour phenotypes, such as decreased social interaction and increased anxiety- and repetitive-like behaviour. Maternal immune activation (MIA) has been identified as an environmental factor for neurodevelopmental disorders, such as autism spectrum disorders (ASDs) and schizophrenia (SZ), in offspring, which occurs during pregnancy when the mother's immune system reacts to the exposure to viruses or infectious diseases. In this study, we investigated the interaction effect of a genetic factor (ZnT3 knockout (KO) mice) and an environmental factor (MIA). We induced MIA in pregnant female (dams) mice during mid-gestation, using polyinosinic:polycytidylic acid (polyI:C), which mimics a viral infection. We administered dams heterozygous for ZnT3 with polyI:C (20mg/kg) or saline, at embryonic day 12.5, as it is translational to the 2nd trimester of pregnancy in humans, a period in which MIA is thought to contribute to neurodevelopmental disorders significantly. At postnatal day 9 (P9), we recorded ultrasonic vocalizations from all pups isolated from the dams and the nest and assessed their calls. Call duration usually reaches a peak between P7-P9 in mice. In adulthood (P60-75), male and female ZnT3 KO and wild-type mice were examined using a battery of behavioural tests commonly used in rodents to assess ASD- and SZ-like behaviour: open field to assess anxiety, marble burying to assess repetitive behaviour, 3-chamber social test to assess social interaction, and pre-pulse inhibition to assess sensory-motor gating. Our results indicate that loss of vesicular zinc does not show enhanced ASD- and SZ-like phenotype compared to wild-type, nor does it show a more pronounced phenotype in male ZnT3 KO compared to female ZnT3 KO. Finally, MIA offspring demonstrated an ASD- and SZ-like phenotype only in specific behavioural tests: increased calls emitted in ultrasonic vocalizations and decreased marbles buried. Our results indicate no interaction between the loss of vesicular zinc and MIA induction in the susceptibility to developing an ASD- and SZ-like phenotype.

Disclosures: K.C. Sandoval: None. **A. Wong:** None. **N. Niewinski:** None. **S.E. Thackray:** None. **R.H. Dyck:** None.

Digital Abstract Session

P025. Autism: Behavioral Analysis

Program #/Poster #: P025.14

Topic: A.07. Developmental Disorders

Support: NIH

Title: Longitudinal study of stool-associated microbial taxa in children with autism and their neurotypical siblings

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[1]Microbiology, [2]Microbiology, Pharmaceut. Sci., Oregon State Univ., Corvallis, OR; [3]Pediatrics (Systems Medicine), Biomed. Data Science, and Psychiatry and Behavioral Sci., [4]Bioengineering, Stanford Univ., Stanford, CA; [5]Second Genome Inc., Brisbane, CA

Abstract: The existence of a link between the gut microbiome and Autism Spectrum Disorder (ASD) is well established in mice, but in human populations efforts to identify microbial biomarkers remain limited due to a lack of appropriately matched controls, stratification of participants within the spectrum, and sample size. To overcome these limitations, we crowdsourced the recruitment of over 100 families with age-matched sibling pairs between 2-7 years old, where one child had a diagnosis of ASD and the other did not. Parents collected stool samples, provided a home video of their children's social behavior, and 365 metadata features assessing inter-and intra- family variabilities. 16S amplicon analysis from stool samples was collected for every subject at three-time points and was analyzed, alongside multi-omics data provided for the initial point of sampling. This extensive study allowed us to identify taxa and metabolic biomarkers specific to ASD, undergoing mice behavior testing.

Disclosures: **A. Phillips:** None. **C. Tataru:** None. **A. Martin:** None. **G.E. Deitzler:** None. **J. Schwartz:** None. **B.S. Chrisman:** None. **E. Rutherford:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Second Genome. **X. Yin:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Second Genome. **K. Sabino:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Second Genome. **R. Hannibal:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds

come to an institution.; Second Genome. **W. Hartono:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Second Genome. **M. Lin:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Second Genome. **E. Raack:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Second Genome. **Y. Wu:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Second Genome. **T. DeSantis:** A. Employment/Salary (full or part-time):; Second Genome. **S. Iwai:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Second Genome. **D.P. Wall:** None. **M.M. David:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Second Genome.

Digital Abstract Session

P026. Autism: Environment and Pathology

Program #/Poster #: P026.01

Topic: A.07. Developmental Disorders

Support: NIMH Grant R01MH083972

Title: Genetic and environmental influences on brain structure in twins with autism

Authors: *J. P. HEGARTY II, J. C. MONTERREY, L. C. LAZZERONI, S. C. CLEVELAND, J. M. PHILLIPS, J. F. HALLMAYER, A. Y. HARDAN;
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Abstract: Brain development is altered in children with autism spectrum disorder (ASD); however, the type and degree of alterations are heterogeneous. This heterogeneity may be due to the variable effects of genetic and environmental influences. This study used a twin model approach to examine multimodal neuroimaging data and compare genetic and environmental influences on brain structure between twins with and without ASD. Same-sex monozygotic and dizygotic twin pairs in which at least one twin was diagnosed with ASD or both were neurotypical controls were recruited. T1 (n=164), diffusion-weighted (n=168), and 1H proton spectroscopy (n=106) MRI data were collected and processed with FreeSurfer, FSL Diffusion Toolbox, and LCModel, respectively, in order to examine brain structure based on grey matter

morphometry (cortical thickness-CT; surface area-SA; volume-VOL; curvature-CURV), white matter microstructure (fractional anisotropy-FA; mean diffusivity-MD), and neurochemical markers of neuronal integrity (N-acetylaspartate-NAA). Intra-class correlations were computed within twin pairs, and ACE modeling for broad sense heritability (a2=additive genetics) and environmental influences (c2=shared family environment, e2=unique environment) was examined. Brain structure was predominantly genetically-mediated in control twins, with variation in cerebral and cerebellar grey matter (CT, SA, VOL), white matter (FA, MD), and neurochemical levels (NAA) primarily associated with genetic factors. The only measures that were predominantly environmentally-mediated in control twins were CURV and some region-specific CT and MD. Similarly, genetic factors also accounted for the majority of variation in brain structure in twins with ASD, potentially to a larger extent for deep brain structures like subcortical VOL and commissural FA, yet there were also greater environmental influences in twins ASD. Variation in global and regional CT as well as cerebellar (VOL and FA) and cerebral white matter (FA in projection and association fibers) were primarily associated with environmental factors in twins with ASD. As previously reported, brain structure appears to be primarily genetically-mediated during typical development, with some regional and measure-specific environmental influences that most likely represent changes from adaptive plasticity. However, children with ASD exhibit a markedly different and more pervasive pattern of environmental influences on brain structure. These results are promising because they suggest that treatments targeting the early environment could potentially alter brain development to provide some clinical benefits.

Disclosures: J.P. Hegarty II: None. **J.C. Monterrey:** None. **L.C. Lazzeroni:** None. **S.C. Cleveland:** None. **J.M. Phillips:** None. **J.F. Hallmayer:** None. **A.Y. Hardan:** None.

Digital Abstract Session

P026. Autism: Environment and Pathology

Program #/Poster #: P026.02

Topic: A.07. Developmental Disorders

Title: Maternal docosahexaenoic acid supplementation effects on prenatal stress mouse model alleviates autistic-like behaviors in offspring

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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by restricted social communication and repetitive behaviors. We previously reported that prenatal stress is critical in neurodevelopment and increases risk for ASD, particularly in those with greater genetic susceptibility to stress. Docosahexaenoic acid (DHA) is one of the most abundant omega-3 fatty acids in mammalian brain, and dietary omega-3 fatty acid affects the development

and maintenance of brain structure. In this study, we investigated whether prenatal supplementation of DHA alleviates autistic-like behaviors in a gene/stress mouse model and how it alters lipid peroxidation activity in the brain. Pregnant heterozygous serotonin transporter knockout (SERT-KO) and wild-type (WT) dams were placed in either non-stressed control condition or chronic variable stress condition and fed either control diet or DHA-rich (1% by wt) diet. Offspring of each group were assessed for anxiety and autism-associated behavior at post-natal day 60, including an open field test, elevated-plus maze test, repetitive behavior, and the 3-chamber social approach test. Male mice from stressed SERT-KO dams showed significantly decreased social interactions in the 3-chamber social approach test compared with male offspring from non-stressed WT dams. DHA supplementation fed to stressed SERT-KO dams mitigated these behavioral changes. This result indicates that maternal supplementation of DHA can ameliorate autistic-like behaviors caused by prenatal stress. Now we are analyzing lipid peroxidation products in the plasma, heart, and cerebral cortex. In addition, brain structures will be examined via MRI in order to identify whether autistic-like behaviors are associated with neurochemical or neuroanatomical changes.

Disclosures: T. Woo: None. **C. King:** None. **M. Will:** None. **D.Q. Beversdorf:** None.

Digital Abstract Session

P026. Autism: Environment and Pathology

Program #/Poster #: P026.03

Topic: I.07. Data Analysis and Statistics

Support: NIH R01MH110630 and R00MH094409 (DPK)
T32HD007475 Postdoctoral Traineeship (LB)

Title: Autism diagnosis prediction from inter-subject similarity of video-evoked fMRI timeseries

Authors: *L. BYRGE[1], D. KLIEMANN[2], U. KELES[3], H. SAARIMÄKI[5], R. ADOLPHS[4], D. P. KENNEDY[6];
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Abstract: Naturalistic viewing paradigms, in which participants undergo functional magnetic resonance imaging (fMRI) scanning while watching videos, have emerged as an important methodological advance toward improving ecological validity in neuroscience. Coupling these paradigms with machine learning approaches may particularly benefit clinical neuroscience, by providing the power to both inform neurobiological differences between clinical and control groups in more naturalistic contexts (Eickhoff et al., 2020), and to pinpoint specific stimulus properties that evoke robust group differences. Here, we present novel analytic approaches for predicting diagnosis using inter-subject similarity of brain responses across epochs of video watching. We used an extensively and densely sampled fMRI dataset from 29 adults with autism

(ASD) and 22 age-, IQ-, and sex- matched controls with 4 ~15-min video-watching scans (813 TR) and resting-state scans; preprocessing is described in Byrge & Kennedy (2018). Results presented here include 36-40 participants per scan (13-15 ASD) after data quality -related exclusions and employ censored timeseries for highest quality from 110 regions of interest (ROIs) from a common anatomical parcellation (Harvard-Oxford). We combine various feature selection and modeling approaches into a cross-validated inter-subject-similarity-based predictive modeling framework (c.f. Shen et al., 2017), using mean pairwise inter-subject correlations between a held-out subject and healthy controls as similarity measures (ISCs) and both permuted group assignments and resting state data as null models. Using brain-wide ISCs across the entire video, we achieve above-chance diagnosis prediction accuracy of 73.7%-80.6% for the 4 video scans. Preliminary analyses using feature selection to model only subsets of timepoints and ROIs from each scan obtain even higher accuracy. In previous work (Byrge & Kennedy, 2020), we were unable to predict diagnosis above chance levels using functional connectivity matrices derived from these same scans. Ongoing work aims to understand whether this dissociation -- diagnosis prediction from evoked brain responses but not from evoked connectomes -- arises due to statistical properties or neurocognitive differences informative about ASD. Next steps will examine whether the most informative video segments and brain regions replicate in an independent dataset, and will characterize stimulus features of those segments, providing important insights into the low-level stimulus properties and/or socio-cognitive processes driving brain response differences in ASD.

Disclosures: L. Byrge: None. **D. Kliemann:** None. **U. Keles:** None. **H. Saarimäki:** None. **R. Adolphs:** None. **D.P. Kennedy:** None.

Digital Abstract Session

P027. Fragile X

Program #/Poster #: P027.01

Topic: A.07. Developmental Disorders

Support: NIH Grant HD052731
NIH Grant MH109026

Title: FMRP ubiquitination and degradation are required for MEF2-induced synapse elimination

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Abstract: Fragile X Mental Retardation Protein (FMRP) is necessary for experience-dependent, developmental synapse elimination and loss of function mutations in FMRP lead to excess dendritic spines and hyperconnectivity of cortical neurons in Fragile X Syndrome, a common inherited form of intellectual disability and autism. We have characterized a model of synapse

elimination in CA1 neurons of organotypic hippocampal slice cultures that is induced by expression of a persistently active transcription factor MEF2 and relies on acute postsynaptic expression of FMRP. FMRP is an RNA-binding protein that represses translation of its target mRNA in dendrites and evidence suggests that its ubiquitination and degradation are necessary for activity-induced translation of target mRNAs to mediate synaptic plasticity. An E3 ligase for FMRP, Anaphase Promoting Complex, along with its co-activator Cdh1 (APC/Cdh1), has been identified (Huang et al. Neuron 2015). Using a combination of pharmacological approaches and mutants of FMRP, that affect ubiquitination or degradation, we provide evidence for a role of APC/Cdh1 and FMRP degradation in MEF2-induced synapse elimination. In wildtype CA1 neurons, pharmacological blockade of APC/Cdh1 with apcin abolishes MEF2-triggered synapse elimination without inhibiting a MEF2 transcriptional reporter, suggesting a role for endogenous APC/Cdh1-mediated ubiquitination downstream of MEF2 activation. APC/Cdh1 interacts with FMRP via a destruction box motif (DBM). Deletion of the DBM of FMRP or mutation of 6 candidate ubiquitination sites in the KH2 RNA binding domain (K6XR-FMRP) prevents MEF2-induced synapse elimination. Dephosphorylation of S499 in FMRP triggers FMRP ubiquitination. Both phospho- and dephospho-mimics of FMRP (S499D/S499A) block synapse elimination. These results suggest that dynamic phospho/dephosphorylaiton of FMRP, APC/Cdh1 mediated-ubiquitination and degradation of FMRP are necessary for MEF2-triggered synapse elimination. Ongoing experiments are exploring whether MEF2 regulates FMRP ubiquitination and degradation in neurons using a bimolecular fluorescence complementation (BiFC) assay.

Disclosures: J.R. Wilkerson: None. **M. Ifrim:** None. **A. Valdez-Sinon:** None. **G.J. Bassell:** None. **K.M. Huber:** None.

Digital Abstract Session

P027. Fragile X

Program #/Poster #: P027.02

Topic: A.07. Developmental Disorders

Support: NIH Grant 1F31NS117178-01 (to MR)
NIH Grant 1U54HD082008 (to IE, KR, DB)

Title: Targeted modification of cortical excitatory neurons during critical developmental period regulates structural and functional circuits in mouse models of Fragile X Syndrome

Authors: *M. RAIS[1], J. W. LOVELACE[2], X. S. SHUAI[1], W. WOODARD[1], S. BISHAY[1], L. ESTRADA[1], A. R. SHARMA[1], A. NGUY[1], P. S. PIRBHOY[1], A. R. PALACIOS[1], D. K. BINDER[1], K. A. RAZAK[2], I. M. ETHELL[1];
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Abstract: Fragile X syndrome (FXS) is a leading genetic cause of autism with symptoms that include sensory processing deficits. Our previous studies have shown that elevated levels of

Matrix Metalloproteinase-9 (MMP-9) contribute to the hyper-responsiveness of auditory cortex in *Fmr1* KO mice by affecting perineuronal net (PNN) formation around parvalbumin (PV)-expressing inhibitory interneurons during third postnatal week. This period between postnatal day (P) 14 and P21 coincides with the critical period of structural and functional circuit development in the rodent auditory cortex driven by sensory input. Abnormal development of PV neurons most likely contribute to abnormal electroencephalography (EEG)-based phenotypes of auditory hypersensitivity in the *Fmr1* KO mice that are remarkably similar to those seen in humans with FXS. Here, we examined whether deletion (cOFF) or rescue (cON) of *Fmr1* in cortical excitatory neurons during the critical developmental period P14-P21 is sufficient to trigger or prevent the development of abnormal phenotypes in the auditory cortex of a mouse model of FXS. FMRP was specifically deleted from the cortical excitatory neurons during the P14-P21 window through Cre-mediated deletion of floxed *Fmr1* gene in excitatory neurons using CaMK2a promoter. We found that similar to global *Fmr1* KO mice, the density of PV/PNN cells and sound-evoked responses were reduced in $Cre^{CaMK2a}/Fmr1^{Flox/y}$ cOFF mice, whereas cortical MMP-9 gelatinase activity and resting EEG gamma power were enhanced. In addition, mTOR/Akt and TrkB phosphorylation were altered in cOFF mice, which also showed increased locomotor activity and anxiety-like behaviors. Remarkably, when FMRP levels were restored in cortical excitatory neurons during the P14-P21 period, cortical MMP-9 gelatinase activity, mTOR/Akt signaling, and resting EEG gamma power were reduced in adult $Cre^{CaMK2a}/Fmr1^{Flox/Neo-y}$ cON mice, whereas the density of PV/PNN cells and sound-evoked responses were improved. In addition, the cON mice also showed an improvement in locomotor activity and anxiety-like behaviors. Taken together, these results indicate that the loss or re-expression of FMRP in cortical excitatory neurons during early postnatal development is sufficient to elicit or ameliorate abnormal cellular, electrophysiological and behavioral phenotypes in mice. These results will have broad implications in terms of prospects for gene reactivation studies by targeting specific cells and identifying optimal treatment windows.

Disclosures: **M. Rais:** None. **J.W. Lovelace:** None. **X.S. Shuai:** None. **W. Woodard:** None. **S. Bishay:** None. **L. Estrada:** None. **A.R. Sharma:** None. **A. Nguy:** None. **P.S. Pirbhoy:** None. **A.R. Palacios:** None. **D.K. Binder:** None. **K.A. Razak:** None. **I.M. Ethell:** None.

Digital Abstract Session

P027. Fragile X

Program #/Poster #: P027.03

Topic: A.07. Developmental Disorders

Support: NICHD R01HD052731

Title: Experience-dependent Silencing of Callosal Synaptic Connections in the Absence of Postsynaptic FMRP

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Abstract: Abnormal functional coupling and reduced white matter integrity between cortical regions is a pathological feature in Autism Spectrum Disorders (ASD) patients. However, little is known about the synaptic and cellular bases for this developmental alteration in the inter-area long-range connectivity. Using optogenetics and slice electrophysiology, we demonstrate that *Fmr1*, which loss-of-function mutations lead to Fragile X Syndrome (FXS), is necessary for the proper development of callosal excitatory synaptic connections between homotopic somatosensory barrel cortices. Postnatal, cell-autonomous deletion of *Fmr1* in a sparse population of Layer (L) 2/3 and L5 neurons results in weakening of excitatory postsynaptic currents (EPSCs) evoked from Channelrhodopsin2-expressing axons from the contralateral hemisphere. In contrast, synaptic inputs from ipsilateral, or local L4 and L2/3 neurons are normal. These results suggest that postsynaptic *Fmr1* specifically promotes long-range synaptic connectivity. Recordings of Sr^{2+} evoked quantal events and isolated NMDA receptor EPSCs reveal a "silencing" or selective loss of AMPA receptors at callosal synapses onto *Fmr1* knock-out (KO) neurons. Interestingly, sensory deprivation of postsynaptic *Fmr1* KO L2/3 neurons, by trimming contralateral whiskers, prevents weakening of callosal synaptic inputs, whereas deprivation of presynaptic callosal projecting L2/3 neurons has no effect. These results suggest that experience driven activity of postsynaptic *Fmr1* KO L2/3 neurons leads to a silencing of callosal excitatory synaptic inputs and reveal a synaptic basis for long-range underconnectivity observed in FXS patients. Ongoing experiments are aimed at determining if activity-dependent plasticity of callosal synapses is regulated by postsynaptic *Fmr1*.

Disclosures: Z. Zhang: None. **K. Huber:** None. **J. Gibson:** None.

Digital Abstract Session

P027. Fragile X

Program #/Poster #: P027.04

Topic: A.07. Developmental Disorders

Title: Correlation between the Fragile X Mental Retardation Protein (FMRP) and the cerebral expression of the metabotropic glutamate receptor subtype 5 ($mGluR_5$) in fragile X syndrome (FXS)

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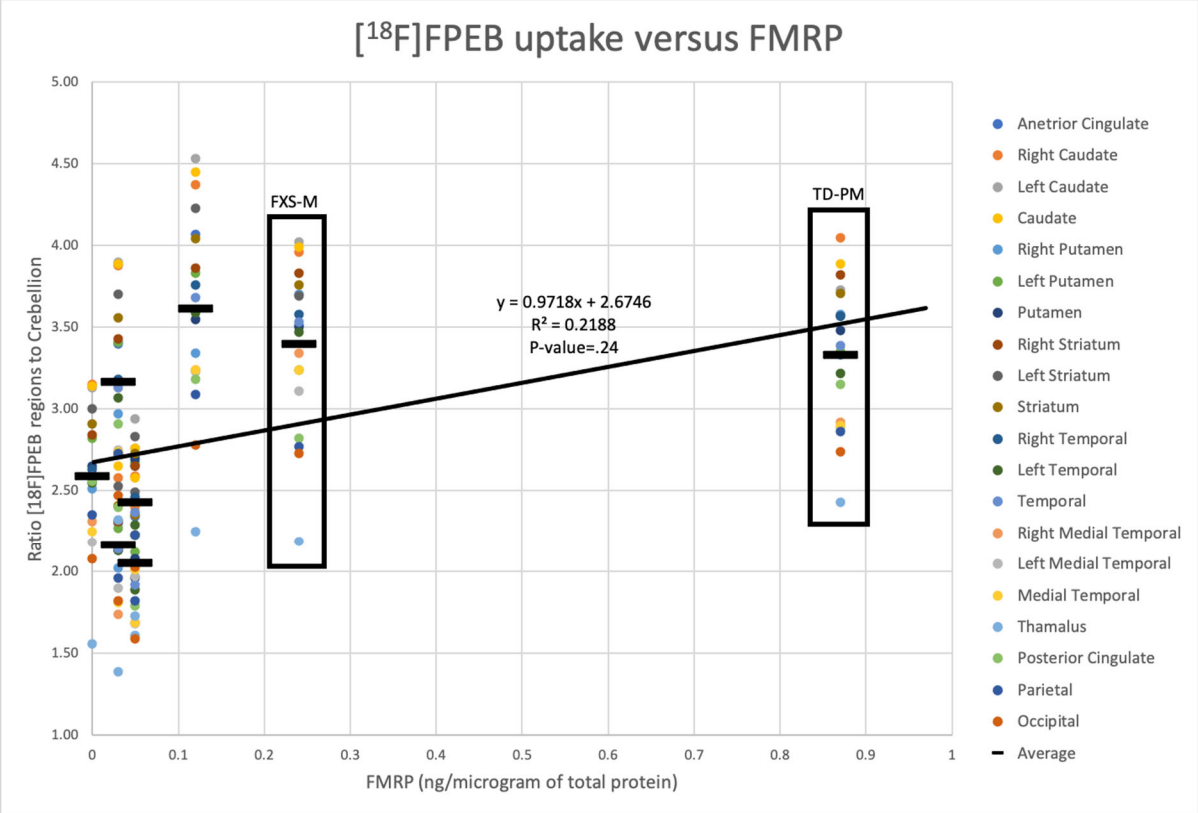
Abstract: Objective: To assess the relationship between the Fragile X Mental Retardation Protein (FMRP) and the cerebral expression of the metabotropic glutamate receptor subtype 5 ($mGluR_5$) in fragile X syndrome (FXS).
Methods: Six men with the full mutation (FM) of the *FMR1* gene aged 21-35 years (mean 27.1, SD 4.7), one male with a mosaicism of the mutation (FXS-M) aged 56.6 years, and one female

with typical development and a premutation (TDPM) of the *FMR1* gene aged 56.3 years, tested by PCR/Southern Blot, underwent positron emission tomography (PET) with 3-[^{18}F]fluoro-5-(2-pyridinylethynyl)benzonitrile ([^{18}F]FPEB), a specific mGluR$_5$ radioligand, to measure the density of mGluR$_5$s as a proxy of mGluR$_5$ expression in key cortical and subcortical brain regions.

Results: FMRP was mean 0.047 ± 0.04 (range 0.00, 0.12) ng/microgram total protein for FXS and 0.24, FXS-M. The FMRP for the participant with TDPM was estimated by utilizing the reference mean for TD (0.87).

The density of mGluR$_5$s was approximately a standard deviation (SD) lower in the men with FXS in contrast to the man with FXS-M and the woman with TDPM (Brasic, *et al.,* Zenodo, v. 1, 2020. https://doi.org/10.5281/zenodo.4101172).Additionally, density of mGluR$_5$ in all brain regions was positively correlated to FMRP levels (P=.24) (Figure 1).

Discussion: These results suggest that marked deficits of both FMRP and mGluR$_5$ characterize men with FXS and are positively correlated. The reductions of FMRP and mGluR$_5$ in cortical and subcortical regions provides an basis for the neurobehavioral phenotype of FXS (Budimirovic, *et al.,* Brain Sci. 2020, 10, 694 https://doi.org/10.3390/brainsci10100694). Understanding this relationship could provide the bases for the development of novel interventions for FXS.



Disclosures: S.D. Martin: None. **E. Berry-Kravis:** None. **D. Russel:** None. **D. Budimirovic:** None. **J. Brasic:** None.

Digital Abstract Session

P027. Fragile X

Program #/Poster #: P027.05

Topic: A.07. Developmental Disorders

Title: Mechanisms of dysfunctional cross modal sensory processing and hypersensitivity in Fmr1 KO mice

Authors: *N. RAHMATULLAH[1], J. ROBLEDO[1], G. CHAUDHARI[2], B. TODISCO[2], C. PORTERA-CAILLIAU[2], A. GOEL[1];
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Abstract: Fragile X Syndrome (FXS) is associated with atypical sensory processing, which may translate into cognitive impairments in learning, memory, social interaction, communication, and emotion. Behavior in an environment replete with cross-modal sensory information, requires that neural circuits selectively attend to salient stimuli, while tuning out irrelevant stimuli. Multisensory processing is influenced by top-down control from pre-frontal areas of the brain and bottom-up subcortical neuromodulation. Hypersensitivity and learning deficits may result from dysfunction in this process. We recently showed impaired sensory processing in FXS contributes to delayed learning of a visual discrimination task consisting of sinusoidal gratings drifting in two orthogonal orientations (Goel et al., 2018). Here we investigated whether *Fmr1* knockout (KO) mice exhibit hypersensitivity to distracting stimuli (visual and auditory) during the same go/no-go visual discrimination task due to atypical cross-modal sensory processing. Preliminary data shows that the presence of distractors - flashing LED lights or loud tones - did not compromise task performance for expert WT mice, but performance significantly declined for trained *Fmr1* KO mice. We then tested the hypothesis that this impairment results from a state of sensory hyperarousal due to dysfunction in the disinhibitory circuit in primary visual cortex (V1), mediated by hyperactive Vasoactive Intestinal Polypeptide (VIP) interneurons. VIP cells integrate functional inputs from the medial prefrontal cortex and cholinergic input from basal forebrain, to ultimately disinhibit V1, thus enhancing visual discrimination. Our preliminary in-vivo two-photon calcium imaging data reveals enhanced visually evoked responses in VIP cells from *Fmr1* KO *mice*. We propose that 1) Elevated basal VIP function prevents detection of change in arousal associated with errors during visual discrimination 2) Reduced top-down modulation of VIP function in *Fmr1* KO mice prevents selective processing of task-relevant stimuli and enhances susceptibility to distractors. Our future goals are to investigate how dysfunctional integration of elevated subcortical input and reduced top-down input to VIP cells contribute to hypersensitivity to distracting stimuli. In addition, this study will provide fundamental insights into the neurobiological mechanisms that contribute to multisensory processing and affect other domains of behavior in FXS and autism, such as social interaction and recognition.

Disclosures: **N. Rahmatullah:** None. **J. Robledo:** None. **G. Chaudhari:** None. **B. Todisco:** None. **C. Portera-Cailliau:** None. **A. Goel:** None.

Digital Abstract Session

P027. Fragile X

Program #/Poster #: P027.06

Topic: A.07. Developmental Disorders

Support: FRAXA Research Foundation
NIH Grant 1U54HD082008-01

Title: Conditional expression of Fmr1 in the inferior colliculus rescues deficits in phase-locking in the Fmr1 mouse model of Fragile X syndrome

Authors: *A. J. HOLLEY, J. R. GIBSON, K. M. HUBER;
Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Rationale: Humans with Fragile X Syndrome (FXS), the most common monogenetic cause of autism spectrum disorder (ASD) and a common cause of intellectual disability, often present with altered sensitivity to sensory stimuli. Recently, it has been shown that patients with FXS and the mouse model of FXS, the *Fmr1* knockout (KO) mouse both show remarkably similar reductions in cortical EEG phase-locking to a chirp-modulated stimulus and a marked increase in resting gamma power (30-100Hz). Both phenotypes are proposed biomarkers of FXS. A recent study has shown that the resting EEG alterations, but not the decreased phase-locking, are prevented when *Fmr1* is deleted in only forebrain excitatory neurons. Given this data, we hypothesized that auditory phase-locking deficits may have a subcortical origin. **Methods:** To determine if *Fmr1* deletion in a subset of brain stem neurons is required for decreased phase-locking, we crossed Ntsr1-cre mice with *Fmr1* conditional on (cON) mice, both on a C57BL6/J background, to produce three functional phenotypes: cON (functionally equivalent to an *Fmr1* KO; n=16), cre-cON (expression of *Fmr1* in the inferior colliculus, or IC; n=18), and two wildtype (WT) conditions (WT and Ntsr1-cre; n=18,17). Because Ntsr1-cre expression in the brainstem is primarily in the IC, an early sensory structure of the brainstem, we can test whether the restoration of *Fmr1* in the IC, in an otherwise *Fmr1* KO mouse, can restore the deficit in phase-locking. We implanted male mice between P45 and P50 with epidural screw electrodes and after recovery measured their response to a “chirp” stimulus that efficiently presents sound from 1-100Hz over a 2-sec period. **Results:** We found that, like *Fmr1* KO mice, the cON mice showed a marked deficit in phase-locking in both auditory and frontal cortices, particularly in the beta (13-30Hz) and high gamma (65-100Hz) frequencies, compared to WT and Ntsr1-cre mice. Moreover, this deficit is at least partially rescued in the cre-cON mice. These results confirm a role for the IC in a potential biomarker for FXS. Our results also reveal a potential target for treatment in FXS and may extend to other forms of ASD where similar deficits have been reported.

Disclosures: A.J. Holley: None. **J.R. Gibson:** None. **K.M. Huber:** None.

Digital Abstract Session

P027. Fragile X

Program #/Poster #: P027.07

Topic: A.07. Developmental Disorders

Support: DGAPA/PAPIIT Grant IA210620

Title: Proteomic analysis of fibroblasts from patients with Fragile X Syndrome to identify new therapeutical targets.

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Abstract: Introduction: Fragile X Syndrome (FXS) is the most common cause of inherited mental disability. The patients exhibit major behavior disorders such as: hyperactivity, impulsiveness, anxiety, poor language performance and severe mental disability. FXS is caused by the absence of Fragile Mental Retardation Protein (FMRP) encoded by *FMR1* gene; this protein has an important role in mRNA regulation, causing deregulations especially in postsynaptic neurons. The silencing of FMRP is due to CGG triplet abnormal repeats in the promoter region of *FMR1* gene; the severity of FXS depends on the number these CGG repeats, below 55 repeats is considered normal, between 55 and 200 repeats is called a premutation where mild symptoms are shown; over 200 repeats it is considered a full mutation presenting severe symptomatology. The studies conducted to understand FXS have been performed mostly in the knockout mouse model; however the results obtained in these animal models are difficult to be translated into humans due to different reactions to drugs and treatments. Moreover, these disorders are human specific. It has been suggested to use peripheral cells such as skin fibroblasts to analyze the physiological characteristics of the disease; these show an opportunity to analyze humans with FXS in a low invasive method. Proteomics allow us to study the protein universe of FXS cells and give us information of the alterations occurring in these group of diseases, indicating important therapeutical targets and allowing to propose new treatments. Method: Fibroblasts of FXS patients and apparently healthy individuals (AHI) were obtained from the Coriell Institute (New Jersey) repository and cultured in Earl MEM salts medium with 15% non-inactivated fetal bovine serum. The cells were characterized by immunofluorescence detection of Vimentin and differential expression of FMRP. The cells were cultured to reach a monolayer on nine T300 flasks to obtain enough protein for the proteomic analysis (aprox. 1 mg). The samples were processed at USAI unit (UNAM Chemistry Faculty) where 2-DE and mass spectrometer shotgun analyses were made. Proteomics results were confirmed using Western blot techniques. Results: Proteomics show a deregulation in pathways involved in protein recycling such as Ubiquitin C protein and Ubiquitin peptidase 5, we also found a deregulation of genes related to gene expression. Conclusion: Proteomic analysis of skin

fibroblasts obtained of FXS patients show alterations in several pathways related to the pathology, meaning this tool is useful in the study and proposal of new therapeutic approaches and biomarkers.

Disclosures: C. Torres: None. **Y. Ramiro-Cortés:** None. **F. Peña-Ortega:** None. **M. Cardenas-Aguayo:** None.

Digital Abstract Session

P027. Fragile X

Program #/Poster #: P027.08

Topic: A.07. Developmental Disorders

Support: NIMH/NICHD Grant U54 HD082008-01

Title: Sex-specific Associations between EEG Frontal Asymmetry and Clinical Features in Fragile X Syndrome

Authors: *J. E. NORRIS[1], L. M. SCHMITT[2], L. A. DE STEFANO[2], E. V. PEDIPATI[3], J. A. SWEENEY[4], C. A. ERICKSON[3], L. E. ETHRIDGE[1];
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Abstract: Fragile X Syndrome (FXS) has a clinical presentation that includes intellectual disability and autism-like symptoms. FXS electroencephalography (EEG) biomarkers of sensory processing have been previously demonstrated by our group, but assessment of neural differences between hemispheres and clinical outcomes is limited, particularly in regard to sex differences. Thirty-eight adolescent and adult participants with the full mutation FXS [Mean age = 25.5, SD = 10.1; 13 females], and forty age- and sex- matched controls [Mean age = 27.7, SD = 12.1; 17 females] listened to an amplitude modulated auditory chirp stimulus which changed in frequency over time during continuous EEG. Relationships between neural stimulus processing measures and neural power between hemispheres in the theta and alpha frequency bands (alpha and theta asymmetry) during the pre-stimulus period were assessed. Asymmetry measures also were correlated with clinical measures. Males and females with FXS trended toward differences on theta asymmetry, $t(34) = 1.59$, $p = .09$. Males with FXS also trended toward difference from controls on alpha asymmetry, $t(43) = -1.77$, $p = .08$. In females with FXS, increased SCQ scores correlated with increased right-hemisphere alpha power ($r = -.796$, $p = 0.01$). Increased Vineland play/leisure behaviors correlated with increased left-hemisphere theta power ($r = -.689$, $p = .03$). In males with FXS, increased left-hemisphere alpha power correlated with increased ABC inappropiate speech ($r = -.450$, $p = .05$) and increased Sensory Profile avoidance scores ($r = -.693$, $p = .01$). Increased alpha power on the right was correlated with increased ABC social avoidance ($r = .468$, $p = .04$). Theta/alpha power ratios are generally atypical in FXS and EEG frontal asymmetry may exhibit sex-effects, though groups did not significantly differ in frontal asymmetry. Alpha asymmetry may play a role in presence of social-communication deficits

associated with autism in females with FXS, whereas alpha asymmetry may play a greater role of behavioral phenotypes more specific to FXS in males. Our findings suggests a complex role for alpha/theta frequencies. Males with FXS may utilize alpha power differently in each hemisphere for specific types of baseline neural processing. Alpha asymmetry may mechanistically underlie the lateralized finding between clinical measures of sensory avoidance and social avoidance in males with FXS by modulating sensory processing differently across the hemispheres. Our study extends previous research findings that address general alpha and theta abnormalities in FXS and may be useful by adding specificity to prior established biomarkers.

Disclosures: J.E. Norris: None. **L.M. Schmitt:** None. **L.A. De Stefano:** None. **E.V. Pedipati:** None. **J.A. Sweeney:** None. **C.A. Erickson:** None. **L.E. Ethridge:** None.

Digital Abstract Session

P027. Fragile X

Program #/Poster #: P027.09

Topic: A.07. Developmental Disorders

Support: USDA 2018-67001-28266
NIH HD097067
NIH HHSN272201400008C
Japan AMED 19fk0108113

Title: Repurposing Fragile X drugs to inhibit SARS-CoV-2 viral reproduction

Authors: *C. J. WESTMARK[1], M. KISO[3], P. HALFMANN[2], P. R. WESTMARK[1], Y. KAWAOKA[2];
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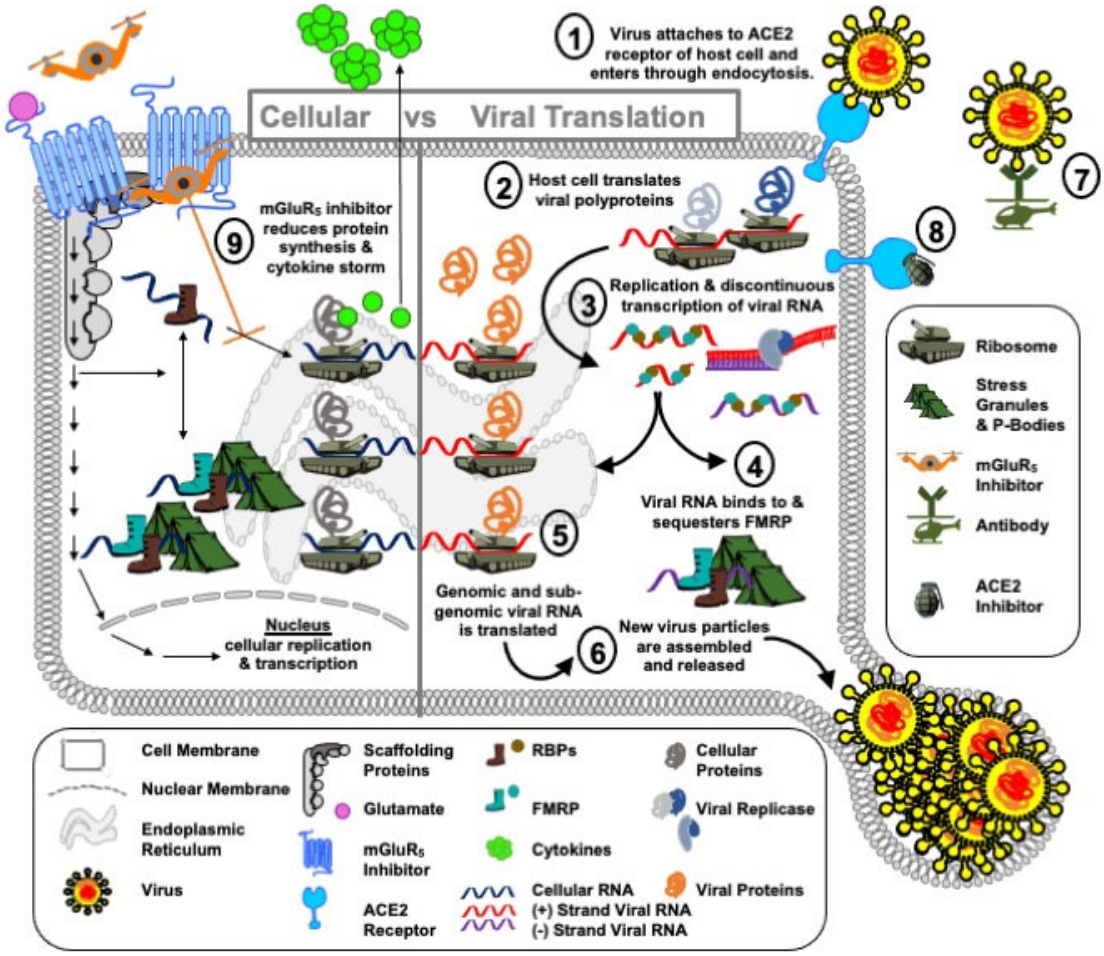
Abstract: The COVID-19 pandemic is a global health crisis that requires the application of interdisciplinary research to address numerous knowledge gaps including molecular strategies to prevent viral reproduction in affected individuals. We propose a novel therapeutic strategy to repurpose metabotropic glutamate 5 receptor (mGluR5) inhibitors to interfere with viral hijacking of the host protein synthesis machinery. Negative allosteric modulators (NAMs) of mGluR5 are potent, non-competitive, selective and systematically active allosteric antagonists that are under study for a range of indications including fragile X syndrome (FXS) where these drugs rescue disease phenotypes in multiple preclinical models and have been safely tested in clinical trials. Herein, based on molecular modeling data, we demonstrate that SARS-CoV-2 RNA has high potential to sequester the RNA binding protein FMRP, which is absent in FXS and leads to exacerbated protein synthesis, thus preventing the normal function of FMRP to inhibit protein synthesis. We further demonstrate that mGluR5 NAMs could be a prophylactic treatment to slow viral protein synthesis in patients infected with SARS-CoV-2. Treatment of VeroE6/TPMRSS2 cells with the mGluR5 inhibitor CTEP reduced SARS-CoV-2 viral plaque load with an IC50 of 13.1 μM. The inclusion of mGluR5 NAMs as part of a drug cocktail

approach to combat COVID-19 offers the advantages of: (1) extensive preclinical research regarding its mechanism of action; (2) prior safety testing in human clinical trials of FXS; (3) numerous mGluR5 NAMs available; (4) orally dosed; (5) protein target ubiquitously expressed including the lungs; (6) less expensive to produce small molecule drugs; and (7) targets a post-transcriptional gene regulatory step in viral production not addressed by other therapies under investigation. In addition, blockade of mGluR5 activity prevents an increase in proinflammatory cytokines and chemokines, which may quell the cytokine storm. Herein, we provide a mechanistic-based hypothesis and data to support testing mGluR5 inhibitors in COVID-19.



Disclosures: **C.J. Westmark:** None. **M. Kiso:** None. **P. Halfmann:** None. **P.R. Westmark:** None. **Y. Kawaoka:** None.

Digital Abstract Session

P028. Rett Syndrome

Program #/Poster #: P028.01

Topic: A.07. Developmental Disorders

Support: CDKL5 - 19 – D-104 - 01

Title: Dna methylation editing of X-linked intellectual disability genes via adeno-associated viruses

Authors: *J. A. HALMAI, C. GONZALEZ, P. DENG, J. J. WALDO, F. BUCHANAN, J. CARTER, C. WELCH, D. CAMERON, K. FINK;
UC Davis, SACRAMENTO, CA

Abstract: Despite only containing about 5% of the human genome, there are approximately 40 known X-linked intellectual disability genes present on the X-chromosome with female phenotypic expression. In females, monogenic disorders with an X-linked dominant pattern of inheritance result in a mosaic of cells expressing the mutant and wild-type alleles. One of the alleles becomes epigenetically silenced via a dosage compensation mechanism called X-chromosome inactivation. A large number of genes are able to escape from X-chromosome inactivation. These escapees have a specific epigenetic signature associated with them, particularly reduced levels of 5-methylcytosine in CpG island promoters. A potential therapeutic approach previously demonstrated by our group for a gene on the X-chromosome is to activate the silenced wild type allele in cells expressing a loss-of-function mutant allele, rewriting the epigenetic signature of an escapee. Here, we demonstrate reactivation of two genes involved in X-linked intellectual disabilities in patient-derived induced pluripotent stem cells and neural stem cells, *CDKL5* and *MECP2*. We demonstrate that gene reactivation is possible via DNA methylation editing of the promoter regions using CRISPR/dCas9. In addition, our group demonstrates that this approach is readily translatable via the employment of intein-mediated trans-splicing of large dCas9 effector fusion proteins, allowing delivery to the central nervous system *in vivo* via adeno-associated viruses. Our approach holds great promise for those suffering from X-linked dominant disorders of the CNS.

Disclosures: **J.A. Halmai:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); receipt of intellectual property rights/patent holder. **C. Gonzalez:** None. **P. Deng:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); receipt of intellectual property rights/patent holder. **J.J. Waldo:** None. **F. Buchanan:** None. **J. Carter:** None. **C. Welch:** None. **D. Cameron:** None. **K. Fink:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); receipt of intellectual property rights/patent holder.

Digital Abstract Session

P028. Rett Syndrome

Program #/Poster #: P028.02

Topic: A.07. Developmental Disorders

Support: Swedish research council

Title: Social and locomotor behaviour in MECP2 mutants: Zebrafish model of Rett Syndrome

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Abstract: Rett syndrome, an X-linked neurodevelopmental disorder exclusively affects females and is characterized by slowed development, loss of purposeful use of the hands, slowed brain and head growth, seizures and intellectual disability. Another feature in children with Rett syndrome are autistic-like behaviours such as loss of social interaction and communication. Deficits in the X-linked gene methyl-CpG binding protein 2 (*MECP2*) has been identified as the genetic cause of Rett syndrome, and *MECP2* mutations are observed in other phenotypic groups such as people diagnosed with autism and X-linked mental retardation disorders. As an *in vivo* model that is easily modified genetically and allows generation of hundreds of test subjects, the vertebrate species zebrafish (Danio rerio) holds many advantages for studying *mecp2* functions. Here, using heterozygous parents, we generated and investigated social behaviour, locomotor activity, and propensity to seizures in zebrafish lacking *mecp2*, compared to wildtype siblings (n > 20). Social preference test was investigated in 21-days-old siblings to determine phenotypes for fish lacking one or both copies of *mecp2*. To determine the locomotor activity in response to a stimulating convulsant, wild-type fish and heterozygous and homozygous mutant fish (n >70) were treated with pentylenetetrazol (PTZ, 0, 5, 10 µMol solution, water immersion) and general locomotor activity and burst-like and circling movements were measured at 7-days of age. Genotypes were confirmed for all subjects following behavioural recordings. Our results show that lack of *mecp2* did not change social preference for visual social stimulus in heterozygous or homozygous mecp2 mutants at 21-days of age. In contrast, locomotor activity in response to 10 µMol PTZ solution was significantly affected in both homozygous *mecp2* mutant fish and in heterozygous *mecp2* mutant fish, compared to wildtype siblings at 7-days of age (but not for 5 µMol PTZ solution). These findings help us characterize the behavioural phenotypes resulting from manipulation of *mecp2* gene and allow modelling of social and non-social behaviours seen in developmental disorders such as Rett syndrome. Our work also highlights the potential of future investigations of zebrafish *mecp2* gene towards generating better models of Rett syndrome and other related disorders.

Disclosures: P. Cronell: None. **S. Shams:** None. **L. Westberg:** None.

Digital Abstract Session

P028. Rett Syndrome

Program #/Poster #: P028.03

Topic: A.07. Developmental Disorders

Support: NIH Grant MH085802

Picower Neurological Disorder Research Fund – PNDRF
James W. (1963) and Patricia T. Poitras Fund
Simons Center for the Social Brain seed grant

Title: Early Molecular and Cellular Deficits in 3D Cerebral Organoid models of Rett Syndrome

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Abstract: Rett Syndrome (RTT, OMIM 312750) is a progressive and pervasive, X-linked neurodevelopmental disorder that predominantly affects girls, who exhibit symptoms by early childhood. The vast majority of typical RTT cases is triggered by a sporadic mutation in the methyl CpG-binding protein 2 (MeCP2) gene. Although most research has focused on postnatal mice and humans displaying clinical symptoms, recent studies have shown that MeCP2 deficiency triggers molecular and cellular defects at very early stage of embryonic brain development, prior to clinical manifestation. We used isogenic RTT patient-derived induced pluripotent stem cells to generate 3D human cerebral organoids to recapitulate these early developmental events and to better understand the molecular and cellular events that underlies RTT pathology. Previously, we showed that MeCP2 deficiency was linked to dysregulation of the AKT/ERK pathway, which led to an increase in neural progenitor proliferation and concomitant decrease in neurogenesis and neuronal migration and maturation (Mellios et al., *Molecular Psychiatry* 2018). In this current study, we further investigated the molecular underpinnings of the deficits in neuronal migration observed in RTT organoids. Using genetic expression of fluorescent markers, and immunostaining, combined with confocal and multi-photon microscopy, we found that, although the morphology and polarity of radial glial cells were mostly preserved, neuronal migration patterns (speed, trajectory, and distance) were disrupted in RTT MeCP2 mutant organoids compared to isogenic controls. We then used transcriptomic, proteomic and phospho-proteomic techniques to screen for dysregulated adhesion molecules downstream of RTT MeCP2 mutations. We found an increase in the phosphorylation of the Reelin signaling adaptor protein, DAB1, at the tyrosine (Y) 232 residue. By introducing a dominant-negative phospho-null Y232F plasmid construct, and an DAB1 overexpression construct in the ventricles of RTT organoids and isogenic controls, here we probe the contribution and mechanisms of DAB1 in migration deficits observed in RTT organoids, through the regulation of cell adhesion. By using small molecules to modulate the AKT signaling pathway, we interrogate the links between DAB1 phosphorylation, adhesion, neuronal migration and signaling. These findings will further clarify the role and mechanisms of early deficits during cortical development in RTT.

Disclosures: C. Delepine: None. **V.A. Pham:** None. **H.W.S. Tsang:** None. **M. Yildirim:** None. **N. Morshed:** None. **X. Adiconis:** None. **S. Simmons:** None. **P. Arlotta:** None. **J. Levin:** None. **F. White:** None. **L. Tsai:** None. **M. Sur:** None.

Digital Abstract Session

P028. Rett Syndrome

Program #/Poster #: P028.04

Topic: A.07. Developmental Disorders

Support: NIMH Fellowship F31MH119699
Rettsyndrome.org Grant 3511
Pitt-Hopkins Research Foundation Grant 23

Title: Investigating the effects of genetically increasing MECP2 dosage in mouse models of typical and atypical Rett syndrome

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Abstract: *De novo* loss-of-function (LOF) mutations in the transcription factor methyl-CpG-binding protein 2 (MeCP2) are the main cause of the neurodevelopmental disorder Rett syndrome (RTT). Seminal studies have shown that clinical symptoms are recapitulated after knock-out of the murine *Mecp2* locus or knock-in of pathogenic mutations. Furthermore, normalizing *MECP2* gene dosage rescues phenotypes in mouse models. However, this approach is complicated by a potentially narrow therapeutic window as a 0.5x over-expression of MeCP2 can cause adverse effects that resemble symptoms of a related disorder known as *MECP2* duplication syndrome (MDS). The therapeutic window is predicted to be further impacted by hypomorphic MeCP2 mutations where expression and function of MeCP2 is not completely ablated. Additionally, there are cases of atypical RTT, wherein the patient is *MECP2* mutation-negative but has another compromised gene, such as the transcription factor 4 (*TCF4*). *TCF4* LOF mutations primarily lead to the diagnosis of Pitt-Hopkins syndrome (PTHS), which has significant overlap in symptomology as RTT. **Therefore, we hypothesize that increasing MeCP2 dosage will (1) lead to MDS-like phenotypes in RTT mice harboring a hypomorphic MeCP2 mutation and (2) improve symptoms in PTHS model mice.**
We interrogated these hypotheses at the preclinical level by crossing MDS model mice (*MECP2*$^{Tg1/o}$) to RTT mice with a hypomorphic MeCP2 mutation (*Mecp2*$^{R133C/y}$ or *Mecp2*$^{R133C/+}$) or PTHS mice (*Tcf4*$^{+/-}$). Behavioral characterization was performed in mice with and without the *MECP2* transgene. In the hypomorphic mutant RTT mice, we observed reversal of RTT phenotypes in the male *Mecp2*$^{R133C/y}$ animals; in contrast, female *Mecp2*$^{R133C/+}$ mice with the *MECP2* transgene exhibited MDS-like phenotypes in anxiety, motor coordination and contextual fear learning and memory. Control experiments with heterozygous RTT mice (*Mecp2*$^{+/-}$) illustrated that these MDS-like phenotypes are due to the *R133C* mutation. Interestingly, expression of the *MECP2* transgene in the PTHS mice rescued behavioral phenotypes of hyperlocomotion, and attenuated anxiety and contextual freezing. Molecular studies showed that MeCP2 does not impact Tcf4 expression, indicating a more complex relationship between these

two proteins, which we are exploring with RNA-sequencing studies. Overall, our data suggest that the effects of a 1x increase in MeCP2 dosage is dependent on *MECP2* mutation, and safety may need to be further considered and evaluated in the context of hypomorphic MeCP2 mutations; in contrast, at present, increasing MeCP2 may induce favorable effects in disorders with overlapping symptoms to RTT, such as PTHS.

Disclosures: S.D. Vermudez: None. **R.G. Gogliotti:** None. **B. Arthur:** None. **A. Buch:** None. **C. Morales:** None. **Y. Moxley:** None. **H. Rajpal:** None. **P.J. Conn:** None. **C.M. Niswender:** None.

Digital Abstract Session

P029. Down Syndrome

Program #/Poster #: P029.01

Topic: A.07. Developmental Disorders

Support: Jerome Lejeune Foundation 1920
KSU BHRI Pilot grant

Title: Dscam overexpression replicates neuronal morphology alterations observed in human Down syndrome iPSC-derived neurons during development.

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Abstract: Down syndrome results from the triplication of human chromosome 21 (HSA21). It affects 1 in every 700 live births within the United States and is the most common genetic cause of intellectual disability. Multiple genes are implicated in this phenotype, including Down Syndrome Cell Adhesion Molecule (DSCAM). *DSCAM* is located on HSA21 and regulates the formation of neuronal connectivity in the developing brain. DSCAM is a member of the immunoglobulin superfamily, a transmembrane protein and can act as a receptor for the axon guidance molecule netrin-1. Our lab has previously shown that *Dscam* mRNA is locally translated within growth cones, and dysregulation of this process interferes with proper axon growth. In the current study, we used two different models: 1) *Dscam* gain-of-function mice, which mimic the DSCAM overexpression occurring in Down syndrome, and 2) neurons derived from human induced pluripotent stem cells (hiPSCs), which were created from apparently healthy individuals and individuals with Down syndrome. In the experiments using *Dscam* gain-of-function mice, hippocampal neurons were isolated from E17 mouse pups and cultured for 2 days *in vitro*. We found that overexpression of *Dscam* leads to impaired axon growth, and reduces axon branching *in vitro*. Using the Dunn chamber axon guidance assay, Dscam-overexpressing neurons did not show axon turning in response to netrin-1, as compared to wild-type neurons. Dscam-overexpressing neurons also showed decreased soma area, and a significant reduction in the number of dendrites and total neurite length. In the experiments using hiPSC-

derived Down syndrome neurons, we observed a reduction in axon length and impaired axon branching. These neurons also had a smaller soma area and growth cone area, and a significant reduction in total neurite length. Thus, axon growth, branching, total neurite length and soma area were impaired in both *Dscam* gain-of-function mice and hiPSC-derived Down syndrome neurons. These types of changes in neuronal morphology can lead to altered neuronal connectivity. Taken together, these results suggest that *Dscam* plays an important role in the development of neuronal networks and should be further studied as a potential contributor to the connectivity deficits that occur in DS.

Disclosures: **M. Agrawal:** None. **K. Welshhans:** None.

Digital Abstract Session

P029. Down Syndrome

Program #/Poster #: P029.02

Topic: A.07. Developmental Disorders

Support: The Medical Research Council
the Health Foundation
Addenbrooke's Charitable Trust
Alzheimer's Research UK – Cambridge Network
Fearnsides Fund
Marmaduke Shield Fund

Title: The auditory mismatch negativity reflects accelerated aging in adults with Down's Syndrome

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Abstract: Down's Syndrome (DS) is associated with premature and accelerated aging and a propensity for early-onset Alzheimer's disease (AD). The early symptoms of dementia in people with DS may reflect frontal lobe vulnerability to amyloid deposition. The Mismatch Negativity (MMN) is a frontocentral event-related brain potential (ERP) component elicited by auditory violations. Predictive coding postulates that the brain creates neural predictions about future events by compiling statistical regularities from incoming stimuli. Within the MMN network, the MMN results from the failure of higher frontotemporal nodes to predict the incoming sensory input, which results in changes of coupling between the sensory and the frontotemporal cortices. In the typically developing (TD) population, the MMN response has been found to decrease with age. In the cross-sectional phase of this study, the MMN was used to investigate the premature neurological aging hypothesis of DS. The MMN ERP and the MMN Global-Field Power (GFP) were elicited with an auditory global-local task and recorded with high-density EEG for 36 (15

females) DS adults aged 20 years or more (mean age 36.81, age range 22-55) and for 39 (21 females) age-matched TD controls (mean age 39.89, age range 20-59). Furthermore, a measure of cognitive impairment was taken in DS with the neuropsychological battery CAMCOG-DS. Bayesian model comparison was carried out on multilevel linear models testing whether the MMN predicted age in the two groups. An interaction between the MMN amplitude, both expressed in ERP and GFP, and group was found. In particular, MMN amplitude predicted age in DS but not in TD. In the longitudinal phase, we evaluated the MMN as a potential predictor of cognitive decline. In a one year follow-up 34 adults with DS underwent the second administration of the CAMCOG-DS. Bayesian model comparison was carried out on multilevel linear and curvilinear models testing whether the MMN obtained at the first time-point was predictive of cognitive decline one year later as measured by changes in CAMCOG-DS scores. However, neither amplitude nor latency of the MMN predicted cognitive decline one year later. Our study shows that in our cohort the MMN amplitude reflects accelerated aging in DS but not in TD, suggesting that MMN amplitude may be a sensitive marker of early onset AD in the DS population. On the other hand, neither MMN amplitude or latency predicts cognitive decline one year later. Gender differences were not explored in our analyses, neither in the cross-sectional nor in the longitudinal phase.

Disclosures: **C. Avancini:** None. **S. Jennings:** None. **S. Chennu:** None. **V. Noreika:** None. **A. Le:** None. **T. Bekinschtein:** None. **M.J. Walpert:** None. **A.J. Holland:** None. **S. Zaman:** None. **H.A. Ring:** None.

Digital Abstract Session

P030. Mechanisms of Vulnerability

Program #/Poster #: P030.01

Topic: A.09. Adolescent Development

Support: CONACyT
FRQ-PBEEE
NIDA Grant R01DA037911
CIHR Grant MOP-74709
NSERCC Grant 2982226

Title: Distinct effects of social defeat stress in adolescence vs. adulthood on the Netrin-1/DCC pathway, prefrontal cortex dopamine and cognition

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Abstract: Exposure to social stress, such as bullying or domestic violence, is a risk factor for psychiatric disorders. Social stress can be especially harmful during adolescence when the brain

is still maturing, rendering it sensitive to environmental influences. An important adolescent maturational process is the gradual unfolding of the dopamine (DA) innervation to the prefrontal cortex (PFC). The guidance cue Netrin-1 and its receptor, DCC, control the growth of DA axons from the nucleus accumbens (Nacc) to the PFC throughout adolescence. Alterations in the Netrin-1/DCC pathway during this period lead to dysregulated PFC DA connectivity and to changes in cognitive performance later in life. Previous studies in rodents have shown that the response of *adult* animals to social stress involves the DA system and DCC. However, the potential age-specific impact of social stress *in adolescence* on the Netrin-1/DCC pathway and the maturing DA system has not been investigated. To this end, we established an accelerated social defeat (AcSD) stress paradigm to expose adolescent and adult male mice to social stress and categorised them as "resilient" or "susceptible" based on social avoidance behaviour. Following AcSD in adolescence, the majority of mice are resilient and exhibit less-anxious/more risk-taking behaviour, while AcSD in adulthood leads to a majority of susceptible mice without altering anxiety-like traits. AcSD in adolescence, but not adulthood, dysregulates DCC and Netrin-1 expression in mesolimbic DA regions and alters DA connectivity in the matured PFC. The nature of these changes differs between resilient and susceptible groups. Following AcSD in adulthood, cognitive function remains unaffected, but both resilient and susceptible mice exposed to AcSD in adolescence show deficits in inhibitory control when they reach adulthood. The distinct effects of social stress on the Netrin-1/DCC pathway, social behaviour, and cognition between adolescence and adulthood highlight adolescence as a unique period for vulnerability and resilience to mental illness and indicate that environmental factors change the developing and the matured brain through fundamentally different mechanisms.

Disclosures: A.H. Pantoja Urban*: None. **P. Vassilev*:** None. **M. Giroux:** None. **D. Nouel:** None. **G. Hernandez:** None. **T. Orsini:** None. **C. Flores:** None.

Digital Abstract Session

P030. Mechanisms of Vulnerability

Program #/Poster #: P030.02

Topic: A.09. Adolescent Development

Support: NIH (DK124727, GM060507, MD006988)
AMRIS (DMR-1644779)
NIH (S10RR025671)
Loma Linda University GRASP Seed Funds to JDF and IDP.

Title: Neuropathological Correlates of Combined Posttraumatic Stress, Overweight, and Obesity

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Abstract: Background: Obesity is a multifactorial disorder that requires environmental influences to manifest. Some individuals are more susceptible than others to gain weight and become obese when exposed to psychosocial stress during adolescence. However, the neurobiological adaptations that contribute to this susceptibility remain unclear. Here we sought to determine the adverse synergy of an obesogenic diet and psychosocial stress (PSS) on cardiometabolic and immune function, brain structure, and behavior. **Methods:** Fifty-six adolescent Lewis rats (postnatal day, PND21) were fed for five weeks with an experimental Western-like high-saturated fat diet (*WD*, 41% kcal from fat) or a matched control diet (*CD*, 13% kcal from fat). Subsequently, a group of rats ($n = 29$) was exposed to a well-established 31-day PSS model composed of a predator exposure in conjunction with social instability starting at PND60. We assessed the effects of the WD and PSS using a comprehensive battery of standard behavioral tests. The brains were collected for neurite orientation dispersion and density imaging (NODDI) and diffusion tensor imaging (DTI) at PND107. **Results:** We found that WD/PSS rats exhibited increased weight gain (25%) relative to WD/Unexposed rats at three-weeks post-PSS. WD rats showed reduced diastolic pressure and heart rate (both 10%) compared to CD animals. WD and PSS synergized to reduce the open arm duration in the elevated plus maze (54% reduction) and social interactions in the social Y-maze (57% reduction) relative to controls. PSS significantly attenuated acoustic startle responses (28% reduction) while heightening the fear-potentiated startle (70% increase) relative to unexposed controls. NODDI/DTI identified unique brain anatomical signatures in WD and PSS rats. **Conclusions:** Our findings confirm the adverse synergy of WD and PSS on health and behavior. This study contributes to enhancing our understanding of potential neurobiological adaptations by which obesogenic environments shape the maturational trajectories of resilience networks. We anticipate that this study will continue to inform needed biomarkers and interventions for improving the quality of psychosocial stress management, particularly in overweight and obese children.

Disclosures: P. Ontiveros-Angel: None. **J.D. Vega-Torres:** None. **V. Williams:** None. **M. Pompilus:** None. **T. Simon:** None. **E. Bayeta:** None. **M. Febo:** None. **C. Viet:** None. **I. De La Peña:** None. **J.D. Figueroa:** None.

Digital Abstract Session

P030. Mechanisms of Vulnerability

Program #/Poster #: P030.03

Topic: A.09. Adolescent Development

Support: NIH Grant F31AA028446

Title: Moderate and heavy alcohol exposure effects on the proteome of fetal neural stem cell-derived extracellular vesicles

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Abstract: Prenatal alcohol exposure (PAE) is the leading cause of neurodevelopment disability worldwide, commonly resulting in growth and neurobehavioral deficits. Neural stem cells (NSCs) are an important target of ethanol during the late first through second trimester, during the peak period of fetal neurogenesis. The NSC microenvironment is rich in sub-200 nanometer-sized extracellular vesicles (EVs), that may function as a mode of intercellular communication, transporting proteins, lipids, and RNAs between NSCs and their progeny. Using fetal mouse derived cortical neuroepithelium, cultured ex-vivo as non-adherent neurosphere cultures, we previously found that ethanol exposure resulted in significant elevation of miRNA cargo like miR-140-3p in EVs, which direct NSCs towards an aberrant lineage. EVs may amplifying PAE's temporal and spatial effects in the NSC niche, resulting in an overall decline in neurogenic capacity. For this study, we further investigated the impact of ethanol on the proteome of NSC-EVs by employing quantitative mass-spectrometry to profile the protein expression across alcohol-treated and control NSCs. Ethanol-exposed NSCs significantly altered the profile of proteins packaged within EVs. Of the 3,617 consistently expressed EV proteins, moderate ethanol exposure (26mM) differentially regulated 65 proteins compared to controls, with >95% being upregulated, while heavy ethanol exposure (70mM) differentially regulated 108 proteins compared to controls, with ~66% being upregulated (Paired t-test, $p<0.05$; effect size, Cohen's $d>0.5$, $\alpha=5\%$, $1-\beta=0.8$**)**. For cells, in contrast, out of 4,698 expressed proteins, moderate ethanol exposure differentially regulated 492 proteins compared to controls, with >92% being downregulated, while heavy ethanol exposure differentially regulated 750 proteins, with >95% again being downregulated. Due to this contrast of protein upregulation in EVs and downregulation in cells, expression of proteins that were significantly altered in EVs by ethanol exposure were compared to the expression of the same proteins in cells. For both moderate and heavy ethanol exposures, the majority of affected proteins were upregulated in EVs but downregulated in cells. Therefore, ethanol exposure results in increased loading of specific proteins into EVs, at the expense of their intracellular levels in NSCs. Ongoing studies are focused on understanding the biological effects of ethanol-exposed EVs on NSC populations.

Disclosures: M. Lai: None. **N. Collins:** None. **D. Chung:** None. **R. Miranda:** None. **S. Weintraub:** None.

Digital Abstract Session

P030. Mechanisms of Vulnerability

Program #/Poster #: P030.04

Topic: A.09. Adolescent Development

Support: NHS
AMT Grant F31AA028446
RCM Grant R01 AA024659

Title: Pathway enrichment analysis of the alcohol-sensitive proteins of fetal neural stem cell-derived extracellular vesicles

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Abstract: Prenatal alcohol exposure (PAE) can result in growth deficits, and is the leading cause of neurodevelopment disability worldwide. Neural stem cells (NSCs) are particularly vulnerable to alcohol (ethanol) exposure during the late first through the second trimester, when they are most extensively involved in neurogenesis. NSCs reside in a complex microenvironment rich in sub-200 nanometer-sized extracellular vesicles (EVs), which are shown to traffic protein, lipid, and RNA cargo between cells, and may serve as a mode of intercellular communication. We used cortical neuroepithelium sourced from fetal mice cultured ex-vivo as non-adherent neurosphere cultures and found a significant increase of miRNA cargo in EVs as a result of ethanol exposure. The NSCs are directed toward an aberrant astroglial lineage following the significant elevation of miRNA cargo such as miR-140-3p in EVs. EVs may amplify PAE's spatial and temporal effects across the NSC niche, to further inhibit their neurogenic capacity. Using quantitative proteomics across treatment groups of 18 EVs and the 18 parent NSCs to profile the protein expression, we further investigated the effect of ethanol exposure on the proteome of NSC-EVs. Our analyses show that NSC-derived EVs express ~86% of proteins needed for eukaryotic translation initiation, implying that EVs carry with them the ability to translate mRNAs in recipient cells. Statistical and pathway overrepresentation analyses showed that moderate ethanol, ~26 mM, resulted in a significant increase in proteins of the Nonsense-Mediated Decay (NMD) pathway in EVs, whereas a higher dose, ~70 mM, resulted in EV overexpression of mitochondrial proteins that constitute a Danger-Associated Molecular Pattern (mito-DAMP) pathway. NMD is an important surveillance pathway that reduces errors in gene expression by eliminating premature stop codon-containing mRNA transcripts. Consequently, NMD pathway proteins sequestered in EVs are hypothesized to transfer neuroprotection to cells where the capacity to correct errors in protein translation may be depleted. Eukaryotic cells under high stress, expel mitochondrial proteins as a 'danger' signal that activates 'pattern recognition' receptors and pro-inflammatory responses in target cells. Consequently, mito-DAMPs in EVs from heavy-ethanol-exposed NSCs are predicted to spread inflammation through the NSC microenvironment, compromising NSC growth and differentiation. Collectively, these studies identify EVs as a novel source for communicating stress responses, in an ethanol dose-related manner, to cells within the fetal neural stem cell niche.

Disclosures: E.A. Payne: None. **K. Le:** None. **M. Kim:** None. **D. Chung:** None. **S. Weintraub:** None. **R.C. Miranda:** None.

Digital Abstract Session

P030. Mechanisms of Vulnerability

Program #/Poster #: P030.05

Topic: A.09. Adolescent Development

Support: CIHR MOP-74709
NSERC 2982226
NIH RO1 DA037911
FRQS

Title: Genetic variation in corticolimbic DCC gene networks predict impulsivity in children

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Abstract: By the time humans reach adolescence, most of the neuroanatomical foundations are set in place. An important exception is the establishment of dopamine connectivity in the prefrontal cortex (PFC), which reaches full maturation in adulthood. Altered PFC dopamine connectivity and function is associated with impulsive phenotypes. Given the central role of the Netrin-1/DCC signaling pathway in the delayed development of the PFC dopamine circuitry, we investigated whether a polygenic score based on *DCC* gene co-expression networks in two main mesocorticolimbic dopamine terminal regions -the PFC and the nucleus accumbens (NAcc) - would associate with impulsivity-related phenotypes in community samples of children. We created a list of genes co-expressed with *DCC* in the PFC and NAcc and explored its enrichment for potential, and then compiled single nucleotide polymorphisms from these genes in a score using the SNP-gene expression association betas described in GTEx. We investigated the ability of this novel genetic score to predict impulsive phenotypes in 3 ethnically diverse cohorts. A lower polygenic score was associated with higher impulsive choice in 6-year old children tested in the Information Sampling Task (n=197). Lower polygenic scores were also associated with greater impulsive action in 6 (n=398) and 10 (n=3318) year old kids, as measured with the Stop Signal Reaction Time Task.This novel biologically informed genetic score, which aims to capture the activity of the *DCC* co-expression networks in two key regions for developing dopamine neurons, can predict elements of impulsivity in humans, suggesting a key role for these gene networks in the proper maturation of PFC dopamine connectivity

Disclosures: J. Restrepo: None. **I. Pokhvisneva:** None. **Z. Wang:** None. **M. Meaney:** None. **P. Silveira:** None. **C. Flores:** None.

Digital Abstract Session

P031. ADHD, SLI, Dyslexia, and Other Specific Disorders of Neurobehavior

Program #/Poster #: P031.01

Topic: A.07. Developmental Disorders

Support: Eunice Kennedy Shriver National Institute of Child Health and Human Development RO1 HD086011

Title: Synching the systems: an executive-functions based training is related to a greater synchronization between the somatomotor and auditory networks in dyslexia

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Abstract: Aim: Dyslexia is a specific reading disorder characterized by phonological, sensory-motor, orthographical, and executive function (EF) deficits. In a series of previous studies, we have demonstrated that a specific EF-based intervention was associated with decreased glutamate levels in EF regions (anterior cingulate cortex, ACC), greater activation within the dorsolateral prefrontal cortex and ACC, increased functional connectivity within and between EF networks, and in event-related potentials associated with error monitoring, all related to reading improvement in children with dyslexia. There we suggested that this intervention “synchs” the functional connections between networks supporting phonological/auditory processing, visual processing, somatomotor functions, and EF abilities. Here, we aim to examine this assumption across tasks and conditions in children with dyslexia. Methods: Thirty one 8-12 years-old children with dyslexia and 46 typical readers were recruited and trained on EF-based reading program for 8 weeks, 3 times a week. Reading ability was assessed pre/post intervention. Several functional MRI tasks were acquired pre/post intervention, including a reading task, a Stroop task, story listening, sentence comprehension tasks and a resting-state fMRI condition. The task-regressed connectivity data was subjected to an omnibus test to determine whether there were whole brain differences (object-oriented data analysis; OODA) using a 300 regions-of-interest-based atlas. Next, block-wise permutation tests were performed on the connectivity matrices to determine which network blocks were driving brain-wide differences. Finally, a regression of reading improvement with network connectivity changes was performed on a subset of significant within/between-network blocks. Results: Training was related to increased word reading accuracy and was associated with greater functional connectivity between the dorsal and lateral somatomotor and auditory networks in children with dyslexia. Better word reading was also related to a greater functional connectivity within the dorsal attention network in typical readers Conclusions: The results suggest that reading improvement following the EF-based reading intervention involves two distinct paths in children with dyslexia and typical readers: whereas in children with dyslexia there is a better synchronization between the networks related

to articulation/phonology (somatomotor networks) and auditory processing networks, typical readers show greater within network connectivity related to visual processing.

Disclosures: T. Horowitz-Kraus: None. **R. Farah:** None. **A. Dworetsky:** None. **R. Coalson:** None. **S. Petersen:** None. **B. Schlaggar:** None.

Digital Abstract Session

P031. ADHD, SLI, Dyslexia, and Other Specific Disorders of Neurobehavior

Program #/Poster #: P031.02

Topic: A.07. Developmental Disorders

Support: NSF
1640909

Title: Comparing age dependent statistical motor biomarkers in neurodevelopmental disorders

Authors: *J. V. JOSE;
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Abstract: We have studied the motor kinematic abilities of individuals with Neurodevelopmental Disorders (NDD). In particular, subjects with Autism Spectrum Disorder (ASD), Attention Hyperactive Deficit Disorder (ADHD) and comorbid ASD and ADHD (ASD+ADHD). We used high definition kinematic sensors to measure the kinematic angular variables of *velocity, acceleration and jerk,* of the arms rotations *as well the linear acceleration and jerk of upper extremity movements. T*he subjects moved their arm towards touch screen targets appearing in the monitor. These angular and linear variables show millisecond timescale fluctuations in the trajectory, away from naked eye detection. We carefully filtered out the electronic noise from the physiological statistical fluctuations in these kinematic variables. We analyzed the probability distribution in the physiological signals during reaching and arm return to its initial lap position in terms of their corresponding nearest-neighbor (NN) kinematic fluctuations in the variables studied. To extract the relevant NDD cognitive physiological information, we introduced a **new** Fano-Factor (FF) type biomarker to describe the NN probability distribution fluctuations. Presently, we have studied 8-ASD, 13-ASD+ASHD and 4-ADHD, subjects. To enhance the analysis of our present results we have implemented a machine learning *Support Vector Machine Learning* tool. This has allowed a separation in the cognitive conditions of their diagnoses for the NDD subjects studied *as a function of age*. As found in our previous study, using a different biomarker, we did not find age changes in the FF biomarker age in the new ASD subjects studied [1]. However, we did find that subjects with ASD+ADHD show age dependent changes, as well as in the small sample of ADHD subjects so far studied. This is consistent with clinical studies that record improvement in clinical ADHD severity and impairment with age. Our preliminary results provide a new biomarker that allow us to characterize the cognitive abilities of subjects with different neurodevelopmental disorders as

seen in their motor abilities.[1] Wu, D., José, J. V., Nurnberger, J. I., and Torres, E. B. Scientific Reports (Nature), 8(1):614, 2018.

Disclosures:

Digital Abstract Session

P031. ADHD, SLI, Dyslexia, and Other Specific Disorders of Neurobehavior

Program #/Poster #: P031.03

Topic: A.09. Adolescent Development

Support: NIH Grant MH62568
NIH Grant MH002954

Title: Motor activity declines across adolescence in humans and monkeys

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Abstract: Background There is emerging evidence for a substantial decline in motor activity (MA), a core feature of several human disorders, across adolescent development. However, elucidation of the mechanisms is complicated by contextual factors that constrain MA in human studies. The availability of accelerometry as an unobtrusive tool to obtain objective indices of MA in both humans and non-human primates provides an ideal opportunity for cross species research on MA without the social restrictions in human samples. The aims of this poster are: (1) to evaluate the contribution of age, sex, pubertal status and contextual factors on changes in MA in a human sample of adolescents; and (2) to examine biologic, genetic, and environmental factors associated with MA in non-human primates in a natural environmental context. **Methods:**Human: 1520 youth (mean age 10.2; range 5-21) from the Healthy Brain Network, a study of a broad range of behavioral, emotional, and cognitive assessments in youth in the New York area. Accelerometry was assessed for one week with the Actigraph (wGT3X-BT). Two features derived from accelerometry including Total Log Transformed Activity Count (TLAC) across 24 hours (TLAC), and Total Sedentary Time (TST).Monkeys: 145 monkeys (n=69 females; 76 males) from the Oregon National Primate Research Center assessed with a neck worn Actical device for 3-10 days at two time points (ages 23-30 mo- pre-pubertal) and (34-43 mo-peri/post pubertal). TLAC and TST were compared at pre-pubertal and post pubertal time points among male and female monkeys. **Results:**In the human sample, there was a monotonic decline in TLAC and an increase in TST in both male and female adolescents across ages 8-12. Analyses yielded significant effects for changes in both age and pubertal status, but pubertal status was more influential in males then females. Likewise, there was a significant decrease in TLAC and an increase in TST across the pre and post pubertal assessments in monkeys, with

females having higher TLAC and lower TST than males at both time points. **Discussion:**These findings demonstrate the importance of the influence of biologic factors involved in pubertal development on the decline in motor activity in adolescents. Use of the common phenotype and measure of motor activity facilitates our ability to pursue cross species studies of the genetic, environmental and developmental influences on motor activity. The adolescent decline in MA may provide etiologic insight for several human disorders that emerge at adolescence.

Disclosures: J.L. Cameron: None. **S. Kang:** None. **V. Zipunnikov:** None. **K. Merikangas:** None. **M. Milham:** None. **L. Alexander:** None. **M. Xiao:** None.

Digital Abstract Session

P031. ADHD, SLI, Dyslexia, and Other Specific Disorders of Neurobehavior

Program #/Poster #: P031.04

Topic: A.07. Developmental Disorders

Support: R21MH101609
L.I.F.E. foundation
University of Cincinnati Dean's Dissertation Completion Fellowship Award

Title: Comparison of constitutive Lphn3 knock-out and Lphn3-Th-Cre conditional knock-out Sprague-Dawley rats on activity, learning, and memory

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Abstract: Comparison of constitutive *Lphn3* knock-out and *Lphn3-Th-Cre* conditional knock-out Sprague-Dawley rats on activity, learning, and memory
Samantha L. Regan, Chiho Sugimoto, Michael T. Williams, and Charles V. Vorhees.*Department of Pediatrics, University of Cincinnati College of Medicine, and Division ofNeurology, Cincinnati Children's Research Foundation, Cincinnati, OH, 45229*
AbstractAttention deficit hyperactivity disorder (ADHD) is a heterogeneous neurodevelopmental disorder affecting ~10% of children in the United States. Latrophilin-3 (LPHN3) is a brain specific G-protein coupled receptor that is associated with increased risk of ADHD and treatment response. We characterized a global *Lphn3* knock-out (KO) rat usingCRISPR/Cas9 to delete exon-3 in rats. Global *Lphn3* KO rats are hyperactive and have egocentric and allocentric learning and memory (L&M) deficits. *Lphn3* KO rats also have altered striatal dopaminergic markers and hippocampal glutamatergic markers. To understand the dopaminergic contributions to the global *Lphn3* KO phenotype, we created a *floxed Lphn3* rat and then created an *Lphn3-TH-Cre* rat (cKO). Rats were tested in home-cage activity, egocentric L&M learning (Cincinnati water maze: CWM), and spatial L&M (Morris water maze (MWM)). *Lphn3* KO and cKO rats were hyperactive in the home-cage test. *Lphn3* KO and cKO rats both had longer latencies to escape in the CWM compared with controls, but the global KO rats were

worse. In the MWM, global KO rats but not cKO rats had reduced path efficiency on acquisition, reversal, and shift trials. LPHN3 is highly expressed in the striatum, and the results suggest a role of *Lphn3* in TH-positive neurons that project to the striatum and result in hyperactivity and egocentric learning and memory deficits.
(Supported by R21MH101609, the L.I.F.E. foundation and University of Cincinnati Dean's Dissertation Completion Fellowship Award).

Disclosures: S.L. Regan: None. **M.T. Williams:** None. **C. Sugimoto:** None. **C. Vorhees:** None.

Digital Abstract Session

P032. Synaptic and Cellular Mechanisms Related To Autism Risk

Program #/Poster #: P032.01

Topic: A.07. Developmental Disorders

Title: Neurodevelopmental risk factors ADNP and POGZ affect convergent transcriptomic and synaptic functions

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Abstract: ADNP and POGZ are two top-ranking high-confidence risk factors for autism spectrum disorder (ASD) and intellectual disability (ID), but how they are linked to these neurodevelopmental disorders (NDD) is largely unknown. Both ADNP and POGZ are chromatin remodelers, which could profoundly affect gene transcription and cellular function in the brain. Using postmortem tissue from patients with ASD, we found diminished protein levels of ADNP and POGZ in the prefrontal cortex (PFC), a region highly implicated in NDD (n=10 pairs). Thus, to understand the functional role of these important risk factors, we designed an exploratory study to investigate how Adnp or Pogz reduction in mouse PFC affects behavioral, transcriptomic, and synaptic function. We used viral-based gene transfer to knock down *Adnp* and *Pogz* in PFC of male and female wild-type mice. Control littermates received virus coupled to GFP only. Stereotaxic injection was done at postnatal week 4, the point of critical PFC synaptic regulation. Behavioral testing revealed impaired NDD-linked cognitive task performance with *Adnp*- or *Pogz*-deficiency (3 batches, n=12-20/group). No sex differences were observed. RNA-sequencing demonstrated transcriptional upregulation induced by *Adnp*- or *Pogz*-deficiency (n=3/group), with significant overlap in upregulated gene targets. Gene ontology and HUB gene analyses indicated that the most affected functional category was inflammation, representing 48% of the commonly upregulated genes, which show remarkable similarity to multiple human findings of increased pro-microglial signaling in NDD. Patch-clamp recording of PFC pyramidal neurons showed impaired glutamatergic signaling mediated by AMPA and NMDA receptors in *Adnp*- or *Pogz*-deficient mice (n=15-30 neurons/group). Taken together, these findings suggest that a reduction in ADNP or POGZ leads to the elevation of

inflammatory genes in the PFC, causing increased microglial activation and impaired synaptic transmission. This results in impairment of PFC-mediated cognitive behaviors. This study has provided novel insights into the convergent actions of two top risk factors for NDD and potential intervention targets.

Disclosures: M. Conrow-Graham: None. **Z. Yan:** None. **J. Williams:** None. **B. Rein:** None. **J.A. Martin:** None. **P. Zhong:** None.

Digital Abstract Session

P032. Synaptic and Cellular Mechanisms Related To Autism Risk

Program #/Poster #: P032.02

Topic: A.07. Developmental Disorders

Title: Developing a neuronal protein interaction network for neurodevelopmental disorder associated genes using proximity-based proteomics

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Abstract: With the rising prevalence of autism spectrum disorders (ASDs), its broad range of encompassed neurodevelopmental disorders (NDDs), and absence of robust treatments, novel techniques are necessary to study the shared pathologies of the highly heterogeneous disorder. Large-scale sequencing studies have identified ~100-200 ASD high-risk genes involved in gene regulation and neuronal communication; however interactions at the protein level are largely unknown in neurons. To identify the shared protein interaction networks (PINs) of these ASD genes we are using the proximity protein-labelling technique, BioID, which we have adapted for screening in primary neurons. Using primary mouse cortical neurons and a number (>50) of unstudied and functionally characterized ASD genes involved in neuronal communication and cellular signaling, many involved in synapse formation and function, we are creating a publicly accessible database for the PINs. Preliminary BioID of 17 ASD-risk genes, including DLG4 (PSD95) a scaffold protein in the excitatory post-synapse, have revealed individual protein interaction networks that possess shared interactors between multiple ASD-risk genes. For many of these genes novel interactions were identified in previously unconnected networks, for example the involvement of TAOK2 in protein translation regulation and cellular respiration. We validated these newly associated TAOK2 PINs, and showed that Taok2 KO mouse cortical neurons have altered protein translation and mitochondrial respiration, which may contribute to excitatory synaptic deficits in Taok2 KO mice. This database also incorporates the impact of patient non-truncating, de novo missense variants, from multiple databases, including SFARI and

MSSNG, on the individual and shared PINs of high-risk ASD genes to study disease pathophysiology. BioID of GRIA1 and two patient de novo missense mutations revealed significant changes in its interaction with synaptic scaffold proteins and AMPA-receptor associated proteins, suggesting a strong impact on AMPA-receptor function. Furthermore, BioID was used to probe abnormal PPIs in compartments of mutant neurons cultured from genetic models of ASD. This highlights the use of BioID as a potential screen for compartments and signaling proteins in different genetic models of ASD and potentially, in human patient iPSC-derived neurons. Identification of core shared ASD-linked PINs will create a large-scale resource to categorize multiple ASD gene subsets and elucidate their relationship with the various neuronal communication and signaling pathways.

Disclosures: **N. Murtaza:** None. **C. Brown:** None. **J. Uy:** None. **A. Cheng:** None. **B. Unda:** None. **N. Pipko:** None. **E. Deneault:** None. **K. Singh:** None. **B.W. Doble:** None. **J. Ellis:** None. **Y. Lu:** None. **S.W. Scherer:** None. **K.K. Singh:** None.

Digital Abstract Session

P032. Synaptic and Cellular Mechanisms Related To Autism Risk

Program #/Poster #: P032.03

Topic: A.07. Developmental Disorders

Support: Wellcome Trust 107687_Z_15_Z
Fondation Santé

Title: Mnk1/2 kinases regulate memory and autism-like behaviours.

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Abstract: Downstream of Erk (extracellular regulated kinase) and p38 (mitogen-activated protein kinase) MAPK pathways, MAPK interacting protein kinases (Mnks), exert a plethora of biological functions in response to external stimuli (e.g. mitogens). Mnk1 and Mnk2 are known to phosphorylate several targets among which the eukaryotic translation initiation factor 4E (eIF4E) on Ser 209. In most tissues and cell types examined, phopsho-eIF4E (Ser209) is considered the key effector of Mnks in the regulation of mRNA translation. Yet, in *Eif4e*^{Ser209Ala} mice, hippocampal CA1 LTP and hippocampus-dependent behaviours (e.g. spatial memory and fear memory) were intact, suggesting that Mnks may possess additional key downstream effectors, other than eIF4E. Herein, we demonstrate that *Mnk1/2* deletion (DKO) in mice impairs synaptic plasticity, hippocampal learning, and memory and engenders autism-like behaviours, in contrast to phenotypes previously shown in *Eif4e*^{Ser209Ala} mice. These phenotypic alterations are accompanied by altered mRNA-specific translation, which is pronounced in synaptic

compartments. We reveal that there is only slight overlap between the brain translatome of *Eif4e*^{Ser209Ala} and Mnk DKO mice, comprising chiefly mRNAs coding for genes of the Extracellular Matrix (ECM), suggesting that substrates other than eIF4E may dictate the effects of Mnks in regulating brain function. Quantitative mass spectrometric analysis in Mnk DKO mouse brain further revealed pervasive changes in the phosphoproteome of whole brain and synaptosomes, including new targets and known syndromic Autism Spectrum Disorders (ASD) risk genes and Fragile X Mental Retardation Protein (FMRP) targets. Together, these data establish a previously unknown link between Mnks memory and autism-related behaviours, which is amenable to pharmacological manipulation.

Disclosures: G. Lach: None. **K. Chalkiadaki:** None. **M. Hooshmandi:** None. **M. Jafarnejad:** None. **A. Khoutorsky:** None. **C.G. Gkogkas:** None.

Digital Abstract Session

P032. Synaptic and Cellular Mechanisms Related To Autism Risk

Program #/Poster #: P032.04

Topic: A.07. Developmental Disorders

Support: NIH Grant NS111965
Schlumberger Foundation Faculty for the Future Award

Title: Identifying Cellular Phenotypes of Idic(15) neurons Using CRISPR-edited iPSC-derived neurons

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Abstract: Dup15q syndrome is a neurodevelopmental disorder that results from duplications in the 11.2-13.1 region of the long arm of chromosome 15. These duplications primarily occur in two flavors: idic(15) in which a supernumerary isodicentric chromosome carries two extra copies of 15q11.2-13.1 and interstitial duplication in which a single extra copy of the region lies in tandem with the normal copy. Autism and epilepsy occur in the vast majority of Dup15q patients. In addition, affected children exhibit a range of intellectual disability and developmental delay. The syndrome only manifests when the duplication occurs on the maternally-inherited, not paternally-inherited allele. UBE3A, a maternally-expressed imprinted gene encoding an E3 ubiquitin ligase, is thought to play a critical role in Dup15q syndrome. To identify cellular phenotypes in Dup15q neurons, we performed patch clamp recordings from patient-derived human induced pluripotent stem cell (iPSC)-derived neurons harboring the idic(15) chromosome. We used CRISPR-Cas9 genome editing in these iPSCs to generate an isogenic line with the idic(15) chromosome completely removed to minimize the confounding effects of genetic variability between individuals. Compared to the corrected line, Dup15q neurons showed increased sodium current, larger action potential amplitude, and a higher firing frequency.

Changes in the sodium current and action potential amplitude appeared at an early point of in vitro development (8 wks), and persisted to 19 wks. Firing frequency was only significantly increased at 19 wks. Although UBE3A overexpression has been used to model Dup15q syndrome in mice, the contribution of this gene to the cellular phenotypes in human neurons is unknown. To determine if UBE3A overexpression is necessary, we knocked it down to control levels in Dup15q neurons using antisense oligonucleotides (ASO). This prevented the increased sodium current, action potential amplitude and firing frequency when UBE3A was knocked down early (at 6 wks), indicating that UBE3A overexpression is required for the development of these cellular phenotypes. In contrast, a late knockdown (at 16 wks) was unable to reverse these phenotypes. Activating the silent copy of UBE3A in neurotypical cells will lead to UBE3A overexpression. This will determine if UBE3A overexpression alone is sufficient for the development of Dup15q phenotypes. Elucidating the role of UBE3A in the electrophysiological phenotypes of Dup15q neurons may shed light on the functions of this ubiquitin ligase in neurons. Overall, these studies may help narrow down the most impactful therapeutic targets for treating Dup15q.

Disclosures: **M. Elamin:** None. **T.M. Robinson:** None. **A. Dumarchey:** None. **C. Stoddard:** None. **S.J. Chamberlain:** None. **E.S. Levine:** None.

Digital Abstract Session

P032. Synaptic and Cellular Mechanisms Related To Autism Risk

Program #/Poster #: P032.05

Topic: A.07. Developmental Disorders

Support: NIMH Grant MH103398
NINDS Intramural Research Program
Simons foundation
McKnight Foundation

Title: Neuroligin1-dependent synaptic signaling is mediated through an interaction with the GEF protein Kalirin-7

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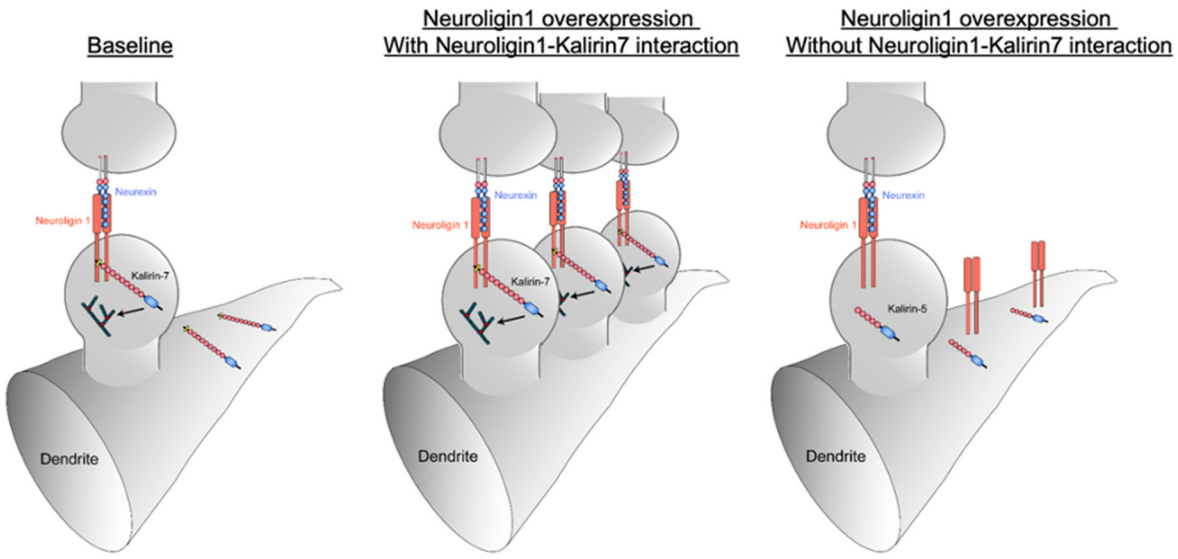
Abstract: Neuroligin1 (NLGN1) is a profoundly synaptogenic cell adhesion molecule that is localized at the postsynaptic side of glutamatergic synapses. However, the mechanism by which NLGN1 promotes the formation of glutamatergic synapses is unknown. We performed an unbiased proteomic analysis of the RhoGEF protein Kalirin and identified a significant interaction between Kalirin-7 and NLGN1. This interaction was validated both in vitro and in vivo. Kalirin mediates the assembly of actin filaments and is essential for synaptic transmission. To determine whether NLGN1-induced enhancement in synapse function is mediated through Kalirin-7, we performed whole-cell patch clamp experiments in cultured hippocampal slices. We

find that increasing postsynaptic NLGN1 expression in CA1 pyramidal neurons promotes glutamatergic synaptogenesis, while eliminating the interaction between the NLGN1 and Kalirin-7 prevents NLGN1's influence on glutamatergic synaptic transmission. Together, our data reveal an intracellular effector of NLGN1 function that is required for NLGN1-mediated synaptogenesis and provide a direct link between NLGN1 and synaptic actin remodeling.



Disclosures: **C. Tian:** None. **J. Paskus:** None. **K. Roche:** None. **B. Herring:** None.

Digital Abstract Session

P032. Synaptic and Cellular Mechanisms Related To Autism Risk

Program #/Poster #: P032.06

Topic: A.07. Developmental Disorders

Title: Understanding The Role of SYNGAP1 in GABAergic Circuit Development and Function

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Abstract: Haploinsufficiency of Syngap1 gene encoding the Synaptic Ras-GTPase Activating protein is associated with intellectual disability, autism spectrum disorder and epilepsy. Syngap1 is a negative regulator of Ras and of AMPA receptor trafficking to the postsynaptic membrane, involved in regulation of synaptic plasticity and neuronal homeostasis. Mouse models of Syngap1 haploinsufficiency show alterations in synaptic plasticity, behavioral abnormalities and cognitive deficits. In particular, several studies have shown that Syngap1 regulates the time course of dendritic spine maturation in excitatory neurons; in contrast, the role of Syngap1 in inhibitory, GABAergic neurons is relatively uncharted. GABAergic neurons are a diverse class

of neurons with different morphology, connectivity and physiological properties. They play an important role in neural circuit development and plasticity. Parvalbumin (PV)-expressing interneurons, one of the major classes of cortical GABAergic interneurons, form synapses onto the soma and proximal dendrites of pyramidal cells and are involved in the synchronization of the firing rate of pyramidal cell populations. Previous in-vitro studies suggest that haploinsufficiency of Syngap1 affects the formation of PV cell synaptic connectivity with reduced inhibitory synaptic activity. In continuation with these previous observations, here we study the role of Syngap1 in PV cell development, in establishing balanced synaptic connectivity and proper network. To this end, we used both germline heterozygous Syngap1 mice, and Nkx2.1Cre dependent Syngap1 conditional knockout mice, to characterize both the excitatory (VGlut1+PSD95+) and inhibitory (PV+ Geph+) inputs to PV+ and non-PV+ cells. We further characterized cognitive behavior such as attention set shifting tasks, novel object recognition and social behavior in these mice. Preliminary data suggest that alteration in synaptic connectivity of PV cells in different cortical regions do contribute to observed overall behavioral and cognitive deficits. A better understanding of the role of Syngap1 in PV cell development and function is necessary to elucidate the cognitive deficits caused by Syngap1 haplosinsufficiency.

Disclosures: V.V. Jadhav: None. **M. Carreno-Munoz:** None. **T. Badra:** None. **G. Di Cristo:** None. **B. Chattopadhyaya:** None. **J. Michaud:** None.

Digital Abstract Session

P032. Synaptic and Cellular Mechanisms Related To Autism Risk

Program #/Poster #: P032.07

Topic: A.07. Developmental Disorders

Support: NIH/NINDS R01 NS051710-11A1

Title: High-resolution identification of engrailed-2 transcript in the ganglionic eminence of the developing mammalian brain

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Abstract: Autism spectrum disorder (ASD), currently estimated to affect 1 in 54 people in the United States, is a neurodevelopmental condition associated with deficits in social interaction and communication, restricted and repetitive behaviors, and aberrant sensory processing. While genetic and environmental factors contributing to ASD vary widely, developmental dysfunction of the γ-aminobutyric acid (GABA) system of the brain has been implicated to underlie ASD. The midbrain-hindbrain patterning homeotic transcription factor, engrailed-2 (En2), is associated with ASD in humans, and a mouse model harboring a null mutation recapitulates behavior and

neuroanatomical abnormalities reminiscent of ASD as well as epilepsy. Interestingly, studies have revealed that En2-null mice show reduced GABAergic marker expression in the hippocampus and cortex, and neural stem cells of GABAergic lineage cultured from these mice display reduced neuron differentiation. The overarching goal of this project is to uncover the role of En2 and its associated signaling networks that drive proper GABAergic circuit development. Our recent *in vivo* work draws attention to the potential role of En2 in radial glial self-renewal and subsequent differentiation, with an emphasis on the birth of GABAergic interneurons. Here, we investigate for the first time the spatial expression of *En2* mRNA at the ganglionic eminences (GE), the major birthplace of GABAergic interneurons, in wild-type E13 mouse embryos. Considering this spatial pattern of *En2* in the GE is completely unknown, we use sensitive RNAscope *in situ* hybridization with widefield and confocal fluorescence microscopy to characterize the distribution of *En2*, guided by tissue co-labeling of GABAergic lineage markers including *Gsx2* and *Dlx2* mRNA. We find that *En2* mRNA is regionally widespread, but sparse in its density across all layers of the major subregions (medial, lateral, caudal) of the GE. In analyzing individual cells of selected radial columns at the lateral GE, we observe *En2* puncta counts consistent with roughly 1-3 transcripts per cell, with ~70% of surveyed cells expressing *En2*. Analysis of *En2* at other GE regions is ongoing, including lineage marker co-expression that may suggest cell-type specific functions. Taken together with results of En2 knockdown in GE via mouse electroporation, widespread but sparse *En2* mRNA presence suggests it may play a role in developmentally important cells of the region, including radial glia progenitors. Our findings raise questions as to the function of En2 in other cell types along the GABAergic maturation trajectory and also whether this has a role in ASD and epilepsy comorbidity.

Disclosures: J.S. Martinez-Fuentes: None. **M. Cogswell:** None. **S.J. Russek:** None.

Digital Abstract Session

P032. Synaptic and Cellular Mechanisms Related To Autism Risk

Program #/Poster #: P032.08

Topic: A.07. Developmental Disorders

Support: KAKENHI 19H05201
KAKENHI 19K08065

Title: Il-17a affects activity and localization of microglia in the embryo cerebral cortex

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Abstract: <META NAME="author" CONTENT="佐々木哲也">Viral infection during pregnancy has been suggested to increase the probability of autism spectrum disorder (ASD) in offspring via the phenomenon of maternal immune activation (MIA). This has been modeled in rodents. Maternal T helper 17 cells and the effector cytokine, interleukin 17A (IL-17A), play a central role in MIA-induced behavioral abnormalities and cortical dysgenesis, termed cortical

patch. However, it is unclear how IL-17A acts on fetal brain cells to cause ASD pathologies. To assess the effect of IL-17A on cortical development, we directly administered IL-17A into the lateral ventricles of the fetal mouse brain. We analyzed injected brains focusing on microglia, which express IL-17A receptors. We found that IL-17A activated microglia and altered their localization in the cerebral cortex. Our data indicate that IL-17A activates cortical microglia, which leads to a cascade of ASD-related brain pathologies, including excessive phagocytosis of neural progenitor cells in the ventricular zone.

Disclosures: T. Sasaki: None. **Y. Taklei:** None.

Digital Abstract Session

P033. Neurodevelopment in Health and Disease: Molecular Mechanisms

Program #/Poster #: P033.01

Topic: A.07. Developmental Disorders

Support: National Institutes of Health (3R35NS097305) (ARK)
NARSAD Young Investigator Award 2018 (27729) (LS)
CURE Taking Flight Award 2018 (P0530360) (LS)

Title: Neurodevelopmental origins of cell types in human epileptic cortical malformations

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Abstract: Mammalian Target of Rapamycin (mTOR) mediated epileptic cortical malformations like Focal Cortical Dysplasia (FCD), Hemimegalencephaly (HME) and Tuberous Sclerosis (TSC) arise due to somatic mutations in the mTOR signaling pathway. Each of these malformations have distinct neuroanatomical and etiologic features, that make it hard to examine the relationship between them. In order to understand the developmental origin and shared molecular features of these mTOR-linked epilepsies, we used single nucleus sequencing strategies in donated patient surgical specimens to characterize the transcriptomic identity, and molecular features of all cell types present within the malformations. We used de-identified tissue from surgical resections, collected with previous patient consent for research in strict observance of legal & institutional ethical regulations. Following resections, brain regions with cortical lesions & epileptogenic foci were flash-frozen & sectioned to preserve cellular architecture. Individual nuclei were isolated from the sections & barcoded according to standard protocols, followed by reverse-transcription, amplification & sequencing to generate single-nucleus expression profiles. We isolated and characterized >30000 cells from patients with cortical malformations (n = 8 FCD, 3 TSC and 2 HME patients). Using unbiased clustering approaches, we generated molecularly distinct transcriptomic clusters of cells, corresponding to identifiable cell types. We identified transcriptomically unique cell types in FCDIIB, HME and

TSC lesions that corresponded to dysplastic balloon cells and bizarre neurons. We established that these cells have a shared molecular identity irrespective of malformation type, co-expressing markers associated with progenitor cells and immature neurons, indicating a shared developmental origin. Using immunofluorescence staining, we validated our findings with marker co-expression in tissue sections. Using gene ontology analyses, we identified shared molecular pathways active in each cell type across malformations. We identified key developmental signatures, normally present in progenitor cells, within the populations of balloon cells and bizarre neurons present in FCD, HME and TSC lesions. Our findings highlight the shared neurodevelopmental origins of these malformations, along disease-relevant changes in all cell types in epileptic regions. The outcome of this study is a thorough transcriptomic characterization of all cell types in mTOR-linked epileptic cortical malformations, that will be an important community resource.

Disclosures: **L. Subramanian:** None. **A. Bhaduri:** None. **D.D. Wang:** None. **K. Auguste:** None. **E. Chang:** None. **M. Paredes:** None. **A.R. Kriegstein:** None.

Digital Abstract Session

P033. Neurodevelopment in Health and Disease: Molecular Mechanisms

Program #/Poster #: P033.02

Topic: A.07. Developmental Disorders

Support: MH116575
T32GM119999
GM054712

Title: Schizophrenia-linked protein tSNARE1 regulates endolysosomal trafficking in cortical neurons.

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Abstract: Schizophrenia is a severe and heritable neuropsychiatric disorder; the etiology of which is the result of genetic variation in many genes. Genome-wide association studies (GWAS) identified 145 genome wide significant (GWS) loci, implicating more than 300 candidate genes as potential risk factors for schizophrenia. However, the impact of these genes in schizophrenia pathogenesis has not been studied. The next critical step is to investigate the function of these genes and how they might be dysregulated in schizophrenia. *TSNARE1*, which encodes the protein tSNARE1, is one of the high confidence candidate genes, but the cellular or physiological function of tSNARE1 is presently unknown. Here we define the major gene products of *TSNARE1* in the human brain and their cytoplasmic localization and function in the endolysosomal system of cortical neurons. We identified four isoforms of tSNARE1 in human brain, all of which contain a syntaxin-like Qa SNARE domain. SNARE proteins are involved in

the fusion of opposing lipid bilayers, and the transmembrane domain of the Qa SNARE is thought to be critical for membrane fusion. However, RNA-sequencing data from adult and fetal human brain suggest that the most abundant isoforms of tSNARE1 lack a transmembrane domain. This suggests an exciting hypothesis in which brain tSNARE1 acts as an inhibitory SNARE (i-SNARE) to negatively regulate membrane trafficking events. We find that brain tSNARE1 isoforms localize to compartments of the endolysosomal network. The non-transmembrane domain containing isoforms, including the most abundant isoform tSNARE1c, most frequently populate compartments of the late endosome. Live-cell three-color trafficking assays suggest that tSNARE1 modulates trafficking to the late endosome and the lysosome in the developing neuron. Determining how tSNARE1 regulates trafficking is critical towards understanding how its dysfunction contributes to schizophrenia pathogenesis.

Disclosures: **M. Plooster:** None. **G. Rossi:** None. **M.S. Farrell:** None. **P.F. Sullivan:** None. **H. Won:** None. **S.L. Gupton:** None. **P. Brennwald:** None.

Digital Abstract Session

P033. Neurodevelopment in Health and Disease: Molecular Mechanisms

Program #/Poster #: P033.03

Topic: A.07. Developmental Disorders

Support: NIEHS ES028202
NICHD HD097093
NIMH MH104184
MH108286
DK 121734
MH 109298

Title: Compounding maternal risk factors exacerbate neurodevelopmental and immune dysfunction and lasting health outcomes

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Abstract: Profound disparities exist in maternal-child health outcomes between racial and ethnic groups. Black women in the United States are significantly more likely to experience preterm birth, fetal growth restriction, and maternal-infant morbidity and mortality than white women. Thus, to examine the hypothesis that neonatal morbidity and lasting health outcomes are the result of multifaceted compounding of insults to the fetus, we established a mouse novel model that takes into account multiple compounding maternal risk-factors on offspring outcomes. After establishing key functional attributes of the human maternal microbiota on lasting outcomes in offspring, we next determined whether a combination of maternal consumption of a high-fat low-fiber diet and a history of bacterial infection during pregnancy influenced fetal development,

subsequent response to postnatal microbial colonization, and lasting health outcomes. Administration of a high fat - low fiber (HFt-LFb) diet resulted in significant differences in pre-pregnancy body weight gain, glucose intolerance, gut microbiota composition relative to females consuming a low fat - high fiber (LFt-HFb) diet, paralleling endophenotypes of pregestational obesity and diabetes in humans. Further, the disruptive effects of the HFt-LFb diet on the maternal gut microbiota persisted across pregnancy, resulted in a marked loss of taxa involved in the production of metabolites essential for offspring development. Surprisingly, the combinatorial effects of maternal diet, infection and the microbiome exhibit a significant impact on the survival of newborn mice, an observation that is consistent with clinical reports on infant mortality. Indeed, triple hit offspring showed a rate of 60% mortality relative to the 100% survival among LFt-HFb offspring. Of the triple hit offspring that survived, single-cell mass cytometry showed a significant expansion of neutrophils in the periphery, with neutrophils accounting for almost 80% of all immune cell in circulation. Tracking of offspring body weight revealed significant acceleration in the rate of body weight gain and increased body weight in the triple hit offspring that survived. Through this mouse model and application of known maternal risk factors, including human cervicovaginal microbiota, we may come closer to estimating combined risks for neurodevelopmental outcomes and to identifying causal mechanisms that may yield novel therapies and biomarkers to be further examined in clinical trials.

Disclosures:

Digital Abstract Session

P033. Neurodevelopment in Health and Disease: Molecular Mechanisms

Program #/Poster #: P033.04

Topic: A.07. Developmental Disorders

Support: RO1

Title: Small molecule and genetic strategies can activate the proteasome in neonatal piglet brain for therapeutic targeting after brain injury

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Abstract: Proteasomes degrade damaged proteins. We show proteasome loss of function in forebrain white matter of neonatal piglets with brain injury from hypoxia-ischemia (HI). The proteasome could be a therapeutic target for white matter protection. Amenability of proteasomes to in vivo manipulation is unknown. We hypothesized that the proteasome in neonatal piglet cortex and white matter can be activated by two small molecules (oleuropein [OLE] and the phenothiazine chlorpromazine [CPZ]) and by virus-mediated gene targeting2-3 day old piglets. OLE sham hypothermia: piglets received general anesthesia with overnight

hypothermia and rewarming. Piglets received OLE or vehicle (IV). Brains were harvested at 29 h (n=6). .OLE after HI received HI, OLE in at 15 min after HI, and emerged from anesthesia 3 h after HI. The brain was harvested at 4 days (n=6).3.CPZ after sham piglets received general anesthesia and CPZ 1, 5, or 10 mg/kg or saline. They emerged from anesthesia after 3 h, and brains were harvested 24 h later. .Genetic targeting with adeno-associated virus (AAV): piglets received stereotaxic microinjections of (AAV-PSME3 [PA28γ]-green fluorescent protein [GFP], AAV-PSME3-short hairpin RNA-GFP, or AAV-eGFP) with to enforce or knockdown the proteasome, or for control (n=2-3 piglets/group). Injections were into white matter and cortical gray matter.Two days later, piglets were for HI. The piglets then received overnight hypothermia, rewarming, and OLE every other hour or vehicle (IV). Brains were harvested at 29 h. subcortical white matter and cerebral cortex were assayed for proteasome activity and proteasome subunits. Values were normalized to GFP in AAV-injected brain. Naïve had higher proteasome activity in white and gray matter than shams, and gray matter had higher ($p= 0.002$) activity than white matter. Alpha-3, beta-5, and PA28γ proteasome subunits were detected in white and gray matter. In sham, proteasome activity was higher in gray matter of OLE-treated piglets compared to vehicle ($p= 0.027$), but OLE had no proteasome agonist activity in white matter., OLE did not activate brain proteasomes after HI and CPZ did not activate proteasomes in shams. Genetic enforcement of PSME3 upregulated proteasome activity in gray matter but not white matter compared to control. PSME3 knockdown inconsistently affected proteasome activity. Proteasome were not altered by AAV. Brain proteasome activity is diminished by anesthesia and hypothermia. OLE activated the brain proteasome in sham but not HI piglets. Brain proteasome activity can be enforced genetically. We conclude that the proteasome can be manipulated in vivo for potential neural protection.

Disclosures: N.M. EL Demerdash: None. **C. O'Brien:** None. **M. W. chen:** None. **S. Adams:** None. **E. KuliKowicz:** None. **L. Martin:** None. **J. Lee:** None.

Digital Abstract Session

P033. Neurodevelopment in Health and Disease: Molecular Mechanisms

Program #/Poster #: P033.05

Topic: A.07. Developmental Disorders

Support: NIH Grant MH0979491
Autism Speaks Grant 11857
NIH 1R01NS110945-01

Title: Disruption of mTor complexes rescues Pten KO-mediated hypertrophy in neurons

Authors: *K. TARIQ[1], W. WANG[1], M. LI[1], M. C. WESTON[2], B. LUIKART[1];
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Abstract: Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a lipid phosphatase that negatively regulates AKT/mTOR signaling pathway and serves as a brake on

unregulated cellular growth. In a non-dividing neuronal cell population, PTEN inactivation leads to hypertrophy, and such neuropathology has been observed in individuals with Autism Spectrum Disorder (ASD). In mouse models of autism, conditional Pten knockout (KO) in hippocampus leads to larger soma size, dendritic hypertrophy, ectopic neuronal migration, and increased spine densities. The underlying molecular mechanisms producing such alterations in neuronal architecture remain incompletely understood. Using well-defined mouse genetic alleles and retroviral systems to specifically target developing neurons, we aimed to determine the individual roles of two mTOR complexes in the development of Pten-KO-mediated autism-associated hypertrophy. mTOR kinase regulates cell growth, proliferation and survival by operating in two multi-protein complexes, mTORC1 and mTORC2. We knocked out Raptor or Rictor, which are unique and obligatory components of mTORC1 and mTORC2 complexes, respectively. We found that disruption of mTORC1 completely rescued Pten-KO-mediated effects on soma size, migration, and dendritic arborization, while partially rescuing increases in spine density. Disruption of mTORC2 generated a partial rescued Pten-KO mediated increase in soma size, migration, spine density and dendritic arborization. The differential impact of mTORC1 and mTORC2 deletion suggests specific mechanistic pathways underlying autism-associated hypertrophy and identifies Raptor and Rictor as potential therapeutic targets for the treatment of ASD.

Disclosures: K. Tariq: None. **W. Wang:** None. **M. Li:** None. **B. Luikart:** None. **M.C. Weston:** None.

Digital Abstract Session

P033. Neurodevelopment in Health and Disease: Molecular Mechanisms

Program #/Poster #: P033.06

Topic: A.07. Developmental Disorders

Title: Caspase-1 inflammasome activation mediates obesity development in NIBP knockout mice

Authors: B. BODNAR, H. SHAN, M. XIN, F. LI, Y. ZHU, Y. LIN, H. WANG, ***W. HU**; Temple Univ. Lewis Katz Sch. of Med., Philadelphia, PA

Abstract: NIBP/TRAPPC9 is a prototypical member of a novel protein family that regulates NFκB signaling and *trans*-Golgi networking. Autosomal recessive mutations of human *NIBP* lead to a novel neurodevelopmental disorder NIBP Syndrome, characterized by intellectual disability and obesity. Previous data from our lab demonstrated that global Nibp knockout mice (*Nibp*$^{-/-}$) spontaneously develop hyperphagia, obesity, and impaired glucose/insulin tolerance, even under normal chow diet. A similar phenotype occurred in hypothalamus-specific *Nibp*$^{-/-}$ mice via stereostatic injection of AAV-Cre into the arcuate nucleus (ARC) of floxed *Nibp* transgenic mice. To elucidate the molecular mechanisms underlying NIBP-deficient obesity, we explored the role of hypothalamic inflammasome activation and microinflammation. RT^2 profiler PCR arrays targeting NFκB signaling and cytokines/chemokines did not identify robust changes

in the mRNA levels of these select genes in the hypothalamus of *Nibp*$^{-/-}$ mice, supporting the existence of hypothalamic microinflammation in NIBP-deficient obesity. IL-1β production is controlled by the activation of Caspase-1 (Casp1) via an inflammasome complex consisting of NLRP3, Asc, and pro-Caspase-1. In particular, the NLRP3 inflammasome is known to contribute to obesity. However, the role of Casp1-inflammasomes in regulating hypothalamic microinflammation and obesity remains elusive. RT-qPCR analysis validated a moderate but significant increase in the mRNA expression of IL-1α (via Casp11) and IL-1β (via Casp1) in the hypothalamus of *Nibp*$^{-/-}$ mice with females showing a more dramatic increase. Interestingly, Casp1 and Asc, the key components of NLRP3 inflammasome, showed significant increase in both male and female *Nibp*$^{-/-}$ mice but mRNA expression of *NLRP3* and *NLRP1* did not change significantly. Further studies using *Nibp*$^{-/-}$/*Casp1*$^{-/-}$ double knockout mice showed that *Casp1* knockout helps ameliorate the weight gain due to *Nibp* knockout, while having no significant impact on the weight of wildtype *Nibp*$^{+/+}$ mice under normal control diet. A high fat diet further aggravated weight gain in *Nibp*$^{-/-}$ mice compared to wildtype mice. In conclusion, this study suggests that (1) Nibp deficiency promotes inflammasome pathways in the hypothalamus and (2) that Casp1 may help mediate obesity development in *Nibp*$^{-/-}$ mice. These findings highlight the importance of Casp1 in obesity development and suggest possible involvement for NIBP in regulating microinflammation in the hypothalamus.

Disclosures: B. Bodnar: None. **H. Shan:** None. **M. Xin:** None. **F. Li:** None. **Y. Zhu:** None. **Y. Lin:** None. **H. Wang:** None. **W. Hu:** None.

Digital Abstract Session

P033. Neurodevelopment in Health and Disease: Molecular Mechanisms

Program #/Poster #: P033.07

Topic: A.07. Developmental Disorders

Title: Bisphenol-a suppresses neuronal chloride exporter (kcc2) expression during early brain development

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Abstract: Bisphenol A (BPA) is a synthetic endocrine-disrupting chemical that can mimic the effects of estrogens. Its widespread presence in the environment has prompted concern that accidental ingestion may disrupt estrogen's critical biological functions, including in early brain development. Animal models have demonstrated that perinatal BPA exposure can affect brain structure, function, and behavior, both acutely and long-term. One mechanism through which BPA could alter neural development and behavior is by modulating the expression of the neuronal chloride exporter, KCC2. The ion transport action of KCC2 maintains low intracellular chloride concentration in neurons, ensuring that GABA transmission activates inhibitory, inward

chloride currents. KCC2 is therefore a critical regulator of neural excitability. Several hormonal factors have been shown to influence KCC2 expression, including sex, treatment with estradiol or testosterone, and ovariectomy, and BPA itself has been shown to modulate KCC2 expression in rodents and cultured human neurons. The current study utilized a novel zebrafish transgenic reporter line, KCC2:mCitrine, to track BPA effects on KCC2 expression in vivo during early brain development. We used CRISPR-mediated transgenesis in zebrafish zygotes to insert a self-cleaving mCitrine sequence before the first exon of SLC12a5b, an ortholog to the human KCC2 gene. KCC2:mCitrine zebrafish show neuron-specific fluorescence from the time of neurogenesis onset and within all nervous system structures as they develop. We first replicated evidence of BPA's estrogenic effects in developing zebrafish by showing that BPA upregulates aromatase B expression in embryonic radial glial cells and causes behavioral hyperactivity at later larval stages. The same BPA treatment also downregulated KCC2 expression as measured by neural fluorescence in KCC2:mCitrine larvae. BPA-induced downregulation of KCC2 could cause GABA to produce paradoxical excitatory effects on neurons with long-term consequences for brain development and behavior. Our KCC2:mCitrine zebrafish provide a new way to track BPA's effects on KCC2 expression and could be used to screen other compounds with a potential to modulate KCC2 expression during early developmental.

Disclosures: E.B. Gahtan: None. **M. Butler:** None. **E.F. Jones:** None. **J.S. Steele:** None. **R.J. McAuley:** None.

Digital Abstract Session

P034. Development of Motor Systems

Program #/Poster #: P034.01

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Title: Performance in the Open Field Test of adult offspring from CD-1 mice fed with a Western Diet during pregnancy and lactation.

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Abstract: Open field test (OFT) has been used to measure the spontaneous motor activity and exploratory behavior in rodents. It has shown changes in the control and mobility in aging mice. In addition to this, it has been observed that disturbances during embryonic development can alter the senescence process, precipitating the deterioration of mobility. One of the teratogenic factors during pregnancy that has become important in recent years is Western diet (WD), characterized as the set of processed foods, which are high in calories, fat and sugar, low in fiber, but pleasant in taste and texture. It has been well explained his relation with cognitive alterations but there are small evidence of long-term effects on mobility. The aim of the study was to evaluate the performance in the OFT of adult offspring from CD-1 mice fed with a WD during pregnancy and lactation. Thirty-six mice were used, 18 from mothers fed a WD (19.8% fat;

16.4% protein; 46.7% carbohydrates; 4.30 (kcal/g)^2), and 18 from mothers fed a control diet (3% fat; 23% protein; 49% carbohydrates; 3.15 (kcal/g)^2) during pregnancy and lactation. At weaning, the offspring of both groups have access to a control diet and water at libitum. Weight and height at birth were registered. At postnatal day (PD) 90, 180 and 360, offspring were evaluated in the OFT. Data were analyzed with Software SPSS. Experimental group were significantly heavier ($P < .0001$) and taller ($P = .019$) than control group at birth. Not differences in distance moved or speed at 90 and 180 were found. At PD 360 control group have a significant greater distance moved ($P = .046$) and were speeder ($P = .046$) than experimental group. In conclusion, the consumption of a western diet during pregnancy and lactation causes an increase in weight and height at birth. And it can have long-term effects on weight, spontaneous motor activity and exploratory behavior in rodents.

Disclosures: R. Pedraza-Medina: None. **N.A. Moy-López:** None. **J. Guzmán-Muñiz:** None. **M.F. Pinto-González:** None. **M. Ortiz:** None.

Digital Abstract Session

P034. Development of Motor Systems

Program #/Poster #: P034.02

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: Grant #NIH NICHD 1R01HD093624
ULTR002649

Title: Exploring neuroplastic changes through diffusion tensor imagining in response to physical therapy intervention in very preterm infants: a case series

Authors: *M. EVANS[1], S. KHURANA[1], M. S. SHALL[1], D. THOMPSON[3], C. E. KELLY[3], D. BESSOM[4], A. HARPER[2], G. VORONA[4], S. C. DUSING[5];
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Abstract: Very preterm infants (<28 weeks gestation) are at high-risk for neurological dysfunction, with 50% of infants experiencing various developmental delays. The Early Intervention Program was created to match children and their families with the support services they need to enhance motor and cognitive development in young children. Despite new advances in neuroimaging, limited research is available on the neuroplastic changes in response to early intervention. Thus the primary purpose of this study was to begin to explore and quantify neuroplastic changes in response to a parent delivered physical therapy intervention, such as Supporting Play Exploration and Early Development Intervention (SPEEDI).Three very preterm infants were chosen from the SPEEDI_Brain_Behavior cohort based on their randomized group assignment, and completion of at least three study visits in a larger study (ClinincalTrials.gov

Identifier: NCT03518736). Intervention was provided as early, (starting before NICU discharge) or late, (starting at 16 weeks post baseline), or usual care. MRIs were done without sedation in a 3T pediatric dedicated scanner within 72 hours after NICU discharge, 3 months post baseline, and 6 months post baseline. Diffusion images were motion corrected, resampled to 1.5mm isotropic voxels, and brain extracted. The diffusion tensor model was fitted, and regions were parcellated using the Melbourne Children's Regional Infant Brain white matter atlas. Diffusion Tensor Imaging allowed for the quantification of neuroplastic changes in white matter tracts over time measured by Fractional Anisotropy (FA) and volumes of eight motor regions. Descriptive statistics were used to describe these cases.The left and right hemispheres were averaged together and the change over time (6-month values - baseline values) values were calculated for FA and volume. The area of most interest, the corticospinal tract (CST), displayed an increase in mean FA values, with the early group showing an increase of 0.063, the late group 0.113, and usual care 0.057. Additionally, there was an increase in the mean CST volumes with the early group reporting a volume increase of 299 mm^3 compared to the 162 mm^3 and 236mm^3 in the late and usual care groups, respectively. Initial evidence suggests that a parent delivered physical therapy intervention may lead to higher increase in FA and volume in the CST compared to usual care. However, the preliminary nature of this research limits external validity until a larger study is completed.

Disclosures: **M. Evans:** None. **S. Khurana:** None. **M.S. Shall:** None. **D. Thompson:** None. **C.E. Kelly:** None. **D. Bessom:** None. **A. Harper:** None. **G. Vorona:** None. **S.C. Dusing:** None.

Digital Abstract Session

P034. Development of Motor Systems

Program #/Poster #: P034.03

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant T32NS086750
NIH Grant R01DC017489

Title: Spatiotemporal Organization of a Central Vestibular Brainstem Nucleus in the Larval Zebrafish

Authors: *D. GOLDBLATT[1], M. GREANEY[2], B. ROSTI[2], V. VOLETI[3], C. PEREZ-CAMPOS[3], K. PATEL[3], W. LI[3], E. M. HILLMAN[3], D. SCHOPPIK[1];
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Abstract: Neural circuits must achieve precise wiring between distinct cell subtypes to produce appropriate behaviors. Identity specification and wiring precision in the circuit that stabilizes gaze is a particular case of this general developmental problem. Vertebrates stabilize gaze using excitatory vestibular interneurons that relay head and body destabilization to motoneurons that

counter-rotate the eyes. Precise connectivity between directionally-sensitive interneuron subtypes and particular motoneurons is therefore crucial for behavior. The developmental mechanisms that specify excitatory vestibular interneuron identity and connectivity are unresolved, though previous work has suggested a role for spatiotemporal cues and/or motoneuron-derived factors. We tested these hypotheses in the tangential nucleus (TAN) of the larval zebrafish, which contains the excitatory vestibular interneurons necessary for vertical gaze stabilization. We first developed an assay using 2-photon and swept, confocally-aligned planar excitation (SCAPE) microscopy to rapidly and reliably define functional nose-up and nose-down vestibular interneuron subtypes. We used this assay to ask whether functional subtypes are spatiotemporally organized and discovered functional organization complementary to spatiotemporal development. Our results are the first demonstration of functional sub-organization within a vestibular nucleus and reflect a putative role for spatiotemporal factors in identity specification. Next, to investigate the requirement of motoneuron-derived signals for TAN identity specification, we used these functional assays to measure changes in TAN identity following constitutive motoneuron knockouts. Our results demonstrate that TAN functional identity and organization is preserved in the absence of motoneurons, arguing against a role for motoneuron-derived cues. Taken together, we predict that spatiotemporally-linked factors organize TAN subtypes into dorsoventral and rostrocaudal microdomains. We propose that functional identity acquisition and organization occurs in a motoneuron-independent manner.

Disclosures: D. Goldblatt: None. **M. Greaney:** None. **B. Rosti:** None. **E.M. Hillman:** None. **V. Voleti:** None. **C. Perez-Campos:** None. **K. Patel:** None. **W. Li:** None. **D. Schoppik:** None.

Digital Abstract Session

P034. Development of Motor Systems

Program #/Poster #: P034.04

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: INFANTPATTERNS H2020-MSCA-IF-2018- 846679
Chalmers Life Science Engineering Area of Advance
MRC Clinician Scientist Fellowship [MR/P008712/1]

Title: Development of functional organization of the resting-state somatomotor network across the human perinatal period

Authors: *S. DALL'ORSO[1,2], T. ARICHI[2,3,4], S. P. FITZGIBBON[5], A. EDWARDS[2,3], E. BURDET[3], S. MUCELI[1,2];
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Abstract: In the mature brain, neural processing related to different parts of the body map to specific locations within the primary motor and somatosensory cortices. This topographical organization is reflected in patterns of functional connectivity (FC), where a given body part is most strongly connected to it's homologue in the opposite hemisphere [1]. Although a crude somatotopic organisation can be identified in the preterm human brain [2], it is unclear whether the underlying FC is similarly organised and what influences its establishment. To investigate the development of FC and its relationship with somatotopy across the perinatal period, we studied changes in inter-regional connectivity in a large group of infants. We hypothesized that maturation would be associated with an increase in inter-hemispheric FC as the brain establishes an adult-like bilateral network representation. We used high temporal resolution resting-state fMRI (TR=0.39s, voxel size 2.2x2.2x2.2 mm3, 15 minutes), acquired and pre-processed as part of the dHCP project (www.developingconnectome.org) [3]. We studied a group of 328 healthy neonates (median postmenstrual age (PMA) 40.7; range 31.4 - 44.9 weeks; 140 female) of which 59 were born and scanned preterm and the others 269 at term. We extracted the mean timeseries from 6 different regions of interest (ROI) identified from previous studies in neonates: 4 body parts (left/right wrist/ankle, localized in the contralateral hemisphere)[2] and 2 control areas (left/right primary visual cortex (V1))[4]. FC was then calculated using partial correlation between each pair of ROIs. A linear regression between partial correlation coefficients and PMA (correcting for postnatal days (pn) and pn-PMA interaction) was computed. The identified FC strength changed significantly ($p<0.01$ Bonferroni corrected) with the increase in PMA as follows: it increased between homologous regions (e.g. left/right wrist) and between adjacent cortical areas in the left hemisphere (right wrist/ankle), while it decreased between distal cortical areas (left wrist/right ankle). There was no effect of PMA on correlation strength between any of the body regions and control regions. We concluded that FC within the primary somatosensory and motor cortices is suborganized into specific patterns related to the somatotopy in the newborn brain. The rapid changes during the preterm period highlight the key importance of this stage for the establishment of the somatomotor resting state network.

References

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[3] Fitzgibbon et al, Neuroimage, 2020
[4] Eyre et al, BioRxiv, 2020

Disclosures: S. Dall'Orso: None. **T. Arichi:** None. **S.P. Fitzgibbon:** None. **A. Edwards:** None. **E. Burdet:** None. **S. Muceli:** None.

Digital Abstract Session

P034. Development of Motor Systems

Program #/Poster #: P034.05

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant F30HD096788
NIH Grant T32GM007250
NIH Grant R00NS085037
NIH Grant R01NS114510
Mt Sinai Foundation

Title: Phrenic-specific transcriptional programs shape respiratory motor output

Authors: *A. N. VAGNOZZI[1], K. GARG[1], C. DEWITZ[2], M. T. MOORE[1], J. M. CREGG[1], L. JEANNOTTE[3], N. ZAMPIERI[2], L. T. LANDMESSER[1], P. PHILIPPIDOU[1];
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Abstract: Breathing is the most essential motor behavior. The frequency and amplitude of breathing movements are controlled by neural networks residing in the brainstem and spinal cord. Degeneration of these networks leads to respiratory disorders, such as central sleep apneas, and, eventually, respiratory failure. In mammals, contraction of the diaphragm muscle is vital for driving airflow into the lungs during inspiration. Diaphragm contraction is controlled by a single motor input, phrenic motor neurons (MNs) in the cervical spinal cord, making this MN population a critical node in the respiratory network. Despite their essential role, the molecular determinants that control the patterns of phrenic MN activity and their integration into respiratory circuits are largely unknown. We show that Hox5 transcription factors are required for robust and efficient breathing. Hox5 loss renders mice vulnerable to respiratory dysfunction in the first two weeks of life, suggesting that Hox5 mutations may contribute to early life respiratory conditions, such as sudden infant death syndrome (SIDS). We show that Hox5 proteins establish phrenic MN clustering and topography through the regulation of a network of cell adhesion molecules. We find that a subset of cadherins are specifically expressed in phrenic MNs and that loss of cadherin function through conditional disruption of downstream β/γ-catenin signaling leads to phrenic MN cell body disorganization and dendrite displacement. Remarkably, after MN-specific deletion of Hox5 genes, phrenic MN firing becomes asynchronous and erratic due to the selective loss of inhibitory inputs to phrenic MNs. Our results demonstrate that Hox5 transcription factors determine phrenic MN topography and connectivity to generate robust breathing behaviors. Our findings support a model where MN-intrinsic transcriptional programs shape the pattern of motor output by orchestrating distinct aspects of MN connectivity.

Disclosures: **A.N. Vagnozzi:** None. **K. Garg:** None. **C. Dewitz:** None. **M.T. Moore:** None. **J.M. Cregg:** None. **L. Jeannotte:** None. **N. Zampieri:** None. **L.T. Landmesser:** None. **P. Philippidou:** None.

Digital Abstract Session

P035. Development of Sensory Systems

Program #/Poster #: P035.01

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: P20-GM-121310-01

Title: A ventral visual pathway in the Xenopus laevis tadpole

Authors: U. G. UDOH, ***K. G. PRATT**;
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Abstract: Visual information in Xenopus laevis is processed in the midbrain, a brain structure that in amphibians comprises two major components along the dorsoventral axis, the tectum and the tegmentum. Retinal ganglion cells (RGCs), carrying visual information from the eye, project to the contralateral midbrain where they form synapses directly onto postsynaptic midbrain neurons. While most of these projections are known to terminate in the well-studied tectum, our electrophysiological recordings show that neurons in the ventral region of the midbrain – the tegmentum – also receive direct RGC input. Here, we compare these ventral and dorsal visual projections. For this, we use an isolated brain preparation that allows access to both tectal and tegmental neurons. RGC axons are activated by placing a stimulation electrode on the optic chiasm. We found that by developmental stage 48/49 (approximately 17-21 days post-fertilization), tectal and tegmental neurons receive similar amounts of direct monosynaptic RGC input. Interestingly, however, the earlier developmental dynamics preceding stage 48/49 were not the same: we observed a marked peak in maximum RGC input onto tectal neurons at developmental stage 45, the time when this circuit is undergoing dynamic refinement. This transient peak in maximum RGC input was not observed in the tegmental neurons, suggesting that perhaps the ventral visual pathway does not refine and that tegmental neurons may receive input from a distinct set of RGCs. We also measured maximum non-visual lateral line inputs received by the same tectal and tegmental neurons. These inputs are activated by placing a bipolar electrode on the hindbrain (HB). We found that for a given tectal neuron, its maximal RGC input is greater than the maximal non- visual (HB) input whereas in ventral tegmental neurons, the maximal HB input is greater than the RGC input. Finally, biocytin fills indicate that axons of tegmental neurons maintain a ventro-medial course, descending ipsilaterally down the hindbrain, while axons of tectal neurons tend to project ventro-laterally. Taken together, our data suggest that (1) the two visual pathways are structurally and functionally distinct, and most likely process different qualities of a visual scene, and (2) like the optic tectum, the midbrain tegmentum is also involved in multi-sensory integration. Currently, in-vivo experiments are being carried out to determine what types of visual stimuli activate the tegmental neurons.

Disclosures: U.G. Udoh: None. **K.G. Pratt:** None.

Digital Abstract Session

P035. Development of Sensory Systems

Program #/Poster #: P035.02

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant R01EY015788
NIH Grant P30EY026878
E. Matilda Ziegler Foundation for the Blind

Title: Directional retinal waves differentially influence the development of direction-selective responses in collicular versus cortical neurons

Authors: *K. ZHANG, X. GE, M. CRAIR;
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Abstract: Before the onset of vision, the developing mouse retina spontaneously generates activity in the form of propagating waves. Previous studies have demonstrated that retinal waves are critical for the development of eye-specific segregation and topographic maps in the mouse visual system. However, it remains unclear whether retinal waves convey information beyond providing correlated activity between topographically neighboring cells and contribute to the development of higher order response properties in downstream visual neurons. Recent findings have shown that retinal waves exhibit a strong directional bias in their propagation during a transient developmental window from P7-P12 that matches an optic flow pattern that would be generated by forward self-motion after eye-opening. Here, we describe experiments that suggest that this specific spatiotemporal property of retinal waves is critical for the refinement of direction-selectivity (DS) in superior colliculus (SC) neurons. We used both pharmacological and genetic approaches to chronically disrupt the directionality of retinal waves during development and used *in vivo* two-photon calcium imaging to measure the responses of SC neurons to drifting grating stimuli immediately after eye-opening (P15). We find that both the tuning strength and direction preference of SC neurons at eye-opening are severely compromised following the loss of directional retinal waves. In addition, previous work suggests that the superficial layers of primary visual cortex (V1) gain independence from retinal drive and generate their own spontaneous activity shortly before eye-opening, while the SC and input layer of V1 faithfully replicate retinal waves throughout development. Retinal waves may therefore play a more critical role in the development of neuronal response properties in areas which are more retina-dependent throughout development, including the SC and input layer of V1, compared to superficial layers of V1. Indeed, we find that chronic manipulation of wave directionality before eye-opening only weakly disrupts the DS responses of V1 neurons in superficial layers and more strongly disrupts the DS responses of V1 neurons in the input layer. Taken together, these results suggest that retinal waves provide ethologically relevant information to the developing visual system by simulating the most likely optic flow pattern animals will receive after eye-opening, which is critical for the refinement of DS responses in

downstream visual neurons, and that retinal waves differentially influence the development of neuronal response properties in different visual areas.

Disclosures: K. Zhang: None. **X. Ge:** None. **M. Crair:** None.

Digital Abstract Session

P035. Development of Sensory Systems

Program #/Poster #: P035.03

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: Research to Prevent Blindness
NIH Grant R01 NS104776

Title: Binocular vs. monocular recovery experience differentially promote recovery of visual deficits in a mouse model of amblyopia

Authors: *J. D. MARTINEZ[1], M. J. DONNELLY[1], D. S. POPKE[1], D. TORRES[1], S. SHESKEY[2], B. C. CLAWSON[1], S. JIANG[1], S. J. ATON[1];
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Abstract: Altered visual experience during monocular deprivation (MD) profoundly changes in ocular dominance (OD) in the developing primary visual cortex (V1). MD-driven changes in V1 are an experimental model of amblyopia, where alterations in vision in early life lead to poor binocular vision in adulthood. Current treatment for amblyopia typically includes patching of the dominant eye, although binocular therapies are also being used. However, the relative impact of monocular vs. binocular recovery experiences have on V1 function is not well understood. Using single-unit recording of neurons in mouse V1, we examined how different forms of recovery visual experience (binocular recovery [BR] or reverse occlusion [RO]) of identical duration and content affects recovery of visual cortical responses after MD. We also tested how the two forms of recovery differentially affect normalization of visually-driven behavior following MD. We find that BR is quantitatively superior with respect to normalization of V1 OD and visually-driven behavior (reflecting improved deprived eye acuity after BR). However, firing rate properties found in principal neuron and fast-spiking interneuron populations undergo changes after MD that do not recovery after visual experience. Additionally, binocular matching of orientation preference remains disrupted after recovery and visual response properties such as visual responsiveness are dramatically decreased after recovery for both experiences. Lastly, we used cFos immunohistochemistry to examine neuronal activity changes in binocular zone of V1 after BR and RO recovery. Thus BR and RO have differential effects on various aspects of vision that will impact the recovery of normal vision in amblyopia.

Disclosures: J.D. Martinez: None. **M.J. Donnelly:** None. **D.S. Popke:** None. **D. Torres:** None. **S. Sheskey:** None. **B.C. Clawson:** None. **S. Jiang:** None. **S.J. Aton:** None.

Digital Abstract Session

P035. Development of Sensory Systems

Program #/Poster #: P035.04

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant EY022730
NIH Grant NS106244

Title: Cortical feedback regulates developing thalamic activity and circuit refinement in the mouse visual system

Authors: *Y. MURATA, M. T. COLONNESE;
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Abstract: Spontaneous retinal activity plays a foundational role in the establishment and refinement of connectivity during the development of the visual system. However, it remains poorly understood how the information from the retina is represented in the developing thalamus and cortex and how it guides circuit formation. Here, we investigate the characteristics and generative mechanisms of thalamocortical activity in the mouse visual system *in vivo* throughout the early postnatal period. We focus on the role of cortical feedback because recent work shows that reciprocal thalamocortical interactions are critical for the generation of activity in the developing thalamus. Using high-density multi-electrode arrays in the visual thalamus and cortex of un-anesthetized neonatal mice, we find that early thalamic firing is correlated in an eye-specific and spatial manner, presumably reflecting the spontaneous retinal wave activity from both eyes. Such correlated thalamic activity is prevalent only until postnatal day (P) 11, one to two days before eye opening. Chemogenetic reduction of cortical activity before P11 revealed that cortical feedback not only amplifies thalamic activity but also coordinates thalamic neuronal firing by increasing correlated activity among same-eye responsive thalamic neurons, thus enhancing their differences from opposite-eye responsive neurons. Furthermore, chronic reduction of cortical activity during the second postnatal week impaired eye-specific segregation of retinal axons in the visual thalamus, consistent with the role of cortical feedback in synchronizing eye-specific thalamic regions. This study shows a crucial role for the unique developmental properties of reciprocal thalamocortical interactions in the transmission of retinal activity and the formation of visual circuits.

Disclosures: Y. Murata: None. **M.T. Colonnese:** None.

Digital Abstract Session

P035. Development of Sensory Systems

Program #/Poster #: P035.05

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: Buffett Early Childhood Institute, University of Nebraska

Title: Inhibition of microglia with PLX5622 prevents developmental pruning of the glossopharyngeal nerve which is then restored following microglia replenishment

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Abstract: The gustatory glossopharyngeal nerve (GL) projects fibers (a 'terminal field') to the nucleus of the solitary tract (NTS) of the medulla. Similar to the developmental pruning observed in other contexts, the volume of GL terminal field in the NTS reduces by approximately 66% between postnatal day 15 (P15) and P40 (Mangold & Hill, 2008). However, the GL terminal field can undergo atypical late life expansion as a result of experimental alterations to input, reversing developmental pruning (Skyberg et al., 2017; Sun, Krimm, & Hill, 2018). It therefore appears as if the GL terminal field not only prunes during development, but is also subsequently actively maintained at a reduced size for the reminder of life, although the mechanism for either are unknown. Evidence from other gustatory nerves suggest that the central macrophages microglia are the mechanism for gustatory developmental pruning (Sun et al., 2020), and possibly active maintenance of the GL terminal field. We first assessed microglia density in the NTS of rats at P11, P17, P24, and P40, observing that microglia density in the region was highest during the pruning period before reducing to adult quantities at P24 (p = .011). To test the hypothesis that microglia are required for GL terminal field pruning and maintenance, we utilized i.p. administration of the colony-stimulating factor 1 receptor inhibitor PLX5622 to deplete microglia by >99% from the developing rat from P1 to P40. Using a biotinylated dextran amine, we labeled the GL terminal field in groups of rats at either P15, P25, P40, or P68 (four weeks after ceasing treatment and allowing microglia to return). As expected, the volume of the GL terminal field was largest at P15 for both PLX5622 and vehicle treated rats. In vehicle controls, GL terminal field volume reduced by 66% by P25 (p = .023) and remained stable through P40 (p= .166). However, no such age-related reduction occurred in PLX5622-treated rats (ps > .1), indicating microglia are required for development GL terminal field pruning. When PLX5622 treatment was ceased at P40, microglia were replenished to age-matched control quantities by P43 (p = .873), and by P68 their GL terminal fields had reduced in volume to control levels (p= .440). This finding suggest that microglia retain their ability to prune well outside the canonical pruning period and may actively maintain the GL terminal field at a reduced volume after developmental pruning.

Disclosures: A.J. Riquier: None. **S.I. Sollars:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Plexxikon Inc..

Digital Abstract Session

P036. Development of Limbic Systems

Program #/Poster #: P036.01

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant R15HD090606
KSU Brain Health Research Institute Blue Award

Title: The role of oxytocin signaling in sex-specific gene expression in the embryonic mouse brain

Authors: *E. A. AULINO, N. M. PLONSKI, H. PIONTKIVSKA, H. K. CALDWELL;
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Abstract: Recent work suggests that the oxytocin (Oxt) system's neuromodulatory actions in adolescent and adult life are contingent on its role in embryonic brain development. In the embryonic brain, there is evidence that Oxt can influence neural cell fate specification and cellular migration, as well as the timing of the GABA switch near birth. These early organizational effects may contribute to the development of brain regions and neural circuits important to the neural regulation of behavior in adulthood. Work from our lab has identified a key developmental timepoint where the Oxt system may begin to exert its effects, embryonic day (E) 16.5. Both males and females first produce Oxtr protein at this time, while females, but not males, produce *Oxt* transcripts. Further, we have evidence that a single, transient disruption of Oxtr signaling at E16.5 has sex-specific effects on adult social behaviors. Taken together, our findings indicate a sex-dependent organizational function for the Oxt system in the embryonic brain. However, what remains unknown is how Oxt's actions functionally organize the brain at this crucial timepoint. One way that we hypothesize that Oxtr signaling contributes to the sex-specific development of neural circuits is by direct effects on gene expression. To test this hypothesis, we re-analyzed available datasets from RNA sequencing experiments performed with brain tissue collected from both male and female C57BL/6J mice between E15.5 and E17.5. The raw data was analyzed using the Automated Isoform Diversity Detector (AIDD) pipeline, which combines alignment, mapping, and differential analysis using DeSeq2. Additional analysis to determine gene set enrichment and functional clustering was performed using the Gene Set Enrichment Analysis and PANTHER on differentially expressed genes identified by AIDD. Preliminary data suggests significant differences between males and females at E16.5 in gene expression associated with pathways unique to Oxtr signaling. These data will inform analysis of an ongoing RNA sequencing experiment which will examine male and female brains at E16.5 from Oxt knockout and wildtype litters, focusing on brain regions known to express the Oxtr at this time.

Disclosures: E.A. Aulino: None. **N.M. Plonski:** None. **H. Piontkivska:** None. **H.K. Caldwell:** None.

Digital Abstract Session

P036. Development of Limbic Systems

Program #/Poster #: P036.02

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: Spanish MINECO Grant BFU2015-68537-R
Spanish MCINN Grant PID2019-108725RB-100
Predoctoral fellowship from Universitat de Lleida i Ajuts Jade Plus
Predoctoral contract from IRBLleida/Diputació de Lleida

Title: A novel telencephalon-opto-hypothalamic embryonic domain produces most of the glutamatergic neurons of the medial extended amygdala

Authors: L. MORALES, E. DESFILIS, *L. MEDINA;
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Abstract: Deficits in social cognition and behavior are a hallmark of many psychiatric disorders. The medial extended amygdala (EAMe), which includes the medial amygdala and the medial bed nucleus of the stria terminalis, is a key component of functional networks involved in sociality. However, this nuclear complex is highly heterogeneous and contains numerous GABAergic and glutamatergic neuron subpopulations with multiple embryonic origins. Deciphering the specific connections of these different neuron subtypes is essential in order to understand how this structure regulates different aspects of sociality, and it is necessary to evaluate their differential implication in distinct psychiatric disorders. Developmental studies are offering new venues to understand the neuronal diversity of the medial extended amygdala, and are helping to establish a relation between the embryonic origin and molecular signature of a neuron with the functional subcircuits in which it is engaged. While the different GABAergic neuron subpopulations of the EAMe have been studied in detail, knowledge about the glutamatergic cells is still scarce. Using an Otp-eGFP transgenic mouse, we found that most glutamatergic neurons of the EAMe originate in a novel telencephalon-opto-hypothalamic embryonic domain, located at the transition between telencephalon and hypothalamus. This new domain produces Otp-lineage neurons co-expressing the telencephalic marker Foxg1 but not Nkx2.1 during development. Furthermore, these glutamatergic cells include a subpopulation of projection neurons of the medial amygdala, which activation has been previously shown to promote autistic-like behavior. Our data open new venues for studying the implication of this neuron subtype in neurodevelopmental disorders producing social deficits.

Disclosures: L. Morales: None. **E. Desfilis:** None. **L. Medina:** None.

Digital Abstract Session

P037. Human Imaging

Program #/Poster #: P037.01

Topic: A.09. Adolescent Development

Title: Neurodevelopmental changes in myelination, cortical thickness and resting state functional connectivity during adolescence

Authors: J. BERO[1], Y. LI[2], A. KUMAR[2], H. LEE[1], W.-Y. AHN[3], S. HAHN[4], H. KIM[5], *D. LEE[6,1];

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Abstract: Changes in morphometric and cytoarchitectural features during development are accompanied by maturation in the functional connectivity. In particular, adolescence represents a time of unparalleled brain development, but the exact nature of these changes is not well understood. Here, we examined how changes in the three facets of brain development, including myelination (T1w/T2w ratio), cortical thickness (CT) and resting-state functional connectivity (RSFC) are coordinated during adolescence in children between the ages of 10 and 16. Consistent with previous studies, we found that the level of myelin in adolescent children was generally lower than in adults. We then investigated the extent of coordinated changes in three measures by computing grayordinate-level correlation matrices for each measure as well as meta-correlations among correlation matrices of different measures. We found that the meta-correlation between myelin and RSFC was more robust than those between myelin and CT and between CT and RSFC. In particular, meta-correlations among myelin, CT and RSFC tended to be maximum in the sensory-motor cortical areas. Nevertheless, compared to the results in adults, the auditory network showed particularly lower meta-correlation between myelin and RSFC in adolescent children, whereas this was higher for the salience network. We also found that the meta-correlation between myelin and RSFC showed higher values in the visual cortical areas than the meta-correlation related to CT. The results from these analyses were largely unaffected when looking at changes in meta-correlation between a young and old groups of subjects, suggesting that the inter-individual variability in the brain growth underlies the meta-correlation observed in this study. By using a permutation test, we further confirmed that there are no significant age-related changes in all three meta-correlations within the population of subjects in our study. These results demonstrate that the development of cortical myelination and thickness is closely coordinated with changes in functional connectivity among multiple cortical areas during adolescence.

Disclosures: **D. Lee:** A. Employment/Salary (full or part-time):; Neurogazer. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Neurogazer. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurogazer. **J. Bero:** A. Employment/Salary (full or part-time):; Neurogazer. **Y. Li:** A. Employment/Salary (full or part-time):; Neurogazer. **A. Kumar:** A. Employment/Salary (full or part-time):; Neurogazer. **H. Lee:** A. Employment/Salary (full or part-time):; Neurogazer. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurogazer. **W. Ahn:** None. **S. Hahn:** None. **H. Kim:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurogazer.

Digital Abstract Session

P037. Human Imaging

Program #/Poster #: P037.02

Topic: A.09. Adolescent Development

Support: NIDA Grant 5R01DA017843-05

Title: Accumbens-orbitofrontal functional connectivity moderates the association between the executive control system and cognitive impulsivity

Authors: *S. D. KLEIN[1], P. COLLINS[2], M. LUCIANA[3];
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Abstract: A preference for immediate smaller rewards over larger delayed rewards reflects impulsive decision-making. Critically, a maturational imbalance between reward and executive networks may underlie the increase in risk-taking observed in adolescence. The present study employed longitudinal analyses of delay discounting data in order to determine the developmental trajectory of cognitive impulsivity. In addition, we examined how changes in functional connectivity between brain regions implicated in reward processing and incentive motivation (i.e. nucleus accumbens [NAc] and medial orbitofrontal cortex [mOFC]) as well as executive-function and goal-directed behavior (i.e. dorsal caudate [DC] and dorsolateral prefrontal cortex [dlPFC]) relate to the developmental trajectory of cognitive control over impulsive decision-making. 197 participants aged 9 to 23 years completed baseline assessments at wave one, and were followed every two years for five waves (10 years total). Participants completed a delay discounting task at each wave. For each trial, participants chose between an immediate amount of money or $10 available after a delay (i.e. 1, 2, 10, 30, 180, and 365 days later). Indifference points were established within participants at each interval and plotted against time (delay), with areas under the discounting curves (AUC) calculated by summing the resulting trapezoids. The imaging protocol included a T1 weighted structural scan beginning at wave one, and resting state functional scans beginning at wave two. We employed a seed-based approach, with ROIs selected from previous work that examined the differential roles of ventral and dorsal striatum. A quadratic effect of age yielded the best fit when comparing linear and quadratic effects of age on cognitive impulsivity, with a plateau emerging in early adulthood (i.e. age 25. NAc-to-mOFC connectivity moderated the relationship between AUC and DC-to- dlPFC connectivity: individuals that displayed coupling between the reward and executive system (i.e. high or low connectivity between both NAc-mOFC and DC-dlPFC) discounted less. Results suggest that adolescents increasingly able to allocate cognitive resources towards reward maximization as they reach young adulthood, consistent with research findings that development of PFC continues into early adulthood. It appears that coupling of executive and reward networks has implications for the developmental trajectory of cognitive impulsivity.

Disclosures: S.D. Klein: None. **M. Luciana:** None. **P. Collins:** None.

Digital Abstract Session

P037. Human Imaging

Program #/Poster #: P037.03

Topic: A.09. Adolescent Development

Support: Quebec Bio-Imaging Network (QBIN) scholarship | 04/08/2020- 04/08/2021 | $5,000
Integrated Program in Neuroscience (IPN) internal returning student Award | McGill University | 27/08/2020- 27/08/2021 | $10,000

Title: Multivariate analysis of cortical morphometry across human brain development

Authors: *H. KALANTAR HORMOZI[1,4], G. A. DEVENYI[4,2], R. PATEL[4,3], A. RAZNAHAN[5], M. CHAKRAVARTY[4,3,2,1];
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Abstract: A major goal for developmental neuroscience is understanding how maturational processes are coordinated between different brain regions. This knowledge can serve to better understand the biological basis of neurodevelopmental disorders. This work is built on recent studies that have examined multiple neuroanatomical features of the cortex that are inter-related during the brain maturational process. However, the results are challenging to interpret as differences across the proposed connectivity measures cannot be interpreted as a function of a specific neuroanatomical measure. This distinction is crucial, as neuroanatomical indices such as cortical thickness (CT) and surface area (SA) arise through separate developmental processes and are differentially affected by disorders. To this end, using a novel multivariate technique known as nonnegative matrix factorization (NMF), we sought to improve our understanding of this inter-relatedness in the context of developing brain anatomy. NMF decomposes cortical features into nonnegative part-based representation to improve interpretability. We used the baseline subset of a unique longitudinal sample of structural magnetic resonance imaging acquired from 776 youths (357 F) aged 5 to 25 (M:12.4, SD:~3), provided through an extensive study conducted at the National Institute of Mental Health (NIMH; Bethesda, MD, USA). The neuroanatomical indices were extracted from preprocessed T1 sMRI scans using CIVET, a fully automated morphometric analysis pipeline. Using CT maps analyzed by NMF, we identified cortical components that were mostly bilateral, and showed local systems that divide anatomical sub-regions such as precentral and postcentral, and occipital components, and a relative resemblance of specific large-scale functional networks, such as fronto-parieto-temporal component as for language network. These components can also act as a normative scaffold for neurodevelopmental disorders. Further, SA metrics will be integrated into this framework to better understand the relationship across cortical metrics.

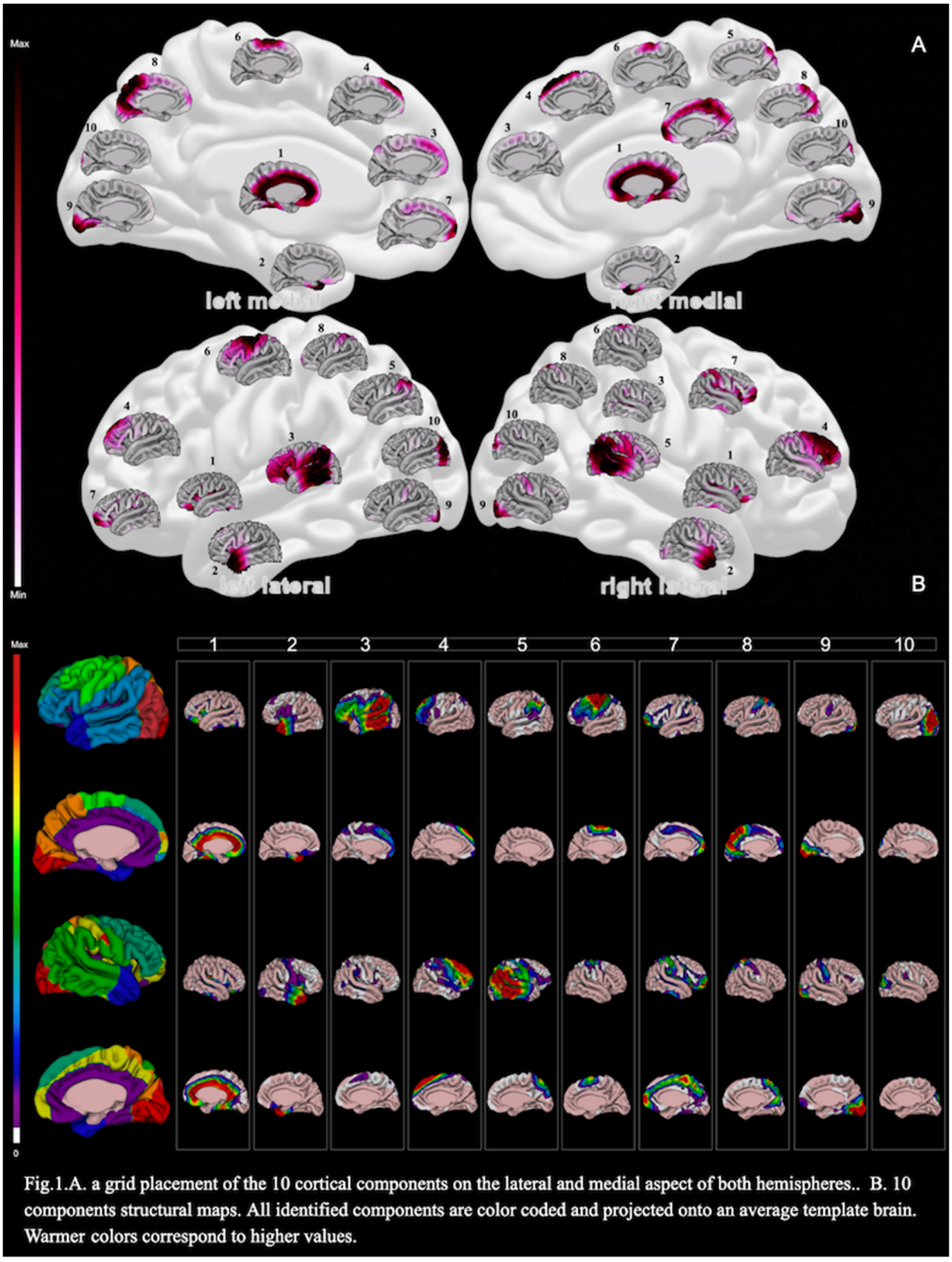

Fig.1.A. a grid placement of the 10 cortical components on the lateral and medial aspect of both hemispheres.. B. 10 components structural maps. All identified components are color coded and projected onto an average template brain. Warmer colors correspond to higher values.

Disclosures: **H. Kalantar Hormozi:** None. **G.A. Devenyi:** None. **R. Patel:** None. **A. Raznahan:** None. **M. Chakravarty:** None.

Digital Abstract Session

P037. Human Imaging

Program #/Poster #: P037.04

Topic: A.09. Adolescent Development

Support: NSF Grant 1940094
NSF Grant 1649865

Title: Impact of the Adolescent Environment on the Resting Connectome and Inhibitory Control

Authors: K. SOLDOZY[1], S. M. PARKS[2], S. J. BROOKS[2], *C. STAMOULIS[3,2];
[1]Neurosci., Princeton Univ., Princeton, NJ; [2]Med., Boston Childrens Hosp., Boston, MA; [3]Med., Harvard Med. Sch., Boston, MA

Abstract: Adolescence is a critical period of development, during which regions of the brain involved in higher-level cognitive processes (e.g., decision-making) continue to maturate and change topologically, in part as a result of positive and negative experiences. A significant body of literature has linked negative behavioral outcomes to youth's adverse environments, which have also been mapped onto differences in cognitive function and underlying neural circuitry. In contrast, few studies have analyzed how typical heterogeneity in the early adolescent environment relates to behavior and brain connectivity. Resting-state fMRI, behavioral and survey data from a sub-cohort of n = 5955 children (~51.0% females; median age = 120.0 months, interquartile range (IQR) = 13.0 months) from the Adolescent Brain Cognitive Development (ABCD) cohort were analyzed, with the goal to elucidate the impact of multiple environmental domains (family, school, neighborhood) simultaneously on the properties of the task-independent (resting) adolescent connectome as well as inhibitory control.
Statistical models were first developed to assess the association between resting connectome properties, demographic data and survey questions on youth environment. Preliminary results indicated a significant positive effect of race ($p=0.02$, white versus non-white) and socioeconomic status ($p=0.03$) and a significant negative effect of parental agreement about whether they "believe in raising their voice" ($p=0.03$) on the brain's overall resting connectivity. A second set of models assessed the impact of the environment on inhibitory control, measured by the Stop Signal Task (SST). Older youth ($p<0.01$ for age) and children from families with higher annual incomes ($p<0.01$) had a higher number of correct "Go" trials, whereas children who agreed that their families "fight a lot" had a lower number of correct "Go" trials ($p<0.01$). Additional preliminary models indicated a significant association between both resting connectome properties and environmental variables with inhibitory control measured in the SST. This included a positive effect of small worldness, a fundamental property of a topologically efficient brain ($p<0.01$) and socioeconomic status ($p<0.01$) on the number of correct "Go" trials. Although neuroscientists have long known that positive and negative youth experiences impact both behavior and the neural circuitry that supports it, to our knowledge this study is the first (at least in size and network approach) to quantify the effects of some aspects of youth environment on both properties of the resting adolescent connectome and inhibitory control.

Disclosures: **K. Soldozy:** None. **S.M. Parks:** None. **S.J. Brooks:** None. **C. Stamoulis:** None.

Digital Abstract Session

P037. Human Imaging

Program #/Poster #: P037.05

Topic: A.09. Adolescent Development

Support: CONACyT 619683/330142
PAPIIT-DGAPA 212219

Title: The development of the functional brain network follows puberty-dependent nonlinear trajectories

Authors: *S. ALCAUTER, **Z. GRACIA-TABUENCA**, M. B. MORENO, F. A. BARRIOS; Inst. De Neurobiologia. UNAM, Queretaro, Mexico

Abstract: Adolescence is a developmental period that dramatically impacts body and behavior, where puberty plays an important role not only in the body changes but also in brain structure and function. However, no previous study has explored the effect of pubertal status on the brain functional organization. This work aims to explore such a relationship in a longitudinal sample of children and adolescents, fitting and testing non-linear trends with pubertal status and chronological age as independent variables. The sample consisted of 98 typically developing subjects (45 male, age: 6.7 - 18.1 years old), of whom 41 and 16 returned to a second and a third session, respectively. Participants filled a 4-level pubertal development scale (PDS) and underwent a resting-state fMRI scan (3Tesla, TR = 2s, 150 volumes). Preprocessing included a rigorous artifact-based regression of 36 head-motion parameters (Satterthwaite et al., 2013). Datasets were normalized to standard space, and average fMRI time series were extracted from 264 regions of interest (Power et al., 2011). Brain network organization was assessed using graph theory measures: degree (D), clustering coefficient (C), and global efficiency (E); which accounts for centrality, segregation, and integration in terms of network organization. Generalized Additive Mixed Models (GAMM) were applied to account for nonlinear trends (smooth splines) and longitudinal effects, including average head-motion as a covariate. In a range of 1-48% of connectivity density, four different models were tested: age, PDS, age-sex, and PDS-sex interactions. The best model was selected according to the lower Akaike Information Criterion (AIC). Spline statistical significance was corrected with a false discovery rate (FDR $q < 0.05$). PDS showed lower AIC at densities above 5%, and significant (FDR-corrected) adjustments for every graph measure. D, C, and E measures showed a nonlinear concave pattern, being the turning point of every curve at level 2 of the PDS, the onset of pubertal signs. Results showed that pubertal status better explains the developmental trajectories of the brain functional network properties, compared to age and sex patterns. Also, we found nonlinear increases of functional centrality, segregation, and integration, particularly after the onset of the pubertal signs (level 2 of PDS). This study points out the importance of considering

longitudinal nonlinear trends when exploring developmental trajectories and emphasizes the impact of puberty on the functional organization of the brain in adolescence.

Disclosures: S. Alcauter: None. **Z. Gracia-Tabuenca:** None. **M.B. Moreno:** None. **F.A. Barrios:** None.

Digital Abstract Session

P037. Human Imaging

Program #/Poster #: P037.06

Topic: A.09. Adolescent Development

Support: NIH Grant R01EY027018
NIH Grant T32GM081760

Title: Cortical morphology of the contralesional hemisphere following pediatric unilateral resection

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Abstract: Neural plasticity is an essential feature of the central nervous system, allowing it to adapt to the demands of typical development. This adaptability becomes especially relevant in cases of early assault on typical brain development, with resultant changes that can be either beneficial or maladaptive. For example, we know that neonatal stroke ultimately results in alterations to the cortical morphology of the healthy hemisphere. What remains unclear is whether similar alterations follow from neural insults later in life, during childhood and adolescence, and whether these changes are contingent on the size and site of the insult. One might hypothesize that the homologous region contralateral to the resection would exhibit alterations, such as an increase in cortical thickness or volume, as is seen in animal models of cortical ablation. In light of this, we examined the cortical morphology in the contralesional hemisphere following pediatric unilateral resection for the management of pharmacoresistant epilepsy. We found largely comparable measures of cortical morphology in the preserved contralesional hemisphere of patients relative to age- and gender-matched controls and largely independent of lesion size or site. Despite the loss of resected tissue in one hemisphere, these patients nevertheless evinced largely intact cognitive behaviors post-surgery. Altogether, this suggests that beneficial plasticity of the contralesional hemisphere may be less dependent on structural changes than on the neuronal populations' malleability to adopt different functions. This is consistent with our previous findings of widespread altered functional--but intact structural--connectivity in the contralesional hemisphere.

Disclosures: **M.C. Granovetter:** None. **A.M.S. Maallo:** None. **C. Patterson:** None. **M. Behrmann:** None.

Digital Abstract Session

P037. Human Imaging

Program #/Poster #: P037.07

Topic: I.07. Data Analysis and Statistics

Title: Age-dependent population norms for resting-state functional connectivity using tolerance intervals.

Authors: ***J. JEONG**, A. MARON-KATZ, M. NARAYAN;
Stanford Univ., Palo Alto, CA

Abstract: There is great interest in understanding what constitutes normal brain function across lifespan and to quantify when individuals deviate from this reference. Tolerance intervals are a statistical tool to answer this question by guaranteeing that a targeted proportion of the population falls within a tolerance interval with statistical confidence. This tool has previously been used to detect abnormal patterns of functional connectivity (Maron-Katz et al. 2020). Here, we employ tolerance intervals to establish age-group specific population norms of resting fMRI features using the NKI resting state study. Resting state connectivity is an effective tool to study the effects of aging on cognition and memory (Ferreira et al. 2013). The NKI sample provides 896 participants between the ages of 18 and 81. Each participant has two fMRI scans at TR=1400 ms and TR=2500 ms. All datasets were preprocessed with fMRIPrep and denoised. Average BOLD signals were extracted for 100, 200, and 300 parcels derived from a previously defined functional parcellation (Schaefer et al. 2018). Using the set of 7 previously identified functional brain networks (Yeo et al. 2011), 28 network-network connectivity measures were extracted by averaging the functional connectivity across all parcel pairs that connect every pair of networks. Age-dependent norms were calculated for each network to network connectivity measure using tolerance intervals at population proportion of 80% and confidence level of 95%. To examine the differences between the age groups, bootstrap estimates of the tolerance intervals were used. For scans with TR=2500ms, nearly all connectivity measures across the age groups (18-25, 26-42, 43-52, 53-63, 64-81) were statistically significantly different, after corrections for multiple testing. The scans with TR=1400ms largely provided similar results with some inconsistencies. The discrepancies between both scan types highlight the need to investigate whether norms for functional connectivity can be generalized across temporal resolutions. In conclusion, the NKI study-specific reference distributions for levels of functional connectivity given by tolerance intervals differ across age groups. These findings are consistent with previous work (Dosenbach et. al. 2010) on the effects of brain maturity on functional connectivity. We leave the development of generalizable study-invariant reference distributions to future work. Our preliminary results demonstrate the feasibility of developing statistically rigorous age-dependent norms of normal functional connectivity which can inform the understanding of pathogenic processes in neurological disorders.

Disclosures: **J. Jeong:** None. **M. Narayan:** None. **A. Maron-Katz:** None.

Digital Abstract Session

P038. Comparative Neuroanatomy

Program #/Poster #: P038.01

Topic: A.10. Development and Evolution

Support: NIH OT2 OD025340
NSERC PGS D3-437918-2013
Duke University University Scholars Program
Duke University Myra & William Waldo Boone Fellowship
Duke University James B. Duke Fellowship
Duke University Pratt School of Engineering Faculty Discretionary Fund

Title: Quantified morphology of the cervical and subdiaphragmatic vagus nerves of human, pig, and rat

Authors: ***N. A. PELOT**[1], G. B. GOLDHAGEN[1], J. E. CARIELLO[1], E. D. MUSSELMAN[1], K. A. CLISSOLD[2], J. A. EZZELL[2], W. M. GRILL[1];
[1]Dept. of Biomed. Engin., Duke Univ., Durham, NC; [2]Histology Res. Core, Univ. of North Carolina, Chapel Hill, NC

Abstract: It is necessary to understand the morphology of the vagus nerve (VN) to design and deliver effective and selective vagus nerve stimulation (VNS) because nerve morphology influences fiber responses to electrical stimulation. We quantified the morphology of nine left cervical and nine anterior subdiaphragmatic VNs from each of three species: humans (4F/5M, 54 to 90+ years), domestic pigs (6F/3M, 10 to 15 weeks), and SD rats (4F/5M, 11 to 38 weeks). We collected samples from each species at standardized locations. The samples were post-fixed in formalin and embedded in paraffin, and transverse sections were stained with Masson's trichrome. To label the perineurium, we conducted anti-claudin-1 immunohistochemistry on human samples and anti-fibronectin immunofluorescence on pig samples. We quantified nerve size, number of fascicles, fascicle size, proportions of endoneurial, perineurial, and epineurial tissue, and perineurium thickness. Human and pig VNs were comparable sizes, but pig nerves had ten times more fascicles than human; rat nerves were ten times smaller in diameter. Human and pig VN perineurium was thicker than previously published human somatic nerve data, with thicker perineurium for larger fascicles and thicker perineurium normalized by fascicle diameter for smaller fascicles; perineurium thickness is known to significantly affect thresholds for electrical stimulation. There were substantial differences in VN morphology across specie and, secondarily, between individuals. Understanding these differences in VN morphology between preclinical models and the clinical target, as well as the variability across individuals, is essential for advancing the therapeutic efficacy of VNS.

Disclosures: **N.A. Pelot:** None. **G.B. Goldhagen:** None. **J.E. Cariello:** None. **E.D. Musselman:** None. **K.A. Clissold:** None. **J.A. Ezzell:** None. **W.M. Grill:** None.

Digital Abstract Session

P039. Molecular Mechanisms in Evolution and Development

Program #/Poster #: P039.01

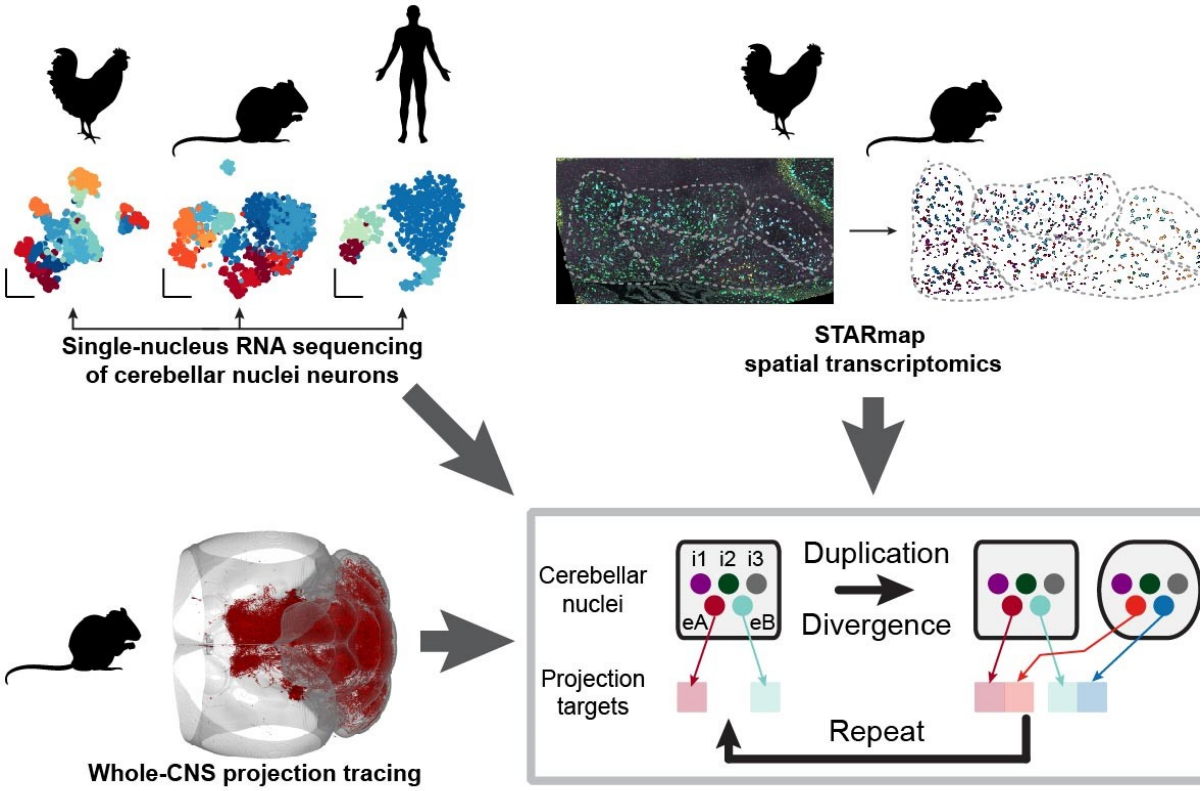
Topic: A.10. Development and Evolution

Support: HHMI
NIH
Jane Coffin Childs

Title: Cerebellar nuclei evolved by repeatedly duplicating a conserved cell-type set

Authors: ***J. M. KEBSCHULL**[1,2], E. B. RICHMAN[1], N. RINGACH[1], D. FRIEDMANN[1], E. ALBARRAN[1], S. KOLLURU[1,3], R. C. JONES[1], W. E. ALLEN[1], Y. WANG[4], S. CHO[1], H. ZHOU[4], J. B. DING[1], H. Y. CHANG[1,5], K. DEISSEROTH[1,5], S. R. QUAKE[1,3], L. LUO[1,5]; [1]Stanford Univ., Stanford, CA; [2]Johns Hopkins Univ., Baltimore, MD; [3]Chan Zuckerberg Biohub, Stanford, CA; [4]UC Davis, Davis, CA; [5]Howard Hughes Med. Inst., Stanford, CA

Abstract: How have complex brain regions, circuits, and cell types evolved from simple origins? Here we investigate brain region evolution at cell-type resolution in the cerebellar nuclei, the output structures of the cerebellum. We applied single-nucleus RNA sequencing in chickens, mice, and humans, STARmap spatial transcriptomic analysis in chicken and mice, and whole-CNS projection mapping in mice. Our work revealed a conserved cell-type set containing three classes of region-invariant inhibitory neurons and two classes of region-specific excitatory neurons. This cell-type set forms an archetypal cerebellar nucleus that was repeatedly duplicated to create new regions. In excitatory neurons, duplication was accompanied by divergence in gene expression and shifts in projection patterns (neofunctionalization). By contrast, inhibitory neurons maintained their gene expression signatures (isofunctionalization). Interestingly, the excitatory cell class that preferentially funnels information to lateral frontal cortices in mice becomes predominant in the massively expanded human Lateral CN. Our data provide the first characterization of CN transcriptomic cell types in three species and suggest a model of brain region evolution by duplication and divergence of entire cell type sets.



Disclosures: **J.M. Kebschull:** None. **N. Ringach:** None. **E.B. Richman:** None. **D. Friedmann:** None. **E. Albarran:** None. **S. Kolluru:** None. **R.C. Jones:** None. **W.E. Allen:** None. **Y. Wang:** None. **S. Cho:** None. **H. Zhou:** None. **J.B. Ding:** None. **H.Y. Chang:** None. **K. Deisseroth:** None. **S.R. Quake:** None. **L. Luo:** None.

Digital Abstract Session

P039. Molecular Mechanisms in Evolution and Development

Program #/Poster #: P039.02

Topic: A.10. Development and Evolution

Support: NIH Grant UO1MH114819
NIH Grant U01 MH114812-02

Title: Human specializations in single nucleus transcriptomic profiling of middle temporal gyrus across the great apes and monkeys

Authors: ***N. L. JORSTAD**[1], T. BAKKEN[3], R. D. HODGE[2], A. YANNY[3], K. SMITH[4], D. BERTAGNOLLI[4], J. GOLDY[4], C. RIMORIN[4], M. TIEU[4], D. MCMILLEN[4], T. PHAM[4], A. TORKELSON[4], K. WARD[4], L. ALYSSA[5], F. KRIENEN[5], E. LEIN[3];
[1]Human Cell Types, [2]Cell Types Program, Allen Inst. For Brain Sci., Seattle, WA; [3]Human Cell

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Abstract: The middle temporal gyrus (MTG) is a cortical brain region involved in language processing and integrating sensory input. Many human-centric neurodegenerative and neuropsychiatric diseases implicate MTG in neuropathological progression. So, what makes human MTG unique compared to our closest living relatives? In this study, we explore this question in the context of transcriptomically-defined cell types. We present a comparative analysis of single nucleus RNA-seq datasets from MTG of human (n = 7), chimpanzee (n = 7), gorilla (n = 4), rhesus macaque (n = 3), and marmoset (n = 3). Brain samples were collected from postmortem adult species of both sexes (except rhesus - female only). Nuclei were stained for NeuN and captured with fluorescence-activated cell sorting (FACS) to enrich for neurons (NeuN+) while also capturing a minority of non-neuronal (NeuN-) cells. Single nucleus libraries were generated with 10x Chromium V3, and ~100,000 nuclei per species passed quality control. Layer-dissected SMART-seq V4 datasets were generated, resulting in ~14,800 human, 4,100 chimpanzee, and 4,700 gorilla nuclei. Datasets were analyzed in R using Seurat 3.2.2. To identify cell types, the datasets were checked for quality, clustered, and curated separately for each species. Curated taxonomies were then integrated to identify a cross-species taxonomy of consensus cell types across the 5 species. We present 6 robust single-nucleus RNA-seq taxonomies; one for each species, and a cross-species taxonomy of conserved cell types. Diversity of MTG cell types, their distributions throughout the cortical layers, and their proportions are remarkably well conserved across species. Despite striking levels of conservation, we report notable species differences in chandelier cell proportions, subclass level expression differences of gene families, and an expansion of diversity in L6 IT Car3 types. Lastly, we explore expression of human neuropsychiatric disease-associated genes across cell types and species.

Disclosures: **N.L. Jorstad:** None. **T. Bakken:** None. **R.D. Hodge:** None. **A. Yanny:** None. **K. Smith:** None. **D. Bertagnolli:** None. **J. Goldy:** None. **C. Rimorin:** None. **M. Tieu:** None. **D. McMillen:** None. **T. Pham:** None. **A. Torkelson:** None. **K. Ward:** None. **L. Alyssa:** None. **F. Krienen:** None. **E. Lein:** None.

Digital Abstract Session

P039. Molecular Mechanisms in Evolution and Development

Program #/Poster #: P039.03

Topic: A.10. Development and Evolution

Title: Genes correlated with increases in neuroanatomical variability through evolution are implicated in neuropsychiatric disorders: a comparative chimpanzee-human neuroimaging and transcriptomic study

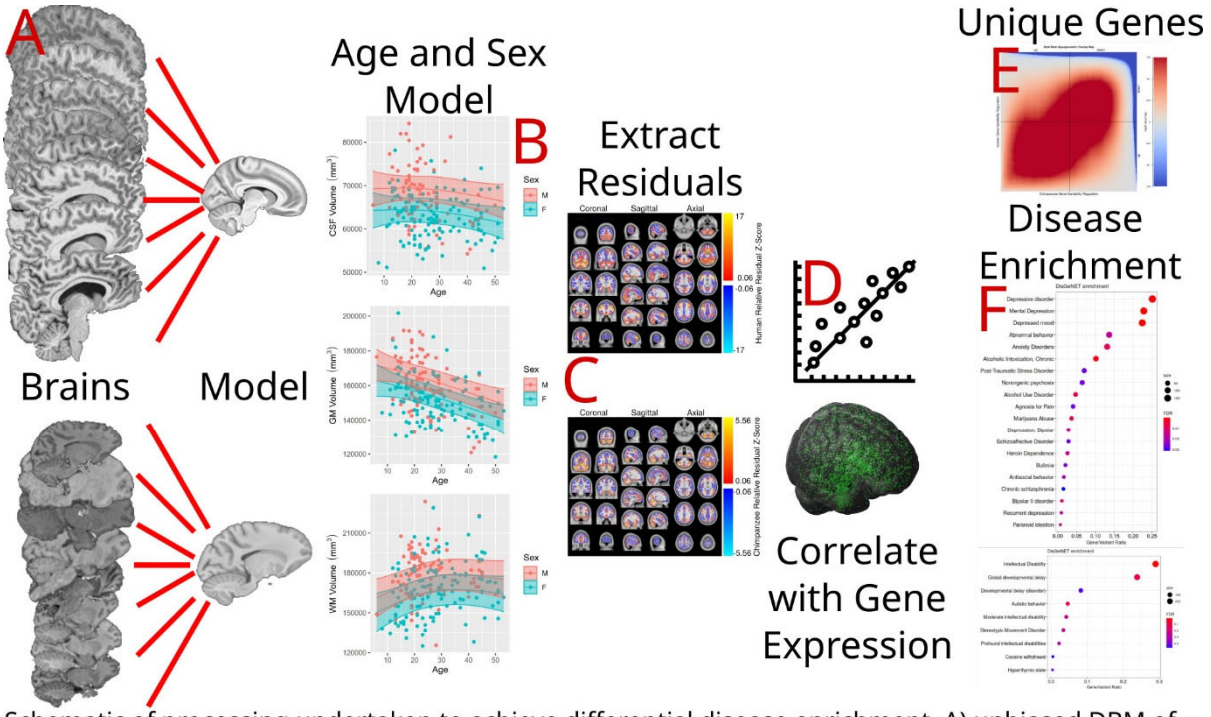
Authors: ***G. A. DEVENYI**[1,2], C. C. SHERWOOD[4], W. D. HOPKINS[5], A. RAZNAHAN[6], M. M. CHAKRAVARTY[1,2,3];

[1]Brain Imaging Ctr., Douglas Univ. Mental Hlth. Inst., Montréal, QC, Canada; [2]Psychiatry, [3]Biomed. Engin., McGill Univ., Montréal, QC, Canada; [4]Anthrop. and Ctr. for the Advanced Study of Human Paleobiology, The George Washington Univ., Washington, DC; [5]Dept. of Comparative Medicine, Michael E Keeling Ctr. for Comparative Med., M D Anderson Cancer Ctr., Bastrop, TX; [6]Section on Developmental Neurogenomics, Human Genet. Branch, NIMH Intramural Res. Program,, NIH, Bethesda, MD

Abstract: Chimpanzees, among the closest living relatives of humans, have remarkably similar brain structure despite 6-8 million years of divergent evolution. Prior work investigating genetic expression differences between chimps and humans (Khrameeva et al., 2020; Pollard et al., 2006; Sousa et al., 2017) identified a relationship between cortical expansion and "Human Accelerated Regions" of the genome (Wei et al., 2019). We present a novel analysis of the human transcriptome related to brain regions that increase in variability through evolution. We develop the concept of “residual variability”, variability remaining in a brain measure after accounting for a population’s age and sex, and use it to examine evolutionary differences between species. Here, we test the hypothesis that brain evolution is accompanied with an increase in neuroanatomically specific variability that has a transcriptional basis.

We apply the residual variability concept to deformation based modelling of healthy adult lifespan chimp and human cohorts yielding residual variability maps, warped these maps into MNI space, correlated them with human gene expression from the Allen Human Brain Atlas, and ordered by strength of correlation. Next, we used Rank-Rank Hypergeometric Overlap, to compare the species lists and extract the uniquely up and downregulated genes. We then subjected these gene sets to gene ontology(Liao et al., 2019) and disease(Piñero et al., 2020) enrichment analyses.

Gene enrichment on molecular function, biological process and cellular components reveal several processes associated with regions of increased variability in humans, in particular, downregulated genes associated with DNA/RNA expression and transcription. Meanwhile, gene disease enrichment analysis reveals associations between upregulated genes and neuropsychiatric disorders such as depressive symptoms (q=0.0002), psychosis (q=0.02) and anxiety disorder (q=0.008). Downregulated genes are associated with neurodevelopmental disorders such as intellectual disability and developmental delay (q=0.01).



Schematic of processing undertaken to achieve differential disease enrichment, A) unbiased DBM of human and chimpanzee population, B) voxel-wise modelling of age and sex trajectories, C) extraction of voxel-wise residuals, D) correlation of residuals with Allen Human Brain Atlas in MNI space, E) rank-rank hypergeometric overlap to extract unique human genes, and finally F) gene list disease enrichment.

Disclosures: G.A. Devenyi: None. **M.M. Chakravarty:** None. **A. Raznahan:** None. **C.C. Sherwood:** None. **W.D. Hopkins:** None.

Digital Abstract Session

P039. Molecular Mechanisms in Evolution and Development

Program #/Poster #: P039.04

Topic: A.10. Development and Evolution

Support: NIEHS ES028202
NICHD HD097093
NIMH MH104184
MH108286

Title: Establishing a Functional Role for UTY in Sex-Specific Fetal Development

Authors: *K. D. ROCK, B. M. NUGENT, L. M. FOLTS, T. L. BALE;
Univ. of Maryland, Baltimore, MD

Abstract: Stress experienced early in pregnancy has been identified as a significant male-specific risk factor for neurodevelopmental disorders. Fetal development relies on a healthy and properly functioning placenta, which can be significantly impacted by changes in maternal

physiology. Primarily made up of cells that are fetal in origin, the placenta expresses fetal sex (XX or XY). Consequently, X and Y-linked genes have the potential to introduce sex-specificity in placental function and fetal development, and in cellular responses to a changing maternal milieu. In our established mouse model of early prenatal stress (EPS), male offspring show increased vulnerability to long-term disruptions in cognition, stress sensitivity, and metabolic dysfunction that are endophenotypes relevant to neurodevelopmental disorders. EPS also induces dramatic changes in the male placental transcriptome that persist throughout the duration of pregnancy. We previously identified the novel X and Y-linked epigenetic regulators, UTX and UTY, of the canonical histone repressive mark, H3K27me3, as potential mediators of male risk and female resilience to stress *in utero*. UTX and UTY are homologous genes on the X and Y chromosome that belong to the H3K27me3 demethylase gene family. While UTX is a demethylase, the exact function of UTY remains unclear. Recent studies suggest that UTY may play some functional role in metabolism, development, and sexual differentiation of the brain. To test the hypothesis that UTY contributes to male-specific developmental outcomes in response to EPS, and improve our understanding of UTY functionality, we utilized two different transgenic approaches in order to overexpress UTY (UTY-OE) either in 1) the placenta or 2) the developing brain. Pregnant dams were randomly assigned to control or EPS conditions and sacrificed at embryonic day 18.5 for fetal brain and placental collections or allowed to litter in order to track changes in offspring growth and stress reactivity. Functional profiling in the placenta revealed a significant overlap in detectable transcripts between wildtype males and UTY-OE females, and highlighted demethylase activity and hormonal signaling as important biological processes regulated by UTY. Partial recapitulation of the male-specific EPS phenotype was observed in female placental UTY-OE offspring that showed a hyperactive stress response. Taken together, these studies demonstrate a novel role for UTY in placental function and fetal neurodevelopment. Further, these studies provide some of the first evidence that UTY, similar to UTX, does function as a demethylase.

Disclosures: K.D. Rock: None. **B.M. Nugent:** None. **L.M. Folts:** None. **T.L. Bale:** None.

Digital Abstract Session

P040. Synaptic Organization and Structure

Program #/Poster #: P040.01

Topic: B.06. Synaptic Transmission

Support: Grant-in-Aid for Scientific Research 20240032, 22110006, 16H01292, 18H04733, and 18H05434 from the MEXT, Japan

Title: Subsynaptic architecture of glutamate receptors and trans-synaptic nanocolumn regulated by CaMKII-mediated liquid-liquid phase separation

Authors: P.-W. LIU[1], T. HOSOKAWA[1], Q. CAI[2], J. S. FERREIRA[3], F. LEVET[3], C. BUTLER[3], J.-B. SIBARITA[3], D. CHOQUET[3,4], L. GROC[3], E. HOSY[3], M. ZHANG[2], Y. HAYASHI[1];

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Abstract: Long-term potentiation (LTP) of synaptic transmission is known to be the basis for memory formation. The regulation of AMPA receptor (AMPARs) at postsynaptic density (PSD) is the major mechanism to maintain LTP. AMPARs form segregated clusters, called nanodomains, in an activity dependent manner and the nanodomains align with presynaptic vesicle releasing sites to form a trans-synaptic nanocolumn, which regulates the efficacy of synaptic transmission. However, the mechanism underlying remains unclear. Here we found that calcium/calmodulin-dependent protein kinase II (CaMKII) undergoes liquid-liquid phase separation (LLPS) with NMDAR subunit GluN2B through its multivalent interaction contributed by its dodecameric structure in a Ca^{2+} dependent manner as possible mechanism of the incorporation of CaMKII into PSD. The initial formation of the CaMKII-mediated LLPS was independent of its kinase activity, however, to maintain the protein condensate, the CaMKII T286 autophosphorylation is required. Interestingly, the incorporation of CaMKII resulted in the segregation of Stargazin, as a proxy of AMPAR, from GluN2B as a phase-in-phase structure. Furthermore, Neuroligin-1 (NLGN1), a neuronal adhesion molecule, which clusters presynaptic neurexin, also segregates together with AMPAR. The segregation of AMPAR and NMDAR was also observed in living neuron with dual-color direct stochastic optical reconstruction microscopy (dSTORM). This segregation was disrupted by membrane-permeable CN21 (tat-CN21) which competes and disrupts CaMKII-GluN2B interaction. Furthermore, tat-CN21 also reduced the segregation between of NLGN1 and NMDAR. These results suggest that the incorporation of CaMKII into PSD and the interaction between CaMKII and GluN2B is critical for the formation of AMPAR nanodomain and the trans-synaptic nanocolumn. We anticipate that Ca^{2+}-induced and persistent formation of LLPS by CaMKII can be a novel mechanism for synaptic plasticity and serves as molecular basis of memory by functioning as an activity-dependent crosslinker for postsynaptic proteins.

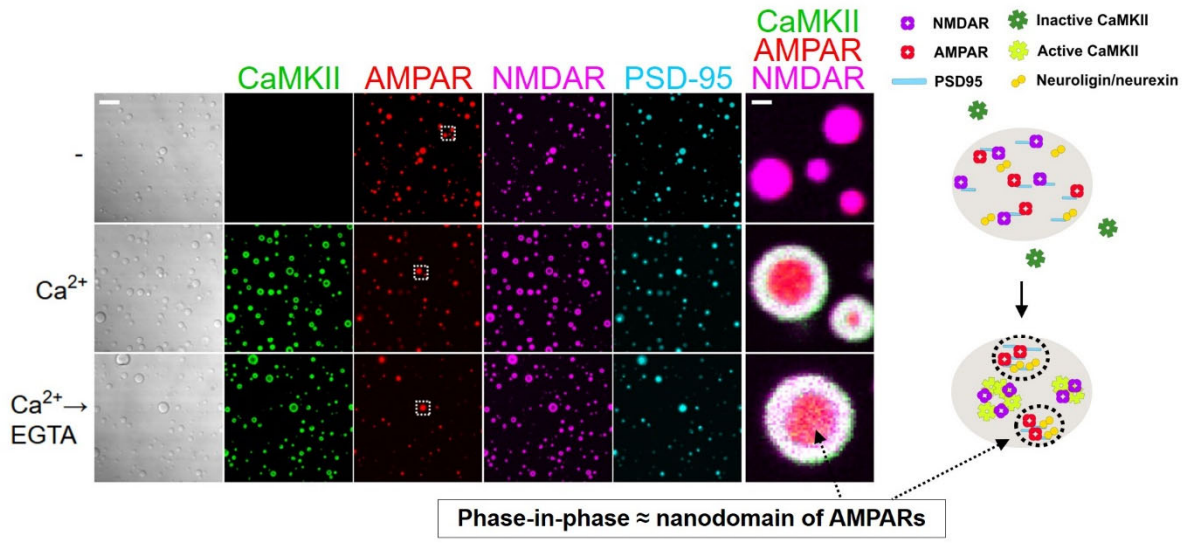

Fig. Incorporation of active CaMKII into protein condensates and the segregation of AMPAR and NMDAR

Disclosures: **P. Liu:** None. **T. Hosokawa:** None. **Q. Cai:** None. **J.S. Ferreira:** None. **F. Levet:** None. **C. Butler:** None. **J. Sibarita:** None. **D. Choquet:** None. **L. Groc:** None. **E. Hosy:** None. **M. Zhang:** None. **Y. Hayashi:** None.

Digital Abstract Session

P040. Synaptic Organization and Structure

Program #/Poster #: P040.02

Topic: B.06. Synaptic Transmission

Support: NIH Grant NS099122

Title: Fast synaptically activated calcium kinetics in pyramidal neuron dendritic spines

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Abstract: Previous studies have examined calcium changes in dendritic spines in some detail. However, there are some problems, which may make some of quantitative conclusions subject to reexamination. One issue is whether 2-photon glutamate uncaging accurately mimics synaptic release of glutamate. Electrical stimulation releases glutamate from vesicles into an area of about 0.1μm^2 while an uncaging pulse releases glutamate into a larger volume. This probably means that more NMDA receptors are activated and for longer time than synaptic stimulation, even when the electrical response to uncaging flashes is matched to mEPSCs made by AMPA receptors. A second issue is whether recordings using relatively high affinity calcium indicators are accurate. Measurements using low affinity indicators, with less buffering, like OGB-5N

should be more precise but may be difficult to record with adequate S/N. To try to overcome these problems we did experiments with minimal electrical stimulation, and detected OGB-5N fluorescence using focused laser illumination and a fast CCD camera. Preparation of mouse hippocampal slices, indicator loading, and patch recordings of CA1 pyramidal neurons were made using standard methods. Sodium transients were measured simultaneously using a high speed multiplexing protocol. We found that: (1) synaptic calcium signals had a rise time of <10 ms, with almost no variation as a function of EPSP size. The same fast rise time was detected with OGB-5N, fluo-5f, or OGB-1. (2) Synaptic sodium signals, detected with SBFI, also had faster rise times than 10 ms, probably faster than the calcium signals. Both of these fast signals were slower that the rise times of bAP calcium and sodium signals, which were both <2ms. This indicates that the EPSP in the spine, while fast, is not as fast as the bAP in the spine. Consistent with previous results, most of the calcium signal (but not the sodium signal) was blocked by APV or CPP showing that it was through the NMDA receptor. Most of the calcium signal also was blocked by NBQX. Since calcium does not enter through these AMPA receptors, this means that the AMPA driven EPSP is the main driver of calcium entry. This is consistent with the fast EPSP voltage relieving the Mg^{2+} block of the NMDA receptor. The synaptic calcium transient recovered more slowly than the calcium transient caused by a bAP (35-50 ms compared with 15-20 ms) but significantly faster than recorded with higher affinity indicators. Since the EPSP in the spine is very fast (implying that the relief of the Mg^{2+} block is short-lived) this suggests that there is an additional brief component of calcium entry through the NMDA receptor after the EPSP potential has returned to rest.

Disclosures: K. Miyazaki: None. **W.N. Ross:** None.

Digital Abstract Session

P040. Synaptic Organization and Structure

Program #/Poster #: P040.03

Topic: B.06. Synaptic Transmission

Support: NIH Grant MH121074

Title: Cell type specific functional impact of NRXN1-alpha splice isoforms

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Abstract: Neurexins are critical pre-synaptic proteins involved in organizing synaptic connections in the brain. Extensive alternative splicing results in diverse and cell-type specific isoform repertoires across brain-regions, which show differential binding to a myriad of postsynaptic ligands. Heterozygous deletions in *NRXN1* (*NRXN1+/-*) are highly penetrant and strongly associated with a variety of neurodevelopmental disorders. Patient-specific *NRXN1+/-* neurons, derived from human induced pluripotent stem cells, show gross dysregulation of

wildtype *NRXN1* alpha isoforms and can express dozens of novel isoforms from the mutant allele. However, the cell-type specific functional impact of such isoform repertoires is poorly understood. We now seek to resolve the cell-type specific functional impact of *NRXN1* alpha splice variants in glutamatergic and GABAergic neurons, using overexpression strategies and advanced gene editing techniques (CRISPR/CasRx). We hypothesize that manipulation of wildtype and mutant *NRXN1* isoforms will produce distinct effects on synaptic neuronal function, transcriptional pathways, and protein-protein interactions. Overall, this study will dissect the cell type specific impact of *NRXN1* alpha splicing and evaluate the genotype specific mechanisms of how *NRXN1*+/- leads to aberrant neurocircuitry and disease.

Disclosures: M.B. Fernando: None. **A.N. Murphy:** None. **E.K. Flaherty:** None. **S. Zhu:** None. **G. Fang:** None. **P.A. Slesinger:** None. **K.J. Brennand:** None.

Digital Abstract Session

P040. Synaptic Organization and Structure

Program #/Poster #: P040.04

Topic: B.06. Synaptic Transmission

Support: NIH Grant EY13079
NIH Grant F31 MH112372
NIH Grant R25GM097634
NSF Grant DBI-1460880
NYU Grant NYU Research Challenge Fund
NYU Grant NYU Dean's Dissertation Fellowship

Title: Gabaergic innervation of dorsal raphe-projecting layer v pyramidal neurons in medial prefrontal cortex correlates with food intake of mice in the activity-based anorexia model

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Abstract: Anorexia Nervosa (AN) is an eating disorder characterized by voluntary food restriction and excessive exercise. With a high mortality rate but no approved pharmacological treatment, research on AN is demanding. We used the Activity-Based Anorexia (ABA) mouse model to study the neurobiology of AN. Previous studies have uncovered the role of dorsal raphe (DR) in regulating feeding behaviors of mice. Interestingly, DR does not receive any direct input from the primary sensory cortices but substantially from the Layer V pyramidal neurons in the medial prefrontal cortex (mPFC). This implies the regulation of feeding by DR may involve higher-order cognitive control, such as the voluntary food restriction even during the 2 hour window of food availability seen in ABA. In this study, we selectively activated pyramidal neurons in Layer V of mPFC projecting to DR with Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) expressed through retrograde cre-dependent gene transfer.

Surprisingly, we found that selective activation of the mPFC-to-DR pathway through sc injection of C21 (1 mg/kg) to activate hM3D(Gq)-mCherry DREADDs did not significantly alter the food intake in the ABA model of mice (N=7, female) compared to the control group (N=13, female) whose mPFC-to-DR pathways were not activated. Electron microscopic immunohistochemistry validated the effectiveness of this chemogenetic method of neuromodulation by identifying the presence of mCherry-immunoreactivity on the plasma membrane and in the cytoplasm of mPFC Layer V pyramidal neurons' cell bodies, apical dendrites, spines and axon terminals forming excitatory synapses. In addition, we found that the degree of gamma-aminobutyric acid-(GABA)-ergic innervation, captured by the percent coverage of the cell body by GABAergic axon terminals, of the DR-projecting pyramidal neurons in mPFC was significantly greater than the non-DR-projecting subgroup by ~60% ($p=0.0312$), and it correlated significantly and negatively with the food intake only on the day when the mPFC-to-DR pathway was stimulated by DREADDs ($p=0.0035$, $r=-0.9181$). These results indicate the potential roles of GABAergic innervation of DR-projecting pyramidal neurons from mPFC in regulating feeding behaviors of mice in the ABA model. It also suggests that local GABAergic interneurons may be recruited during the chemogenetic manipulation of the mPFC-to-DR pathway, which results in a counteracting effect. Overall, this research sheds light on the potential etiology of anorexia nervosa by identifying the involvement of GABAergic innervation of specific subgroup of pyramidal neurons in mPFC in murine vulnerabilities to ABA.

Disclosures: M. Du: None. **A.N. Santiago:** None. **C.J. Aoki:** None.

Digital Abstract Session

P041. Glutamate

Program #/Poster #: P041.01

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant R01MH115188-01

Title: The effect of 14-3-3 proteins on NMDAR synaptic trafficking in hippocampal and cortical neurons

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Abstract: One of the core pathogenic mechanisms for schizophrenia is believed to stem from dysfunction in glutamatergic synaptic transmission, particularly hypofunction of N-methyl d-aspartate receptors (NMDARs). Previously we have shown that 14-3-3 functional knockout (FKO) mice exhibit schizophrenia-associated behaviors accompanied by reduced synaptic NMDARs in forebrain excitatory neurons. Based on these findings, we hypothesize that 14-3-3 proteins may act as a key component of post-synaptic NMDAR complex with a positive role in regulating synaptic levels of NMDARs. To ascertain the role of 14-3-3 proteins in regulating synaptic targeting of NMDARs, we tested whether 14-3-3 proteins affect synaptic localization of

NMDARs by expressing 14-3-3 peptide inhibitor in primary neurons. We found that inhibition of 14-3-3 proteins in these neurons leads to decreased synaptic localization of NMDAR subunits. We then investigated the underlying mechanism by using a variety of biochemical and imaging assays in heterologous cells. Using surface biotinylation and immunocytochemistry, we determined that inhibition of 14-3-3 proteins leads to decreased surface expression of NMDAR subunits. In addition, we found that several 14-3-3 isoforms directly interact with GluN2A and GluN2B subunits. We will further identify 14-3-3 protein binding sites and generate 14-3-3 binding deficient mutant constructs to establish a link between 14-3-3 protein binding and NMDAR surface delivery. As NMDAR hypofunctionality is known to act as a convergence point for progression of symptoms of schizophrenia, our findings may contribute to the understanding on molecular mechanism(s) that leads to NMDAR hypofunctionality at the synapses.

Disclosures: G.S. Lee: None. **Y. Zhou:** None.

Digital Abstract Session

P041. Glutamate

Program #/Poster #: P041.02

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: RGPIN-2020-04269

Title: Glutamate-induced tonic excitatory currents in melanin-concentrating hormone neurons

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Abstract: Glutamate (Glu) transporters play a major role in glutamatergic transmission by regulating the extracellular Glu levels. We previously found using whole-cell patch clamp that a robust tonic inward current (TIC) is induced by a non-specific Glu transporter inhibitor TFB-TBOA in melanin concentrating hormone (MCH) neurons in the hypothalamus. This suggests that MCH neurons, known to promote positive energy balance and sleep, are tightly regulated by Glu transporters. TBOA-induced TIC is largely blocked by NMDA receptor antagonist D-AP5, suggesting an involvement of NMDAR. This was somewhat surprising as our recording condition (holding potential = -70 mV) should not be permissive to postsynaptic NMDAR activation due to Mg2+ block. Thus, to investigate the underlying mechanism, we performed patch clamp recording on MCH neurons in rat and mouse brain slices. We first compared TBOA-TIC recorded in the absence or presence of extracellular Mg2+ while holding the cell at -70 mV and found that there was no significant difference. This confirms that the involvement of postsynaptic NMDAR is unlikely. However, Mg2+ insensitive metabotropic NMDAR signaling may be a probable mechanism. Hence, we tested MK-801 (NMDAR open channel blocker) and found that it largely blocks TBOA-TIC to a similar extent to D-AP5, indicating that ionotropic, not metabotropic signaling of NMDAR is involved. It is possible that TBOA-induced increase in

ambient Glu could indirectly excite MCH neurons by activating NMDAR on other cells. As TBOA-TIC is insensitive to tetrodotoxin that blocks neuronal activity, it may involve astrocytic NMDAR activation followed by release of gliotransmitters such as ATP, which can in turn activate excitatory P2X receptors on MCH neurons. To test this, PPADs (P2XR antagonist) was used, which blocked TBOA-TIC to a similar extent as D-AP5 or MK-801, indicating that indeed ATP mediates NMDAR-dependent TIC. Taken together, our results are consistent with the idea that ambient Glu acts on astrocytic NMDAR to trigger ATP release, which in turn stimulates MCH neurons via P2XR. This may represent a novel mechanism of astrocyte-neuron interaction that regulates the excitability of MCH neurons. Given the known role of MCH neurons, this may have functional implications on sleep and energy homeostasis.

Disclosures: **M. Chowdhury:** None. **S. Bowes:** None. **M. Hirasawa:** None.

Digital Abstract Session

P041. Glutamate

Program #/Poster #: P041.03

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: Lundbeck Foundation

Title: Protons as a putative second messenger in synaptic plasticity

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Abstract: The hippocampus is a complex structure involved in learning and memory. Its main cell type are pyramidal neurons which are glutamatergic neurons. It has been reported that upon glutamatergic, NMDA, AMPA or KCl stimulation, these neurons undergo a brief period of cytosolic acidification, reaching pH 6-6.5. The underlying mechanism of this phenomenon remains unknown, although it has been shown to be dependent on extracellular calcium. pH homeostasis is known to be crucial for proper neuronal functioning, however, it is rarely considered in neuronal activity; There is evidence showing that activity-induced changes in extracellular and intracellular pH are linked to altered neuronal excitability. Moreover, its relevance in synaptic plasticity has also been suggested. Therefore, due the potential implications of this stimulation-dependent acidification, we have investigated the role of intracellular calcium in this phenomenon, as well as exploring the source of H^+. Additionally, we have tested whether this acidic response occurs not only during neuronal stimulation but also during synaptic plasticity. To this end, we employed a novel dual biosensor approach; we used cyto-pHluorin, a pH sensing GFP variant, together with the Ca^{2+}-sensor R-GECO1.2, which allows us to correlate changes in pH to increases in cytosolic Ca^{2+} levels in response to neuronal activation within individual cells. These sensors were transfected into hippocampal primary cultures and live imaging experiments were performed under different drug conditions. To test the role of cytosolic calcium we treated the neurons with Ionomycin, Verapamil, and BAPTA-AM. These

approaches showed that a rise of intracellular calcium itself is not sufficient to trigger the pH drop and depending on the source of stimulation, cytosolic calcium can be a requirement or not for the acidification to occur. Moreover, by alkalization of the extracellular environment and therefore removing the H^+ gradient, we further obtained results suggesting that glutamate receptors might have a conductance for H^+. Finally, to test whether this activity-induced acidification occurs during synaptic plasticity, we switched to a more physiological set up; acute brain slices from adult rats transduced with the sensors were electrophysiologically stimulated to induce LTP. The recordings from CA1 neurons showed a transient cytosolic acidification under the LTP conditions. All together, these findings reveal that the activity-induced acidification mechanism and its requirements varies depending on the stimulation source and this phenomenon is taking place not only in regular activity but also during synaptic plasticity.

Disclosures: A. Konomi Pilkati: None. **S. Pedersen:** None. **K. Madsen:** None.

Digital Abstract Session

P041. Glutamate

Program #/Poster #: P041.04

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NSF1655365
SUNY Albany
SUNY Research Foundation

Title: The neuronal glutamate transporter EAAC1 reduces the excitability of D1 medium spiny neurons

Authors: *M. A. PETROCCIONE, L. D'BRANT, N. AFFINNIH, H. CHESBRO, S. ZAHID, A. SCIMEMI;
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Abstract: The neuronal glutamate transporter EAAC1 is expressed at central excitatory and inhibitory synapses. At excitatory synapses, EAAC1 limits glutamate escape from the synaptic cleft and extra-synaptic receptor activation. At inhibitory synapses, EAAC1 promotes GABA synthesis and release. The dorsolateral striatum is a region of the basal ganglia where EAAC1 is most abundantly expressed. Here, D1- and D2-dopamine receptor expressing medium spiny neurons (D1- and D2-MSNs, respectively) receive excitatory inputs from the thalamus and cortex, and inhibitory inputs from other MSNs and local interneurons. Here, regulating the time course and strength of excitation and inhibition is important to ensure coordinated movement execution. It is currently unknown whether and how EAAC1 controls various sources of excitation and inhibition. Here we use electrophysiology and optogenetics approaches to show that EAAC1 only strengthens inhibition at a subset of inhibitory synapses formed between D1-MSNs. By using compartmental models of D1-MSNs, we show that this effect leads to a multiplicative scaling of the output firing of D1-MSNs that is most pronounced at high

frequencies of incoming synaptic excitation. Together, these findings underscore some of the fundamental mechanisms that allow neuronal glutamate transporters to shape cell excitability by limiting spillover and by promoting neurotransmitter biosynthesis and release.

Disclosures: **M.A. Petroccione:** None. **L. D'Brant:** None. **N. Affinnih:** None. **S. Zahid:** None. **H. Chesbro:** None. **A. Scimemi:** None.

Digital Abstract Session

P042. Small-Molecule Neurotransmitters: Release, Reception and Uptake

Program #/Poster #: P042.01

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH project ZIA AA000407

Title: Stimulation-evoked acetylcholine and choline release in striatum detected with genetically-encoded sensors

Authors: *D. M. LOVINGER, J. A. NADEL, L. VOYVODIC, A. G. SALINAS;
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Abstract: Acetylcholine (ACh) is a prominent neurotransmitter in the striatum derived from cholinergic interneurons (CINs) and brainstem afferents. ACh effects on striatal physiology and related behaviors are well characterized. Less is known about ACh release dynamics and extracellular levels. The ACh metabolite choline can activate α7 subunit-containing nicotinic ACh receptors. Recent development of genetically-encoded periplasmic binding protein-based (iAChSnFR) and G protein-coupled receptor-based (GRAB-ACh3.0, ACh3.0) ACh sensors allows for real-time extracellular ACh measurement. We virally expressed these sensors in dorsomedial striatum, and used brain slice photometry to examine release dynamics. Spontaneous increases in fluorescence lasting a few sec were observed in slices expressing either sensor. In slices expressing iAChSnFR, single intrastriatal electrical stimuli evoked fluorescent transients lasting 10s of seconds. Similar stimuli produced much shorter-lasting transients in slices expressing ACh3.0. The transients observed with both sensors were blocked by tetrodotoxin and reduced in low extracellular calcium. The ACh esterase inhibitor Tacrine prolonged stimulus-induced transients measured with either sensor. We examined mechanisms that might contribute to the prolonged increase observed with iAChSnFR. The transients detected with iAChSnFR showed a biphasic decay with time constants of a few seconds and 10s of seconds. Tacrine converted this to a single decay component lasting 10s of seconds. Microstimulation from a pipette electrode evoked long-lasting transients, but responses at the lowest stimulus intensities persisted for only a few sec. Optogenetic stimulation of CINs using Chrimson produced fluorescence transients persisting for 10s of sec. Unlike ACh3.0, iAChSnFR is activated by choline at uM concentrations. We observed fluorescence increases upon extracellular application of ACh (1-1000 uM) or choline (10-1000 uM) in slices expressing iAChSnFR, with sensitivity higher for ACh. To determine if enhancing choline levels leads to

fluorescence increases we applied the choline transporter inhibitor ML-352. This compound enhanced fluorescence measured with iAChSnFR. Both ACh sensors can detect stimulus-induced ACh increases with comparable sensitivity. However, iAChSnFR may also detect stimulus-induced choline increases. Stimulation-induced choline levels appear to be in the lower end of the range that can activate α7-containing nAChRs. Thus it will be interesting to determine if striatal α7 nAChRs (e.g. on corticostriatal glutamatergic terminals) can be activated by this type of stimulation.

Disclosures: D.M. Lovinger: None. **A.G. Salinas:** None. **J.A. Nadel:** None. **L. Voyvodic:** None.

Digital Abstract Session

P042. Small-Molecule Neurotransmitters: Release, Reception and Uptake

Program #/Poster #: P042.02

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: Faperj
CNPq
Capes
INNT

Title: Adenosine A3 receptors couple to vitamin C transporter SVCT2 and regulate neuronal redox balance

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Abstract: Adenosine is a vital neuromodulator involved in several aspects of CNS physiology and development such as regulation of neurotransmitter release, myelin formation, neurite outgrowth, and neuronal survival. Adenosine activates A1, A2a, A2b and A3 receptors, widely expressed in CNS, which are all G protein-coupled receptors that classically modulate adenylyl cyclase activity. A1 and A3 receptors are usually coupled to inhibitory G proteins to decrease intracellular cAMP, while A2a and A2b receptors usually couple to stimulatory G proteins and increase intracellular cAMP. A3 receptors signal through Gi or Gq proteins, leading to adenylyl cyclase inhibition or phospholipase C stimulation and increased diacylglycerol, IP3 and intracelular calcium. Vitamin C is an antioxidant that exerts several neurophysiological functions such as the formation of the Schwann cell myelin sheath, regulation of acetylcholine release, regulation of NMDA receptor function, and acts as a co-factor in a plethora of enzymatic reactions such as the conversion of dopamine to norepinephrine and the synthesis of neuropeptides. The vitamin C oxidized form, dehydroascorbate (DHA), is taken up by glucose transporters while ascorbate, its reduced form, is taken up by the Sodium Vitamin C coTransporters SVCT1 and 2. Our previous work showed the presence of adenosine and vitamin

C systems in avian retinal cultures. Here we studied the interplay between the adenosinergic system and ascorbate transport in the cultures. We found that selective activation of A3, but not A1 or A2a adenosine receptors, modulates ascorbate transport, decreasing intracellular ascorbate content. Förster resonanse energy transfer (FRET) analysis showed that A3 receptors associate with the ascorbate transporter SVCT2, suggesting tight signaling compartimentalization between A3 receptors and SVCT2. The activation of A3 receptors increases ascorbate release in an SVCT2-dependent manner, largely altering the neuronal redox status without interfering with cell death, glycolytic metabolism and bioenergetics. We conclude that, by regulating Vitamin C transport, the adenosinergic system (via activation of A3 receptors) can regulate ascorbate bioavailability and controls the redox balance in neurons.

Disclosures: R. Paes-de-Carvalho: None. **C.C. Portugal:** None. **T.G. da Encarnação:** None. **M.A. Sagrillo:** None. **M.R. Pereira:** None. **J.B. Relvas:** None. **R. Socodato:** None.

Digital Abstract Session

P042. Small-Molecule Neurotransmitters: Release, Reception and Uptake

Program #/Poster #: P042.03

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: Beijing Municipal Science & Technology Commission (Z181100001318002)
grants from the Peking-Tsinghua Center for Life Sciences
grants from the State Key Laboratory of Membrane Biology at Peking University School of Life Sciences

Title: Molecular, localization and proteomic profiling reveal a putative vesicular transporter for UDP-glucose

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Abstract: Vesicular neurotransmitter transporters (VNTs) mediate selective uptake and enrichment of small molecule neurotransmitters into synaptic vesicles (SVs), and are therefore one major determinant of synaptic output for specific neurons. All the known VNTs belong to solute carrier (SLC) transporter family. Given that SLC contains more than 450 members, with ~one third of which are still orphan or poorly characterized, we hypothesize there exist novel VNTs in the SLC family, and their cognate substrates are potential novel neurotransmitters or neuromodulators. To identify putative new SV transporters, we molecularly cloned 361 human SLC transporters to fuse them in frame with a fluorescent protein, then carried out a localization profiling in cultured neurons by co-expressing SLCs with a known SV marker as well as other organelle markers. Using known VNTs' localization score as a benchmark, we identified ~30

novel transporters capable of localizing to SVs. To validate their in vivo existence, we immune-isolated native SVs from mouse brains. Proteomic analysis revealed the presence of 7 (out of 20) transporters, with a subset of orphan SLC35 subfamily transporters, SLC35D3, SLC35F1 and SLC35G2. Further ultrastructural analysis by electron microscopy confirmed SLC35D3's ability to localize on SVs. Using mass spectrometry-based metabolite profiling and radioactive transport assay, we identified and confirmed UDP-glucose, a nucleotide-sugar, as a specific substrate for SLC35D3. Finally, we performed radioactive transport assay using native SVs derived from mouse brain, and observed UDP-glucose, but not its close analog UDP-galactose, was able to be selectively uptaken into SVs in a way sensitive to vesicular proton electrochemical gradient. In sum, our localization and proteomic analysis identified unexpected rich vesicular transporter candidates and among them SLC35D3 as a novel vesicular UDP-glucose transporter. These efforts would yield new insights to the function of SLC family genes and provide better understanding of the molecular diversity of chemical neurotransmitters.

Disclosures: C. Qian: None. **Z. Wu:** None. **R. Sun:** None. **H. Yu:** None. **J. Zeng:** None. **Y. Rao:** None. **Y. Li:** None.

Digital Abstract Session

P042. Small-Molecule Neurotransmitters: Release, Reception and Uptake

Program #/Poster #: P042.04

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: 2P50MH100023-06

Title: Vagal nerve stimulation modulates levels of select monoamines in primate CSF

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Abstract: Vagal nerve stimulation (VNS), used successfully to treat epilepsy, depression, chronic pain, and even neuro-inflammation, brought to light evidence that increasing afferent traffic along the vagus nerve elevates the level of monoamines in the brain, increases regional blood flow in key brain areas for affect, and alters cellular activity and plasticity. Similar effects are observed in response to behavioral practices that increase vagal tone. These effects appear associated with, but not causally explained by, peripheral autonomic changes. To explore the neurochemical effects of VNS, we harvested CSF from an Ommaya reservoir implanted in an anesthetized monkey instrumented for vagal nerve stimulation. We measured the levels of monoamines in the CSF before and after low-frequency stimulation, expected to increase afferent vagal inputs to the brain, and high-frequency stimulation, expected to block traffic along the vagal nerve. Using ion-pair high performance liquid chromatography with electrochemical detection (HPLC-ECD), we monitored changes induced by VNS in the levels of dopamine and

its metabolites (HVA, DOPAC, 3-MT), norepinephrine and its main metabolite (MHPG) and the main metabolite of serotonin (5-HIAA). The baseline levels of these neurotransmitters and metabolites were comparable to those detected in CSF collected from other NHPs (from cisterna magna and lumbar puncture), as well as to those reported in the literature. Low-frequency VNS increased the CSF levels of dopamine and its metabolites, as well as 5-HIAA and MHPG, but not of norepinephrine. Following high-frequency VNS, the levels of all CSF analytes appeared to decrease. These findings replicate previously reported increases in serotonin (but not norepinephrine) metabolites induced by VNS and bring to light a significant elevation of dopamine metabolites that have been rarely reported in humans and animals undergoing VNS. Future experiments in awake, behaving monkeys will determine whether the observed elevation of dopamine metabolites was related to anesthesia or represents an outcome of low-frequency VNS.

Disclosures: B.T. Seaton: None. **M.J. Avila:** None. **D. O'Neill:** None. **A.J. Fuglevand:** None. **M.L. Heien:** None. **K.M. Gothard:** None.

Digital Abstract Session

P042. Small-Molecule Neurotransmitters: Release, Reception and Uptake

Program #/Poster #: P042.05

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant MH105094 to RDB

Title: Kappa opioid receptor antagonism normalizes biochemical and behavioral phenotypes in mice expressing the ADHD-associated dopamine transporter variant Val559

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Abstract: Extracellular dopamine (DA) is tightly regulated by the presynaptic dopamine (DA) transporter (DAT). The rare DAT Val559 variant, identified in individuals with attention-deficit hyperactivity disorder (ADHD), bipolar disorder and autism, mediates a constant, transporter-mediated outwardly directed leak of DA. As a result thereof, DAT Val559 KI mice reveal behavioral alterations, including waiting impulsivity, hyperactivity in response to imminent handling, elevated motivation for reward and blunted responses to psychostimulants. At a molecular level, the DAT Val559 variant in the dorsal striatum of male mice displays elevated surface trafficking, augmenting effects of efflux prone transporters and arising from the tonic stimulation of D2-type autoreceptors (D2ARs). Since kappa opioid receptors (KORs), in addition to D2ARs, regulate DAT-surface trafficking, we explored whether pharmacological manipulation of KOR-activity might normalize the aberrant trafficking of the Val559 variant and

thereby present a novel opportunity to relieve mutation induced hyperdopaminergia. Using acute coronal slices, we find that both homozygous Val559 DAT animals and WT animals are amenable to regulation via KOR, i.e. activation of KOR increases surface trafficking regardless of genotype and that this increase in surface trafficking coincides with an increase in phosphorylation at threonine 53 in Val559 DAT and WT mice. Remarkably, antagonism of KOR reduced surface trafficking of DAT Val559 but remained without significant effect on WT DAT, ultimately normalizing surface expression of Val559 DAT. Finally, using fiber photometry and microdialysis, we show that systemic administration of the KOR-antagonist nor-binaltorphimine (norBNI) normalizes DA-release in Val559 mice and reverses deficits observed in working memory (Y-maze) and anxiety-like behavior (open field test). Together, our findings support KORs as a target for the treatment of disorders that arise from tonically-elevated extracellular DA.

Disclosures: F.P. Mayer: None. **M. Velez:** None. **A. Stewart:** None. **R.A. Vaughan:** None. **R.D. Blakely:** None.

Digital Abstract Session

P042. Small-Molecule Neurotransmitters: Release, Reception and Uptake

Program #/Poster #: P042.06

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant MH105094
NARSAD Young Investigator Grant #28731

Title: Sexually dimorphic behavioral outcomes in DAT Val559 mice derive from sex-specific homeostatic perturbations at pre- and post-synaptic sites

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Abstract: The release, reuptake and signaling of the powerful neurotransmitter dopamine (DA), a major determinant of reward, motivation, attention, and motor activity, and the architecture underlying dopamine-engaged brain circuitry, are increasingly understood to be modulated genetic/gonadal sex and sex hormones. Animal models expressing neuropsychiatric-disease associated variants in the DA transporter gene (DAT, *SLC6A3*), such as the Ala559Val substitution, have provided a platform to investigate the impact of sex on neurochemistry, neurophysiology and behavior in a construct-valid disease model. Here, we report a series of sexually dimorphic homeostatic DA disruptions that arise in the DAT Val559 homozygote. In male DAT Val559 mice, presynaptic nigrostriatal D2 autoreceptors (D2ARs) are constitutively-activated, driving enhanced DAT phosphorylation and surface trafficking in the dorsal striatum, an effect that is absent in DAT Val559 females. However, females show constitutive D2AR

activation of mesolimbic D2ARs, an effect that is absent in males. Multiple DAT Val559 behavioral alterations also differ by sex including male-specific blunting of acute amphetamine (AMPH)-induced locomotion, faster extinction following AMPH place preference conditioning, and decreased anorexigenic actions of AMPH following repeated exposure. Interestingly, the ability of AMPH to provoke DA elevations in the dorsal striatum was unaltered in DAT Val559 males, which we speculate may result from DAT Val559 leak antagonism in the context of AMPH-induced DA vesicular release, whereas AMPH drives DA release via DAT-mediated efflux in females. Finally, we noted that direct stimulation of postsynaptic D1Rs with the agonist SKF83822 resulted in sex-specific behavioral alterations with DAT Val559 males displaying decreased horizonal locomotion and stereotypy whereas females display reduced vertical locomotion and center occupancy. Thus, we hypothesize that loss of acute AMPH-induced locomotion in DAT Val559 males may result from desensitization, uncoupling or other disruption of D1R-dependent signal transduction. In females, the locomotor response to AMPH is likely maintained, despite reductions in AMPH-induced DA elevations, via increased sensitivity of DA-responsive striatal neurons. Overall, these studies provide striking evidence of a sexually dimorphic impact of functional DAT coding variation and reveal an important opportunity to gather insights into the sex-dependence of behavioral trajectories that attends neuropsychiatric disease risk.

Disclosures: A. Stewart: None. **F.P. Mayer:** None. **R. Gowrishankar:** None. **G.L. Davis:** None. **R. Peart:** None. **K. Spiess:** None. **R.D. Blakely:** None.

Digital Abstract Session

P042. Small-Molecule Neurotransmitters: Release, Reception and Uptake

Program #/Poster #: P042.07

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH XXXXXX

Title: Mitochondrial fatty acid synthesis pathway sustains energy metabolism necessary for the function and health of dopamine neurons

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Abstract: Dopamine (DA) modulates neural signaling across phylogeny. Disruption of DA signaling in humans has been implicated in multiple brain disorders including schizophrenia, addiction, ADHD, and Parkinson's disease. In C. elegans, loss of the DA transporter (DAT-1) results in a phenotype termed Swimming-induced paralysis (Swip). We previously reported the isolation of several mutant lines using a Swip-based mutagenesis screen, which has proved

effective in identifying molecular determinants of DAT activity, DA synaptic transmission and DA neuron degeneration. Here, we report the molecular basis of a Swip line involving mutation of the mitochondrial gene dhs-11. Loss of DHS-11 result in reduced mitochondrial respiration, changes in mitochondrial structure and biogenesis, elevated oxidative stress, and age-dependent DA neurodegeneration. Sequencing analysis reveals that dhs-11 is an ortholog of the human mitochondrial enzyme HSD17B8, a member of the highly conserved mitochondrial fatty acid synthesis pathway (mtFAS), which supports the synthesis of α-lipoic acid (α-LA), a key substrate for the metabolic posttranslational process of protein lipoylation. Consistent with our attribution of dhs-11 as the locus for mutation, mutants in other worm mtFAS genes resulted in DA-dependent Swip. LC-MS/MS analyses of dhs-11 extracts vs wildtype (N2) animals revealed metabolomic signatures of mitochondrial dysfunction, manifested by alteration of metabolites involved in glycolysis, TCA cycle, amino acid anaplerosis, fatty acid β-oxidation. In support of this idea, enhancing energy production via overexpression of the monocarboxylate transporter MCT-1/2, or by supplementation of α-LA, lactate or pyruvate, rescued the Swip of dhs-11 mutants. Mutation of the lipoamidase SIR-2.3/SIRT4 to stabilize mitochondrial protein lipoylation rescued dhs-11 Swip, suggesting that reduction in mitochondrial lipoylation contributes to dhs-11 Swip. Pharmacological and genetic studies also support a role for dhs-11 in regulating normal DAT activity and DA neuron excitability. Moreover, exogenous α-LA suppression of Swip requires the DA transporter DAT-1, suggesting a contribution of the mtFAS pathway in DAT-1 regulation. Additionally, α-LA treatment significantly ameliorated signs of DA neurodegeneration. Together, our results uncover an essential role for dhs-11/mtFAS pathway in regulation of DA neuron function and neuroprotection, suggesting the molecule and its regulators as potential targets for the development of therapeutics for DA-linked disease.

Disclosures: **O. Refai:** None. **P. Rodriguez:** None. **V. Kalia:** None. **S. Mellish:** None. **Z. Gichi:** None. **J. Hardaway:** None. **C. Rongo:** None. **E. Park:** None. **G.G. Miller:** None. **R. Blakely:** None.

Digital Abstract Session

P042. Small-Molecule Neurotransmitters: Release, Reception and Uptake

Program #/Poster #: P042.08

Topic: B.05. Neurotransmitter Release

Support: NIH Grant DA035714
NIH Grant DA041932

Title: Novel allosteric modulator attenuates HIV-1 Tat protein-induced inhibition of dopamine transporter and alleviates cognitive and cocaine rewarding effects in HIV-1 Tat transgenic mice

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Abstract: Cocaine abuse has been shown to increase the incidence of HIV-1 associated neurocognitive disorders (HAND). We have demonstrated that HIV-1 Tat protein allosterically modulates dopamine (DA) reuptake via DA transporter (hDAT). This study determined whether a novel allosteric modulator, SRI-32743, pharmacologically blocks Tat binding to DAT and alleviates Tat-potentiated cocaine rewarding effects in inducible HIV-1 Tat transgenic (iTat-tg) mice. The iTat-tg mouse model recapitulates many aspects of neurocognitive impairments observed in HIV infected individuals. SRI-32743 inhibited [^{3}H]DA uptake (IC_{50}, 9.9 μM) with a 17-fold greater inhibition than the potency of [^{3}H]WIN35,428 binding (IC_{50}, 168 μM) with 68.4% and 71.4% of its E_{max}, respectively. Tat (140 nM) induced 30% and 20% reductions in [^{3}H]DA uptake and [^{3}H]WIN35,428 binding, respectively, which were attenuated by 50 nM SRI-32743. SRI-32743 and indatraline, a competitive DAT inhibitor, increased the cocaine IC_{50} values of [^{3}H]DA uptake by 164% and 280%, respectively. The cocaine-induced dissociation rate of [^{3}H]WIN35,428 binding was similar to that induced by 50 nM SRI-32743; however, SRI-32743 slowed the cocaine-induced dissociation rate. Pharmacokinetics study shows that SRI-32743 is BBB-permeable with a 2.52 and 2.08 ratio of brain to plasma concentration (ng/ml) at 15 and 60 min, respectively. Following a 14 day-doxycycline to induce Tat protein, the iTat-tg mice exhibit a 2-fold potentiation of cocaine-CPP which was dose-dependently ameliorated by pretreatment of SRI-32743 (1 or 10 mg/kg/d, i.p.) prior to CPP. These preliminary data raise the exciting possibility of potential therapeutic interventions for neurocognitive dysfunction in HAND, particularly effective for those with concurrent cocaine abuse.

Disclosures: J. Zhu: None. **C. Zhan:** None. **J.P. McLaughlin:** None. **S. Ananthan:** None.

Digital Abstract Session

P043. Amino Acids and Other Neurotransmitters

Program #/Poster #: P043.01

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Title: Neuromodulatory influences in the hindlimb representation of the developing rat somatosensory cortex

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Abstract: Neuromodulators control information processing in cortical microcircuits by regulating the cellular and synaptic physiology of neurons. Computational models and detailed simulations of neocortical microcircuitry offer a unifying framework to analyze the role of neuromodulators on network activity. In the present study, we quantified the axonal anatomy of catecholaminergic, serotonergic and cholinergic systems using immunocytochemical staining

and stereological techniques to gain insights into how the structural organization of neuromodulatory projections in the cortical neuropil regulate the emergence of network states. We systematically integrated biological data into a detailed computational model of the rodent somatosensory cortex and simulated the effects of ascending neuromodulatory projections. We predict that dose-dependent effects of neuromodulators on diverse neuron types and synapses reveal a fine-grained control of a spectrum of network activity states. Low levels of neuromodulatory signaling drive microcircuit activity into slow oscillations and network synchrony, whereas high concentrations govern fast oscillations and network asynchrony. The models and simulations thus provide a unifying in silico framework to bridge how the anatomical organization of neuromodulatory projections reconfigure the physiology of network activity in the neocortex.

Disclosures: C. Colangelo: None. **A. Muñoz:** None. **J. DeFelipe:** None. **S. Ramaswamy:** None. **A. Anton-Fernandez:** None.

Digital Abstract Session

P043. Amino Acids and Other Neurotransmitters

Program #/Poster #: P043.02

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Title: Laterality effect of chronic restraint stress on TPH2 expression and serotonin turnover in adrenal glands of rats.

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Abstract: Background The adrenal gland is an essential stress-responsive organ that is part of both the hypothalamic pituitary-adrenal axis and the sympathy-adrenomedullary system (Gamallo et al.,1986). Previous studies using the viral trans neuronal tracing technique demonstrated central autonomic circuits more intense in the left adrenal gland (LAG) than the right adrenal gland (RAG) (Tóth et al., 2008). Our Previous studies, Chronic restraint stress (CRS) has been shown to magnify acute stress-induced corticosterone (CORT) secretion in rats through a mechanism involving serotonin (5-HT) via activation of 5-HT7 receptors and apparently, dissociation from ACTH secretion (García-Iglesias et al., 2013). The results of the present study are consistent with the notion that the ectopic expression of 5-HT7 receptors and 5-HT levels in adrenocortical steroidogenic cells is a glucocorticoid dependent phenomenon (Saroj et al., 2019). Furthermore, serotonin is an important neurotransmitter that broadly participates in various biological processes. As a result of evidence for the sensitivity of tryptophan hydroxylase-2 (TPH-2), the rate-limiting enzyme of the serotonin (5-HT) pathway, (Patel et al., 2004). **Abstract -** In present results we have demonstrated that the exposure of the animals to CRS for two weeks, one day 15 animals were sacrificed and both adrenal glands was collected. Adrenal weight, thymus weight and body weight gain were recorded. TPH-2 expression was

determined by immunohistochemistry, PCR and western blot and 5-HT turnover was measured by HPLC in both adrenals. Although CRS significantly increases mRNA and protein expression of TPH2 and (5-HT) turnover in the LAG as compare to RAG. These results conclude that CRS might be prompt more sensory and sympathetic neurons innervate in LAG to be the cause of more perception to the stress and increase stress related protein expression in the LAG than the RAG.

Disclosures: N. Saroj: None. **S. Shanker:** None.

Digital Abstract Session

P043. Amino Acids and Other Neurotransmitters

Program #/Poster #: P043.03

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Title: Taurine plays a key role as a morphogen in the neurogenic process of Subventricular Zone progenitor cells by GABA receptors interaction

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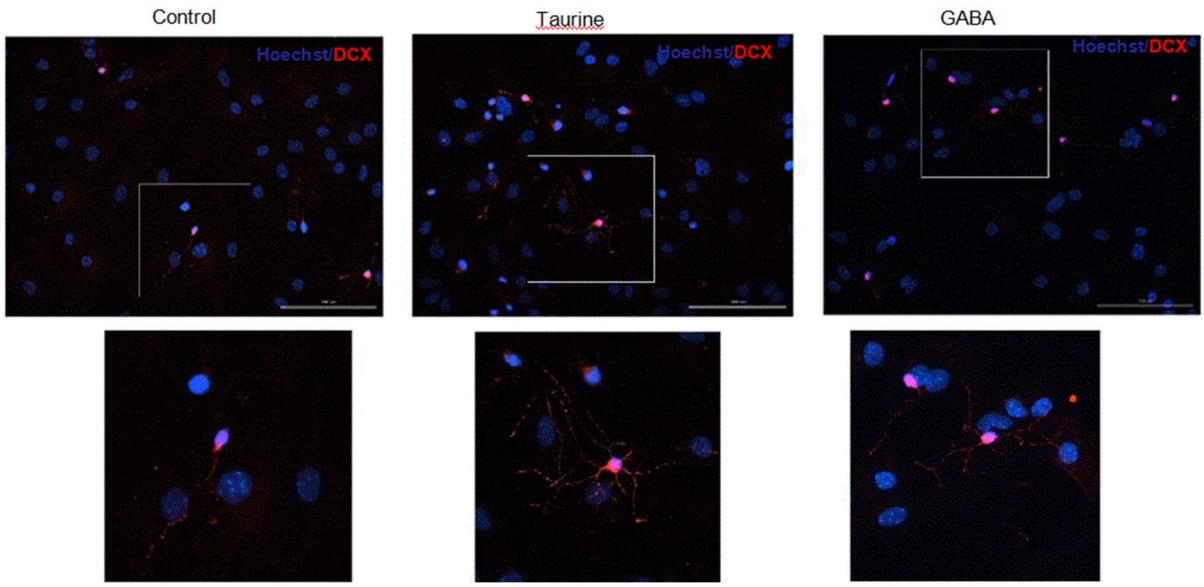
Abstract: The process of neurogenesis occurs throughout the life of mammals, including humans, in the subgranular zone of the hippocampal and the subventricular zone (SVZ) of the lateral ventricles. The participation of extrinsic factors, which regulate this process, has been determined. These factors include neuroactive molecules such as taurine, which play an important role in the processes of proliferation, differentiation, and migration of neural progenitor cells. The mechanism through which it participates in the neurogenesis process is not completely understood, one possibility is through the interaction with inhibitory neurotransmitters receptors, expressed in progenitor cells. Therefore, we aimed to evaluate the effect of taurine on the differentiation process of neural progenitor cells in the subventricular zone through the interaction with GABA receptors. We carried out immunofluorescence assays on cells isolated from SVZ of CD1 mice (P8) and exposed to differentiation conditions, with or without taurine. The results showed that the neural progenitor cells express Sox-2 and Nestin, both progenitor cells markers, nonetheless no signal for DCX was observed. This observation confirms that the cells obtained from the SVZ are neural progenitors. In the cultures exposed to differentiation conditions with taurine, increase the number of cells DCX+. The morphometric analysis showed a significant difference in cell morphology; the cells treated with taurine, presented a greater number of secondary and tertiary neurites, compared to the control and GABA conditions. This effect of taurine was sensitive to picrotoxin, an antagonist of GABA receptors. In conclusion, this result provides us information regarding the role of taurine as a morphogen in the neurogenic processes throughout the interaction with GABA receptors. This

work represents an advance in the morphometric effect of taurine in the neuronal differentiation process.



Disclosures: **N.E. Gutiérrez-Castañeda:** None. **J. Gonzalez:** None. **E.J. Galvan:** None. **H. Sanchez-Castillo:** None. **L.D. Ocho-de la Paz:** None.

Digital Abstract Session

P043. Amino Acids and Other Neurotransmitters

Program #/Poster #: P043.04

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Title: A tale of two tracers: [11C] Raclopride and [18F] Fallypride produce related estimates of dopamine receptor binding potential across a wide range in BMI under identical, controlled clinical conditions

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Abstract: Dopamine is involved in a number of diverse behaviors and experiences including mood, memory, habit formation, reward, and eating behavior. Imaging via Positron Emission Tomography (PET) allows investigation of various aspects of dopamine system function in the human brain. However, use of different radiotracers may lead to challenges interpreting and comparing results across studies. For example, though both are antagonists to the D2/D3 receptor subtypes, [18F]fallypride is selective for D2 receptors over D3 receptors, while [11C]raclopride is non-selective in terms of D2R and D3R. Furthermore, [18F]fallypride has higher affinity for

receptor while [11C]raclopride is more readily displaced from receptor by endogenous dopamine. Here, we present preliminary results of an ongoing PET study measuring striatal dopamine D2/D3 receptor binding potential (D2BP) across a wide range in BMI using two different dopamine receptor antagonist radiotracers, [11C]raclopride and [18F]fallypride, in a within-subjects design under controlled clinical conditions (ClincalTrials.gov, NCT03648892). To control the influence of recent diet and weight fluctuation on D2BP, 13 of a planned 39 healthy, weight-stable adults (8 female; age 28.4±2.2 y, BMI 30.2±2.3, range 20.9-45.1 kg/m2) completed a 5-day standardized eucaloric outpatient diet stabilization followed by a 5-day inpatient stay. Participants completed [11C]raclopride PET and [18F]fallypride PET scans in semi-random order under identical testing conditions (i.e., overnight fast, AM scan time). T1-weighted MR images were collected for coregistration of D2BP and neuroanatomical data. Freesurfer-generated region of interest masks were used to assess D2BP via time-activity curves in the caudate, putamen, accumbens, and pallidum. Preliminary analyses show a positive correlation between D2BP as measured by [11C]raclopride and [18F]fallypride in the pallidum ($r=0.851$, $p=0.002$) and a trending relationship in the caudate ($r=0.524$, $p=0.066$) suggesting a concordance between the two tracers in the dorsal and ventral striatum across a wide range in BMI. Preliminary data in the putamen and accumbens show a weak positive relationship but are not significant with $n=13$ ($r=0.383$, $p=0.197$; $r=0.358$, $p=0.230$). These preliminary findings may facilitate interpretation and comparison of results across [11C]raclopride and [18F]fallypride studies and may be important to inform methodological design of future studies of dopamine receptor availability.

Disclosures: **A. Schick:** None. **V.L. Darcey:** None. **J. Guo:** None. **I. Gallagher:** None. **M. Stagliano:** None. **L. Milley:** None. **A. Courville:** None. **S. Yang:** None. **S. Turner:** None. **P. Herscovitch:** None. **K.D. Hall:** None.

Digital Abstract Session

P044. Neuropeptide Signalling

Program #/Poster #: P044.01

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Title: Rifaximin modulates TRH and TRH-like peptide expression throughout the brain and peripheral tissues of male rats

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Abstract: Abstract Mental health and protection from neurodegenerative disorders, such as Alzheimer's and Parkinson's disease and major depression depends on the gut microbiome and is mediated by the vagus nerve. This is evident in the behavioral abnormalities of germ-free rodents. The antibiotic rifaximin (RF), which does not cross the gut-blood barrier, is a standard treatment for traveler's diarrhea and hepatic encephalopathy. It also has therapeutic benefit in the treatment of prostatitis. TRH and TRH-like peptides, with the structure pGlu-X-Pro-NH_2, where

"X" can be any amino acid reside, have reproductive, caloric-restriction-like, anti-aging, pancreatic-β cell-enhancing, cardiovascular, and neuroprotective effects. The TRH/TRH-R1 receptor signaling pathway is an important mediator of brain-gut axis communication via the brain medulla. TRH and TRH-like peptides occur not only throughout the CNS but also peripheral tissues, with particularly high levels in rat and human prostate. This is particularly noteworthy given the vulnerability of humans to prostatitis and prostate cancer. Bacterial infections are involved in prostate diseases. Female rats were not studied because TRH and TRH-like peptide levels fluctuate 10- to 100-fold in response to the estrus cycle. For this reason 16 young adult male Sprague-Dawley rats were divided into 4 groups. The control (CON) group remained on ad libitum chow and water for 10 days. The acute (AC) group received ad libitum chow and water for 9 days and then 1 g rifaximin (RF)/500 g rat chow for 24 h. The chronic (CHR) animals received RF in chow for 10 days. The withdrawal (WD) rats received RF chow for 8 days and then normal chow for 2 days. TRH and TRH-like peptide levels were measured in medulla oblongata (MED), frontal cortex (FCX), hypothalamus (HY), amygdala (AY), hippocampus (HC), piriform cortex (PIR), nucleus accumbens (NA), entorhinal cortex (ENT), striatum (STR), cerebellum (CBL), anterior cingulate (ACNG), posterior cingulate (PCNG), prostate (PR), liver (L), testis (T), heart (H), pancreas (PAN), adrenals (AD) and epididymis (EP). Highly significant changes in the levels of TRH and TRH-like peptides occurred throughout the brain and peripheral tissues in response to RF treatment. In brain the number of these changes (in parentheses)
declined in the order: MED(17), NA(13), PIR(12), STR(11), AY(10), FCX(9), ENT(6), ACNG (6), HC(5), PCNG(5). In peripheral tissues the corresponding order was: PR(11), AD(11), L(9), T(6), H(3), PAN(3). We conclude the high responsiveness of MED, FCX, ACNG, PCNG in brain and PR, AD, L is consistent with TRH and TRH-like peptides participating in the therapeutic effects of RF.

Disclosures: A.E. Pekary: None. **A. Sattin:** None.

Digital Abstract Session

P044. Neuropeptide Signalling

Program #/Poster #: P044.02

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: MSCA-ITN 764860

Title: Cgrp dose-dependently influences neuronal excitability and cortical spreading depolarization (csd) in rodents

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Abstract: The neuropeptide CGRP plays an important nociceptive role in migraine, and modern CGRP antagonists are effective against migraine pain. CGRP has been suggested to increase neuronal excitability and therefore we tested its influence on ongoing brain activity and CSD in mice and rats. In anesthetized adult rats (sodium thiopentone, 100 mg/kg, i.p.) CSD-related DC deflections and changes in extracellular potassium concentration ($[K^+]_e$) were recorded in CGRP-treated and untreated areas of the cortex. CSD was elicited by a microinjection of 1 M KCl, and 100 µl of CGRP at concentrations of 10^{-8} M, 10^{-7} M, 10^{-6} M, or 10^{-5} M were applied topically for four hours onto the brain surface in the treatment area. In both cortical areas rCBF was measured. Leakage of the blood-brain-barrier was monitored by extravasation of intravenously injected Evans Blue. Size and shape of glial and microglial cells were evaluated by immunohistochemistry (Iba 1 and GFAP). In mouse cortical and cerebellar slices (300 µm) electrophysiological recordings were performed with multi-electrode arrays before and after application of CGRP at 10^{-7} M and 10^{-6} M. In the treated but not in the untreated area, amplitudes of CSD were reduced by CGRP (10^{-5} M to nearly 50 % of controls; 10^{-8} M to nearly 70 % of controls). CGRP slowed the propagation velocity of CSD (10^{-5} M from 3.0 to 2.4 cm/s; 10^{-8} M from 2.6 to 2.4 cm/s). Assessment of Evans Blue fluorescence confirmed blood-brain-barrier leakage. AC-filtering of the electrocorticogram revealed trains of abnormal discharge activity (series of spikes up to focal ictal discharges) in 67 % of the rats treated with 10^{-7}-10^{-5} M of CGRP in treated area only with no obvious dose-dependence. In cortical but not in cerebellar slice preparations CGRP evoked repetitive and discharging network activity that was not removed by wash. Immunostaining of Iba 1 showed fragmented glial cells in the treated area, but no change in astrocytes. Pretreatment with the antagonist $CGRP_{8-37}$ at 10^{-7} M prevented the effects of CGRP at 10^{-5} M on CSDs, and in co-application $CGRP_{8-37}$ acted as a partial agonist. Similar results were obtained when $CGRP_{8-37}$ was used in slice recordings. Thus the neuropeptide CGRP appears to affect microglia and excite neurons up to spike-like discharging, but only rarely ignites CSD waves. It slows down the propagation velocity of CSD waves possibly via plasma extravasation. Whether this decrease is a result of the increased neuronal activity or a specific action of CGRP on receptors for excitatory amino acids is currently elucidated in ongoing experiments.

Disclosures: F. Richter: None. **F. Gimeno-Ferrer:** None. **A. Eitner:** None. **R. Bauer:** None. **H. Schaible:** None. **A. Lehmenkuhler:** None. **M. Kress:** None. **M. Edenhofer:** None.

Digital Abstract Session

P045. Nicotinic Acetylcholine Receptors

Program #/Poster #: P045.01

Topic: B.02. Ligand-Gated Ion Channels

Support: Texas A&M University

Title: Proline mutations in first transmembrane domain suggest important extrinsic interactions during biogenesis of extracellular domain α4β2 nicotinic acetylcholine receptors

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Abstract: **Background**: Extracellular domain (ECD) receptors from α4 and β2 nicotinic receptor subunits truncated after the first transmembrane domain (M1) have functional and structural similarity to full length α4β2 nicotinic acetylcholine receptors (nAChRs). The smaller size of ECD α4β2 nAChRs is advantageous for structural studies. Their reduced complexity also might reveal contributions from ECD and transmembrane domains to the structure, function, and biogenesis of nAChRs. Mutations of the first proline of M1 greatly reduce expression of ECD α4β2 nAChRs. X-ray and cryo-EM structures of full length α4β2 nAChR suggest that interactions between this first proline and the Cys loop might contribute to that reduced expression. **Objective**: To determine whether adding a valine mutation at the second proline of M1 rescues the reduced expression caused by a valine at the first proline. Mutation of the second proline is not expected to affect expression because of the presumed lack of physical interaction between the two sites and the hypothesized importance of interactions of the first proline and Cys-loop. **Methods**: Human α4 and β2 cDNAs were truncated after M1 (α4M1 and β2M1). Along with 5 inserted residues from proximal M1 at the ECD/M1 interface, the first and second prolines of M1 in α4M1 and β2M1 were mutated to valine singly and together, leading to subunits denoted α4PP, α4PV, α4VP, α4VV and β2PP, β2PV, β2VP, β2VV. Subunits were expressed in *Xenopus laevis* oocytes. Immunoblotting and immunoisolated [^{3}H]epibatidine binding sites assessed expression of subunits and ECD α4β2 nAChRs. **Results**: Expression of α4PP/β2PP and α4M1/β2M1 nAChRs were comparable. For the second proline site, α4PV/β2M1, α4M1/β2PV, and α4PV/β2PV nAChRs showed expression comparable to α4M1/β2M1 nAChRs. For the first proline site, expression of α4VP/β2VP nAChRs was severely reduced compared to α4M1/β2M1 nAChRs. α4VP/β2M1 and α4M1/β2VP showed moderately reduced expression. Contrary to expectation, mutating both the first and second prolines, leading to α4VV/β2VV nAChRs, rescued expression. **Conclusions:** The importance of intrinsic interactions within an ECD subunit between the first proline and the Cys-loop to the expression of ECD α4β2 nAChRs needs further investigation. Because of the presumed physical isolation of the second proline site within the membrane bilayer, the rescue of expression by the second valine suggests interactions that are extrinsic to the ECD subunits also may affect the expression of ECD α4β2 nAChRs. Extrinsic interactions might involve cellular machinery that identifies and translocates transmembrane domains for correct topology during biogenesis of α4β2 nAChRs.

Disclosures: **G.B. Wells:** A. Employment/Salary (full or part-time):; Texas A&M University. F. Consulting Fees (e.g., advisory boards); AveXis. **A.M. Person:** A. Employment/Salary (full or part-time):; Texas A&M University.

Digital Abstract Session

P045. Nicotinic Acetylcholine Receptors

Program #/Poster #: P045.02

Topic: B.02. Ligand-Gated Ion Channels

Title: Signaling mechanisms underlying nicotine-induced upregulation of α7 nicotinic acetylcholine receptor (nAChR)

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Abstract: α7 nicotinic acetylcholine receptors (nAChRs), the second most abundant nAChR in brain, have been shown to contribute to nicotine addiction, in part through upregulation by nicotine. Previously, nicotine-upregulation of α7 nAChRs has been reported in several studies, though the mechanisms remain unclear. We have shown ~2-fold functional and numerical upregulation of α7 Rs in *Xenopus* oocytes following 100 μM nicotine treatment. Further, we found that the nic-upregulation of α7 nAChRs was dependent upon intracellular calcium ($[Ca^{2+}]_i$) (being abolished by BAPTA-AM). In various cell types, G protein signaling regulates α7 nAChR function via modulation of $[Ca^{2+}]_i$. α7 nAChRs contain a canonical RMKR G-protein binding cluster (GPBC) within their transmembrane (TM) 3-4 cytosolic loop. Here, we demonstrate a critical role of α7 nAChR/$G\alpha_q$ protein interaction in activation of PLC β - produced increases in $[Ca^{2+}]_i$ that, in turn, inhibits endocytic pathways to cause nic-upregulation of α7 nAChRs. Mutation at GPBC completely abrogated nic-upregulation of α7 Rs. Furthermore, when exogenous wt $G\alpha_q$ was co-expressed with α7 Rs, we saw that it produced ~2X upregulation. But when dominant negative $G\alpha_q$ was co-expressed, attenuation of nic-upregulation of wt α7 Rs was observed. Anti-sense oligonucleotides (ASOs) against endogenous Gq also blocked nic-upregulation. Additionally, we found that the calcineurin inhibitor cyclosporine A produced 2X-upregulation of wt α7 Rs and occluded nic-upregulation. Other endocytic inhibitors (PitStop2 and DynaSore) produced ~2X upregulation of both wt and mutant α7 Rs and occluded nic-upregulation. Brefeldin A (BFA, inhibitor of ER-Golgi protein-transport) failed to affect nic-upregulation. Conversely, methyllycaconitine (MLA)-upregulation of α7 was independent of $[Ca^{2+}]_i$ but was inhibited by BFA. Serine-threonine kinase and tyrosine kinase-phosphorylation events have inhibitory effects on forward trafficking of α7 Rs. Nic-upregulation was unaffected when serine-365 or tyrosine-442 (from TM3-4 loop) was mutated, further supporting the hypothesis that α7 nic-upregulation arises from sustained GPCR-Ca^{2+}-signaling by α7 Rs, inhibiting constitutive endocytosis of α7 Rs.

Disclosures: J. Panchal: None. **M. Islam:** None. **K. DeBoeuf:** None. **J. Farley:** None. **J.B. Anderson:** None.

Digital Abstract Session

P045. Nicotinic Acetylcholine Receptors

Program #/Poster #: P045.03

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH NIDA Grant U19-DA027990

Title: The contribution of the two agonist binding sites in the function of human nicotinic cholinergic receptor possessing the α4, β2, and β4 nAChR subunits

Authors: C. C. SWAIN, H. M. GRAY, T. T. OLSON, K. J. KELLAR, R. P. YASUDA; Pharmacol. & Physiol., Georgetown Univ., Washington, DC

Abstract: The contribution of the two agonist binding sites in the function of human nicotinic cholinergic receptor possessing the α4, β2, & β4 nAChR subunits.
Caroline C. Swain, Hayley M. Gray, Thao T. Olson, Kenneth J. Kellar, and Robert P. YasudaDepartment of Pharmacology & Physiology, Georgetown University, Washington DC 20057Disclosures: C.C. Swain: None. H.M. Gray: None. T.T. Olson: None. K.J. Kellar: None. R.P Yasuda: None.Brain regions that express the α4, β2, and β4 nicotinic acetylcholine receptor (nAChR) subunits include the hippocampus, medial habenula, interpeduncular nucleus, and cerebellum. We designed 3 cDNA plasmids that express human nAChR concatemers composed of the α4 & β2, α4 & β4, α4, β2, & β4 nAChR subunits linked together by hydrophilic amino acids and expressed in heterologous cells. The order and stoichiometry of these concatemers are β4α4β4α4β4 (β4ALL), β4α4β4α4β2 (β4β2), and β2α4β2α4β2 (β2 ALL) with the amino-terminus found on the first subunit and the carboxyl-terminus found on the fifth subunit. Each of these concatemers produces a full-length protein (>300 kDa). [^{3}H]-epibatidine binding competition curves in cells expressing the β4β2 concatemer reveals a significant 2-site fit for both sazetidine-A (Saz-A; which has higher affinity for β2-containing receptors) and AT-1001 (which has higher affinity for β4-containing receptors), while binding competition curves with these two ligands in cells expressing the β4 ALL and β2 ALL concatemers fit best to a single site with K_i values that agree with those in the literature. To study the function of these concatemers, nAChR mediated calcium (Ca^{++}) influx will be measured using an aequorin based assay. Here we describe preliminary results of our aequorin assay using heterologous cells that were transfected with the α3β4, α4β2, or α4β2α5 nAChR subunits. ACh-stimulated dose response curves resulted in EC_{50} values consistent with the literature. We will use cells expressing the concatemeric nAChRs to examine concentrations of Saz-A expected to stimulate only the α4β2 binding site or AT-1001 expected to stimulate only the α4β4 binding site and compare the responses to a maximal concentration of ACh (1 mM). These experiments should allow us to determine the degree of activation of the nAChR via a single α4β2 or α4β4 binding site in a nAChR concatemer possessing both binding sites.

Disclosures: C.C. Swain: None. **H.M. Gray:** None. **T.T. Olson:** None. **K.J. Kellar:** None. **R.P. Yasuda:** None.

Digital Abstract Session

P046. Ionotropic Glutamate Receptors

Program #/Poster #: P046.01

Topic: B.02. Ligand-Gated Ion Channels

Support: NINDS R01 NS088479 (LPW)

NINDS Diversity Supplement (NC)
Dr. W. Burghardt Turner Dissertation Fellowship (NC)

Title: Role of auxiliary subunits in the biogenesis of AMPA receptors

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Abstract: AMPA receptors (AMPARs) transmit the majority of fast excitatory synaptic transmission. Changes to the composition and number of AMPARs at the postsynaptic membrane underlie synaptic plasticity, a cellular correlate of learning and memory, and are implicated in various brain disorders. The molecular mechanisms that regulate the pools of available AMPARs to drive changes in synaptic transmission remain unclear. Native AMPARs associate with auxiliary subunits that can affect the availability receptors, but how they do so is not well understood. Here, we addressed the role of auxiliary subunits in the assembly of AMPARs using blue native gel-electrophoresis, immunocytochemistry, and electrophysiology. We find that cornichon-2 (CNIH-2) and cornichon-3 (CNIH-3) enhance the tetramerization process, whereas stargazin (TARP-2) is less able to do so. To study the basis for this, we mutated key residues in the transmembrane domain which attenuate but do not remove tetramerization and co-expressed mutant AMPARs with auxiliary subunits. When the tetramer was destabilized this led to increased receptor dimers and significantly reduced tetramer formation. When the AMPAR mutants were co-expressed with CNIH-2 or CNIH-3, the tetramer fraction was efficiently rescued compared to co-expression with TARP-2. However, when TARP-2 was in tandem with the AMPAR mutant, the tetramer fraction was restored comparable to wildtype AMPARs. We also find that auxiliary subunits influence receptor tetramerization through the transmembrane domain with CNIHs capable of stabilizing the transmembrane region and rescuing disrupted receptor assembly. In contrast, TARP-2 has a much weaker influence on tetramerization compared to CNIHs unless in tandem when the auxiliary subunit is forced to interact with the receptor during the assembly process. We conclude that CNIHs and TARPs differ in their ability to influence the stages of AMPAR biogenesis.

Disclosures: **N. Certain:** None. **Q. Gan:** None. **L.P. Wollmuth:** None.

Digital Abstract Session

P046. Ionotropic Glutamate Receptors

Program #/Poster #: P046.02

Topic: B.02. Ligand-Gated Ion Channels

Support: R21MH116315 to JAG
d R01MH117130 to JAG

Title: Increased excitation-inhibition balance and loss of GABAergic synapses in the serine racemase knockout model of NMDA receptor hypofunction

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Abstract: There is substantial evidence that both NMDA receptor (NMDAR) hypofunction and dysfunction of GABAergic neurotransmission contribute to schizophrenia, though the relationship between these pathophysiological processes remains largely unknown. While models using cell-type-specific genetic deletion of NMDARs have been informative, they display overly pronounced phenotypes extending beyond those of schizophrenia. Here, we used the serine racemase knockout (SRKO) mice, a model of reduced NMDAR activity rather than complete receptor elimination, to examine the link between NMDAR hypofunction and decreased GABAergic inhibition. The SRKO mice, in which there is a >90% reduction in the NMDAR co-agonist D-serine, exhibit many of the neurochemical and behavioral abnormalities observed in schizophrenia. We found a significant reduction in inhibitory synapses onto CA1 pyramidal neurons in the SRKO mice. This reduction increases the excitation/inhibition balance resulting in enhanced synaptically-driven neuronal excitability and elevated broad-spectrum oscillatory activity in *ex vivo* hippocampal slices. Consistently, significant reductions in inhibitory synapse density in CA1 were observed by immunohistochemistry. We further show, using a single-neuron genetic deletion approach, that the loss of GABAergic synapses onto pyramidal neurons observed in the SRKO mice is driven in a cell-autonomous manner following the deletion of SR in individual CA1 pyramidal cells. These results support a model whereby NMDAR hypofunction in pyramidal cells disrupts GABAergic synapse development leading to disrupted feedback inhibition and impaired neuronal synchrony.

Disclosures: S.A. Jami: None. **S. Cameron:** None. **J.M. Wong:** None. **E.R. Daley:** None. **K.A. McAllister:** None. **J.A. Gray:** None.

Digital Abstract Session

P046. Ionotropic Glutamate Receptors

Program #/Poster #: P046.03

Topic: B.02. Ligand-Gated Ion Channels

Support: NSERC Discovery Grant

Title: Differential expression of GluN2A, GluN2B and GluN2D NMDA receptor subunits in the dorsal horn of male and female rat spinal cord

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Abstract: N-methyl-D-aspartate receptors (NMDARs) are excitatory ionotropic glutamate receptors expressed throughout the CNS, including in the dorsal horn (DH) of the spinal cord. The GluN2 subtypes of NMDAR subunit, GluN2A, 2B and 2D, confer NMDARs with structural

and functional variability, enabling heterogeneity in synaptic transmission and plasticity. Despite essential roles for NMDARs in physiological and pathological pain processing within the DH, the distribution and function of specific GluN2 isoforms across DH laminae remains poorly understood. Surprisingly, there is a complete lack of knowledge on GluN2 expression in female rodents. We therefore investigated the relative expression of specific GluN2 variants in the L4/L5 lumbar DH of both male and female rats. In order to detect synaptic GluN2 isoforms that are expressed in the DH (GluN2A, 2B and 2D), we used pepsin antigen-retrieval to unmask these highly cross-linked protein complexes. We found that GluN2B and GluN2D were preferentially localized to superficial regions of the DH in males, while only GluN2B was predominantly localized in the superficial DH of female rats. Furthermore, unexpectedly, we identified an enhanced expression of GluN2B in the medial division of the superficial horn versus the lateral division, in males only. These sex-specific localization patterns of GluN2-NMDAR subunits have significant implications for the understanding and treatment of pain and are relevant for future translational studies in human spinal cord tissue.

Disclosures: S. Temi: None. **C. Rudyk:** None. **J. Armstrong:** None. **J. Landrigan:** None. **C. Dedek:** None. **N. Salmaso:** None. **M.E. Hildebrand:** None.

Digital Abstract Session

P046. Ionotropic Glutamate Receptors

Program #/Poster #: P046.04

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH IRP
Portuguese Foundation for Science and Technology (FCT) post-doctoral fellowship SFRH/BI/106010/2015
Australian Research Council Discovery Project grant DP190101390
Australian National Health and Medical Research Council Senior Research Fellowship APP1136021

Title: Understanding the functional implications of NMDA receptor subunit rare variants associated with neurodevelopmental disorders

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Abstract: Despite intensive research and groundbreaking advances in the genetics of neurological disorders such as autism spectrum disorders (ASDs) and epilepsy, a profound knowledge of their underlying cellular mechanisms remains elusive. Accumulating evidence from genetic analyses has uncovered two crucial factors: many of the associated genes in

epilepsy are also affected in ASDs/intellectual disability, suggesting a common mechanism of dysfunction; and specific clusters of proteins are preferentially affected at the gene level indicating that disorders arise from an overall dysfunction of protein networks. Rare variants identified in the cluster of synaptic proteins, including the N-methyl-D-aspartate receptor (NMDAR) subunits GluN2A and GluN2B, are thought to be causative in some cases of epilepsy and ASD, respectively. Because GRIN2 rare variants are heavily implicated in neurodevelopmental disorders, we investigated the functional consequences of *de novo* single nucleotide mutations in the C-terminal domain of GluN2A and GluN2B subunits of NMDARs. Since the C-terminal domain has preponderant roles in the regulation of receptor surface expression and trafficking, protein-protein interactions and in many cases excitatory postsynaptic currents (EPSCs), we hypothesized a functional impact of these variants in these parameters. We characterized the functional consequences of GluN2A/B rare variants using a combination of biochemical and imaging approaches. One particular variant, GluN2A-S1459G, led to reduced receptor surface expression, as well as concomitant spine density alterations in cultured hippocampal neurons. Using pull-down and co-immunoprecipitation assays, we found that the epilepsy variant leads to impaired binding with scaffold proteins like PSD-95, as well as with the endocytic protein SNX27. Finally, we observed that expression of GluN2A-S1459G leads to a decrease in spontaneous EPSCs. Notably, we also identified GluN2A-S1459 as a phosphorylation target, mediated by CaMKII, that modulates receptor trafficking and protein-protein interactions. Overall, our results demonstrate that the epilepsy mutant GluN2A-S1459G is a loss-of-function variant. Our results highlight the importance of understanding the functional implications of individual disease-associated NMDAR subunit variants.

Disclosures: **M.M. Vieira:** None. **T.A. Nguyen:** None. **K. Wu:** None. **J.D. Badger:** None. **B.M. Collins:** None. **V. Anggono:** None. **W. Lu:** None. **K.W. Roche:** None.

Digital Abstract Session

P046. Ionotropic Glutamate Receptors

Program #/Poster #: P046.05

Topic: B.02. Ligand-Gated Ion Channels

Support: NINDS R01 NS088479
T32 GM127253

Title: Using calcium dynamics to assess the clinical outcome of NMDA receptor variants

Authors: *G. MOODY, A. MUSCO, L. P. WOLLMUTH;
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Abstract: Channelopathies are clinical disorders arising from alterations in ion channels. As genetic panels become more accessible in the clinic, there is a growing list of identified missense and nonsense mutations or ‘variants’ underlying these channelopathies and potentially driving neurological disorders such as epilepsy. The NMDA receptor (NMDAR) is a ubiquitously

expressed glutamate-gated ion channel that plays key roles in numerous brain functions including the plasticity underlying learning and memory. Not surprisingly, a variety of disease-associated variants have been identified in genes encoding NMDAR subunits. A critical first step to assess whether these variants contribute to their associated disorder is to characterize their effect on receptor function. Using this characterization, variants are categorized as loss- or gain-of-function to potentially guide treatment. This categorization, however, is not ideal since it often misses the complexity of ion channel function and the complex effects of variants, which rarely just affect one biophysical property, instead altering many in potentially subtle and opposing ways. As a first approximation, one can assume that the central signaling mechanism of NMDARs is to regulate Ca^{2+} influx at synapses with the magnitude of this Ca^{2+} influx modulated by their diverse biophysical properties such as voltage-dependent block by Mg^{2+}, receptor kinetics (rates of activation, deactivation, desensitization), small molecules (H^{+}, Zn^{2+}, polyamines), and post-translational modifications. Here, we characterized a diverse set of epilepsy-associated variants. We developed a means to combine multiple affected biophysical properties, including conflicting properties, into a single parameter based on the magnitude of Ca^{2+} influx. Notably, to capture the dynamics of NMDARs at synapses, we applied glutamate pulses at 100 Hz to derive a current integral and transformed this integral into a 'Ca^{2+} index' using fractional calcium data. We then compared which properties most greatly affected Ca^{2+} influx and assigned a severity to the variant depending on changes in Ca^{2+} influx. We further correlated this Ca^{2+} index across difference clinical phenotypes and identified which biophysical properties have the greatest impact on Ca^{2+} influx.

Disclosures: G. Moody: None. **A. Musco:** None. **L.P. Wollmuth:** None.

Digital Abstract Session

P047. Ionotropic GABA Receptors and Glycine Receptors

Program #/Poster #: P047.01

Topic: B.02. Ligand-Gated Ion Channels

Support: Conicyt-21171549 Fellowship (COL)
UCO-1866 (COL)
FONDECYT-1170252 Grant (GEY)
VRID-219.033.111-INV (GMC)
FONCYT-PICT20171120 (CB)
UNS (CB)

Title: Exploring the action mechanisms underlying the effects of positive allosteric modulators of glycine receptors

Authors: *C. O. LARA[1], G. MORAGA-CID[1], A. M. MARILEO[1], V. P. SAN MARTÍN[1], A. E. SAZO[1], J. CORRADI[2], J. FUENTEALBA[1], L. GUZMÁN[1], P. CASTRO[1], L. G. AGUAYO[1], C. BOUZAT[2], G. E. YÉVENES[1];

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Abstract: Glycine Receptors (GlyRs) are pentameric anion-permeable ligand-gated ion channels. The glycinergic neurotransmission regulates critical physiological processes, such as respiration, motor coordination, muscle tone and sensory processing. In addition, hypo-functional GlyRs have been related to several pathological states, such as chronic pain and hyperekplexia. The modulation of GlyRs by positive allosteric modulators (PAMs) has recently emerged as a promising therapeutic approach against chronic pain. However, the GlyR pharmacology is still limited and only few studies have explored the action mechanisms of glycinergic PAMs at the functional level. Here, we studied the mechanisms of modulation of GlyRs by two glycinergic PAMs that displayed analgesic actions in behavioral studies. By using single-channel and whole-cell electrophysiology of recombinant GlyRs, we focus our functional analyses on the effects of the propofol analog 2,6-di-tert-butylphenol (2,6-DTBP) and of the tricyclic sulfonamide AM-1488. Whole cell recordings show that both 2,6-DTBP and AM-1488 exerted concentration-dependent potentiation of homomeric GlyRs. However, while AM-1488 enhanced the function of homomeric and heteromeric GlyRs in a similar fashion, 2,6-DTBP displayed minor effects on heteromeric GlyRs. Single-channel recordings revealed that the effects of 2,6-DTBP and AM-1488 are associated with different biophysical mechanisms of modulation. Kinetic analysis show that 2,6-DTBP generated longer burst time and longer channel openings, whereas AM-1488 also produced longer burst time but without modifications on the open time. Our results reveal different biophysical mechanisms of modulation for PAMs targeting GlyRs. These findings may contribute to the development of novel glycinergic PAMs with differential modulatory profiles.

Disclosures: C.O. Lara: None. **G. Moraga-Cid:** None. **A.M. Marileo:** None. **V.P. San Martín:** None. **A.E. Sazo:** None. **J. Corradi:** None. **J. Fuentealba:** None. **L. Guzmán:** None. **P. Castro:** None. **L.G. Aguayo:** None. **C. Bouzat:** None. **G.E. Yévenes:** None.

Digital Abstract Session

P047. Ionotropic GABA Receptors and Glycine Receptors

Program #/Poster #: P047.02

Topic: B.03. G-Protein Coupled Receptors

Support: Research to prevent blindness

Title: Picrotoxin increased the excitability of mouse retinal ganglion cells

Authors: *W. LI, J. WEILAND;
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Abstract: Title: Picrotoxin increased the excitability of mouse retinal ganglion cells
Abstract:Glycine and γ-aminobutyric acid (GABA), one of the major inhibitory neurotransmitters in the retina, is secreted from amacrine cells and regulates ganglion cells

function. This study focuses on the effect of GABA on the mice retinal ganglion cells (RGCs) to identify the regulatory role of amacrine cells in RGCs. We used *ex vivo* mouse retina and the whole-cell current clamp technique to study the effect of picrotoxin (antagonist of GABAa receptor) on the RGCs baseline and electrically evoked responses. RGCs were classified into ON, OFF, and ON-OFF types using 10 second light exposure. No significant difference in resting membrane potential was noted amongst these groups. Electrical stimulation pulses were applied via an epiretinal electrode (platinum, 75 micron diameter). Pulses were 0.5 ms, cathodic first, biphasic, with a train of 20 pulses delivered at 2 pulses/second. To obtain response data, pulse amplitude was increased in steps. Then the number of pulses that evoked spikes was counted and threshold was set at the 50% response rate. No difference was noted in threshold based on cell type. After administration of picrotoxin (100 μM), threshold decreased (control: 73.6±15.02μA; 100 μM picrotoxin: 62.36±13.95 μA, n=25, p=0.02782). RGCs spontaneous spikes rate increased after picrotoxin (control: 13.16±3.85 spikes/s; 100 μM picrotoxin: 25.27±4.65 spikes/s, n=24, $p=8.581x10^{-5}$). These implied that blocking amacrine cells GABAa receptor increased the excitability of RGCs as reflected by decreases in action potential threshold.

Disclosures: W. Li: None. **J. Weiland:** None.

Digital Abstract Session

P048. Neuromodulator and Neuropeptide Receptor Signaling

Program #/Poster #: P048.01

Topic: B.03. G-Protein Coupled Receptors

Title: Serotonergic effects on orbitofrontal cortex parvalbumin neurons expressing cre recombinase are unchanged after withdrawal from cocaine self-administration in rats

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Abstract: Cocaine is the second-most used illicit drug and is responsible for the second-highest number of overdose deaths in the United States, a number that has sharply increased in recent years. As a non-specific monoamine re-uptake inhibitor, cocaine modulates a variety of brain areas. One region involved in many use disorder behaviors is the orbitofrontal cortex (OFC). This highly interconnected associative learning structure is crucial for higher order function that underlies goal-directed learning and comprehension of consequences, both of which are disrupted by cocaine self-administration (CSA). The OFC is densely innervated by serotonergic (5-HT) projections from the dorsal raphe nucleus (DRN), and 5-HT function is necessary for goal-directed OFC behaviors. Notably, 5-HT function is also altered in principal cells in the OFC several months after withdrawal from CSA in rats. However, little is known about the effects of CSA on 5-HT control of inhibitory parvalbumin fast-spiking interneurons (PVNs) in the OFC. PVNs act as regulators of cortical gamma oscillations that underlie OFC-dependent behaviors and also express 5-HT_{1A} and 5-HT_{2A} receptor mRNA in the nearby medial pre-frontal cortex

(mPFC). Here, 5-HT function was assessed via a novel parvalbumin-Cre rat using electrophysiology and optogenetics, including sex as a biological variable. Direct application of 5-HT to OFC PVNs in brain slices elicited 5-HT_2-dependent inward depolarizing currents in PVNs, in line with previous frontocortical studies. In a minority of cells (4/28, 14.3%) from male rats, 5-HT produced outward, inhibitory currents that were putatively 5-HT_{1A}-dependent, while PVNs from female rats demonstrated no outward currents under similar conditions. Furthermore, whereas 5-HT treatment markedly increases glutamatergic transmission in principal cells, it had no effect on glutamatergic afferents to PVNs, suggesting 5-HT selectively regulates OFC network control in a target-specific manner. In contrast to our previous study with OFC principal cells, neither the magnitude nor the proportion of 5-HT effects in PVNs were altered following withdrawal from CSA, demonstrating a cell-type specific perturbation by cocaine. This variable impact on excitatory output cells and inhibitory PVNs likely alters the overall excitatory/inhibitory balance in the OFC, and this may underlie the behavioral abnormalities seen following withdrawal from CSA.

Disclosures: A.M. Wright: None. **A.F. Hoffman:** None. **B.K. Harvey:** None. **C.R. Lupica:** None.

Digital Abstract Session

P048. Neuromodulator and Neuropeptide Receptor Signaling

Program #/Poster #: P048.02

Topic: B.03. G-Protein Coupled Receptors

Support: Tata Institute of Fundamental Research, Department of Atomic Energy, Government of India, under project no. RTI4003

Title: 5-ht_{2a} receptor stimulation: a sirt-ain target to enhance mitochondrial biogenesis and function in cortical neurons

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Abstract: In neurons, mitochondria are essential to regulate bioenergetics, facilitate synaptic transmission and plasticity, as well as modulate survival under stress. We have recently demonstrated a direct link between the neurotransmitter serotonin (5-HT) signaling and the regulation of mitochondrial metabolism, biogenesis and function in cortical neurocircuits. We further characterize early events following 5-HT_{2A} receptor stimulation, which are a precursor to enhance mitochondrial biogenesis. 5-HT_{2A} receptor stimulation with the agonist DOI, results in enhanced mitochondrial biogenesis, ATP production and spare respiratory capacity in rodent

cortical neurons. Conversely, 5-HT_{2A} receptor blockade or genetic loss of function abrogates the effects of 5-HT on mitochondrial biogenesis and ATP production. 5-HT_{2A} receptor stimulation of cortical neurons, resulted in a dose and time-dependent increase in *Sirt1* levels, which was blocked by the 5-HT_{2A} receptor antagonist MDL100,907. The increase in *Sirt1* was mediated via the PLC and MAPK signaling pathways. The increase in mitochondrial biogenesis and function, via 5-HT_{2A} receptor stimulation required SIRT1. Chronic 5-HT_{2A} receptor stimulation with the agonist DOI, increased cortical mitochondrial DNA and ATP levels in a SIRT1-dependent manner. Seahorse analysis performed on isolated mitochondria derived from cortices of vehicle- and DOI-treated rats revealed enhanced state-2 (via complex-I/II) and state-3 (complex II-dependent) respiration and ATP production rate following DOI administration, demonstrating an increase in OxPhos efficiency. Further, 5-HT_{2A} receptor stimulation was found to be neuroprotective and mediate stress adaptation against excitotoxic and oxidative stressors, an effect that required SIRT1. Our present work highlights the 5-HT_{2A} receptor-SIRT1 axis as a potential therapeutic target to ameliorate mitochondrial dysfunction, which has implications in aging, neuropsychiatric and neurodegenerative diseases.

Disclosures: S.E. Fanibunda: None. **U. Ghai:** None. **S. Deb:** None. **B. Maniyadath:** None. **N. Weisstaub:** None. **J. Gingrich:** None. **A.D. Vaidya:** None. **U. Kolthur:** None. **V.A. Vaidya:** None.

Digital Abstract Session

P048. Neuromodulator and Neuropeptide Receptor Signaling

Program #/Poster #: P048.03

Topic: B.03. G-Protein Coupled Receptors

Support: National Institute of Mental Health Intramural Research Program, Project 1ZIAMH002386

Title: Transcriptional investigation of the effects of PACAP loss: Acute versus developmental effects

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Abstract: Since the advent of gene knockout technology in 1987, insight into the role(s) of neuropeptides has been gleaned by examining loss or alteration of function in animals constitutively engineered to be deficient in transcription of either neuropeptide prohormones or their cognate G-protein coupled receptors. These results are often interpreted as indicating that expression of the gene in question *at the time of the experiment* is required for normal function, complementing lines of experimentation in which the peptide is infused into the brain or transcription blocked acutely. In the case of PACAP, the effect is generally assumed to be via the PAC1 receptor, to which PACAP binds with high affinity. However, neuropeptides such as PACAP play numerous developmental roles: When a gene is constitutively knocked out, long-

term effects of its loss may alter the baseline physiology of the animal. A way to support 'real-time' interpretations of neuropeptide gene knock-out is to demonstrate that the wild-type transcriptome, except for the deliberately deleted gene(s), in tissues of interest, is preserved in the knock-out mouse. Here, we show in adult mice that there is a cohort of genes (constitutively PACAP regulated genes, or cPRGs) whose basal expression is affected by constitutive knock-out of the Adcyap1 gene in multiple neuroendocrine tissues of C57Bl6/N Adcyap1 knockout mice, and additional genes whose expression is altered acutely (aPRGs) in response to physiological challenge. Further, we uncover a surprising lack of transcriptional phenocopy between the PACAP knockout and PAC1 knockout models, mirroring their puzzling divergent behavioral phenotype: While PACAP KO mice perform stereotyped repetitive jumping, PAC1 KO mice do not. Distinguishing constitutive and acute transcriptomic effects of neuropeptide deficiency on physiological function and behavior in mice may prompt a reevaluation of the mechanisms of actions and actual functions of neuropeptides themselves, in the central nervous system, throughout the lifespan.

Disclosures: D. Bakalar: None. **H. Zhang:** None. **S. Sweat:** None. **Z. Jiang:** None. **B. Samal:** None. **L.E. Eiden:** None.

Digital Abstract Session

P048. Neuromodulator and Neuropeptide Receptor Signaling

Program #/Poster #: P048.04

Topic: B.03. G-Protein Coupled Receptors

Support: Universidad Nacional de Colombia, Hermes No. 41821.

Title: Acute lithium administration enhances Gq/PLC-mediated signaling

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Abstract: Although lithium has long been one of the most widely used pharmacological agents in psychiatry, its mechanisms of action remain poorly understood. Biochemical observations link Li to inositol lipid metabolism, but physiological evidence of effects on phosphoinisitide-mediated signaling is lacking. Having garnered robust preliminary evidence on the potentiating effect of Li^+ on Ca mobilization in HEK-293 cells, we turned to SHSY5Y, a human neuroblastoma cell line, as a more pertinent model to probe the effects of acute Li exposure in a neuronal context. This line expresses key elements of the PLC pathway, and in response to carbachol it produces an increase of cytosolic Ca, monitored with Fluo-4, that is largely unaffected by removal of external Ca and is suppressed by U-73122, thus reflecting PLC-dependent release from internal stores. Low millimolar Li significantly enhanced the Ca-fluorescence signal in a reversible way. Cholinergic stimulation also evokes an inward current mostly carried by Na, that is suppressed by PLC inhibition. The amplitude of this current was also robustly enhanced by Li, and its time course accelerated. In an attempt to narrow down the

site of action of this effect, we examined the impact of Li exposure when the muscarinic pathway was directly activated at selected downstream links. The enhancement was still evident when the current was stimulated by GTP-γ-S, but disappeared if direct PLC activation was induced by m-3M3FBS. Comparison of reversal potentials and the use the inert form o-3M3FBS strengthened the contention that it was the same current triggered by carbachol. The observations indicate that Li effects occur at the level of the G_q protein and its interaction with PLC-β. The generality of such results is being explored in primary vertebrate neurons, where preliminary experiments indicate a modulatory action of Li on the metabotropic glutamatergic response of Purkinje cells isolated from the cerebellum. These observations document an up-regulation of Gq/PLC/IP3-mediated signaling by acute exposure to lithium at near-therapeutic levels, manifesting itself in defined physiological responses monitored in individual cells.

Disclosures: **C. Sanchez:** None. **F. Mantilla:** None. **C. Guevara:** None. **M. Gomez:** None. **E. Nasi:** None.

Digital Abstract Session

P048. Neuromodulator and Neuropeptide Receptor Signaling

Program #/Poster #: P048.05

Topic: B.03. G-Protein Coupled Receptors

Support: National Science Centre, Poland (2016/20/S/NZ7/00424)

Title: The effects of TC-G 1008, GPR39-zinc receptor agonist, on hippocampal free zinc and serum zinc concentrations in the pentylenetetrazole model of epilepsy

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Abstract: Introduction: The G-protein coupled receptor 39 (GPR39) is activated by zinc ions and has been suggested as a novel drug target for epilepsy. Our *in vivo* results mostly argue against the hypothesis that activation of GPR39 alleviates seizure. We have observed that TC-G 1008, a potent and selective agonist of this receptor, facilitated the development of pentylenetetrazole (PTZ) kindling and inhibited phosphorylated cyclic-AMP response element binding (p-CREB) and high-affinity tropomyosine-related kinase B receptor (p-TrkB). There is an association between total serum zinc and epilepsy in patients and between free zinc and status epilepticus in experimental animals. Unlike total serum zinc, the free zinc is responsible for signaling. We examined these fractions of zinc following administration of TC-G 1008 in the PTZ model of epilepsy. **Methods:** The PTZ kindling was performed in male Albino Swiss mice (bodyweight range 25-30 g) (approval no 38/2017). The mice received TC-G 1008 (10 mg/kg i.p.) 30 min (based on previous pharmacokinetic analysis), a non-selective GPR39 agonist, zinc

(given as zinc chloride, $ZnCl_2$) (8 mg Zn/kg) or valproic acid (VPA) (150 mg/kg) before each dose of PTZ. Control mice received the respective doses of drugs and vehicle (VEH) instead of PTZ. 24 h after the last dose of PTZ, tissue was harvested. Serum zinc levels were measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Free zinc levels were measured in hippocampal sections using membrane-permeable fluorescent probe Zinpyr-1. The sections were double stained with DAPI. Additional sections were treated with membrane-permeable zinc chelator, TPEN, before staining with Zinpyr-1 and DAPI. The sections were imaged using Leica DM6000 B microscope. Data were analyzed using Image J and Graph Pad Prism v. 5.03, by the Two-way ANOVA followed by a Bonferroni post hoc test.**Results:** The changes in serum zinc were not parallel to changes in hippocampal free zinc concentration. Chronic administration of $ZnCl_2$ but not of zinc-receptor agonist or VPA, increased serum zinc in both control and PTZ-kindled mice. The PTZ-kindling increased free zinc in the CA3 and CA1 areas of the hippocampus, compared to mice that received VEH instead of PTZ. Treatment with TC-G 1008 or $ZnCl_2$ decreased free zinc in the CA1 of PTZ-kindled mice, but not in control mice. **Conclusion:** Our data show the association between epileptogenesis and free zinc in the PTZ model. The changes in free zinc may have consequences for signaling as they may be associated with changes in hippocampal p-CREB and p-TrkB, which we have previously observed following treatment with TC-G 1008 in this model.

Disclosures: U.M. Doboszewska: None. **J. Sawicki:** None. **B. Szewczyk:** None. **A. Rafalo-Ulinska:** None. **M. Mackowiak:** None. **K. Socala:** None. **M. Pierog:** None. **D. Nieoczym:** None. **I. Sowa:** None. **P. Wlaz:** None.

Digital Abstract Session

P048. Neuromodulator and Neuropeptide Receptor Signaling

Program #/Poster #: P048.06

Topic: B.03. G-Protein Coupled Receptors

Support: DA25267
DA48353

Title: The combined effects of the kratom (*Mitragyna speciosa*) alkaloid mitragynine and its *in vivo* metabolite 7-hydroxymitragynine

Authors: *A. PATEL[1], J. D. ZUARTH GONZALEZ[1], S. OBENG[2], V. L. C. PALLARES[1], R. PATEL[1], M. PATEL[1], L. R. GAMEZ-JIMENEZ[1], N. P. HO[1], F. LEÓN[3], M. MOTTINELLI[3], C. R. MCCURDY[3], L. R. MCMAHON[1], T. HIRANITA[1];
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Abstract: Kratom (*Mitragyna speciosa*) is a widely used botanical that contains numerous alkaloids. One of the most abundant, mitragynine (MG), is converted into an *in vivo* metabolite: 7-hydroxy-MG (7-OH-MG). Both alkaloids bind to mu-opioid receptors (MOR); MG has lower

MOR efficacy (i.e., intrinsic activity) than 7-OH-MG. We hypothesized the difference in efficacy between MG and 7-OH-MG imparts a difference in the degree to which they exert agonist and antagonist activity. In a [35S]GTPγS functional assay at human MOR, we confirmed the difference in efficacy: MG did not exhibit agonist activity up to 100 μM, whereas 7-OH-MG produced a maximum stimulation of 41.3% of DAMGO (i.e., was a partial agonist). When combined with 7-OH-MG, 10-fold higher concentrations than the Ki values of MG (7.09 μM) and the opioid antagonist naltrexone (18.4 nM) produced 22- and 69-fold rightward shifts, respectively, in the 7-OH-MG concentration-effect curve. In rats, MG (up to 56 mg/kg, i.p.) did not significantly increase latency to respond on a hot plate warmed to 51°C and 52°C. 7-OH-MG robustly increased hotplate response latency (respectively, ED50s= 6.44 and 8.66 mg/kg, i.p.) at both temperatures, as did the MOR agonist morphine. The low efficacy agonist buprenorphine produced full antinociception (100%) at 51°C, and partial antinociception (67.2%) at 52°C (respectively, ED50s= 0.173 and 0.692 mg/kg, i.p.). In contrast to the antagonism obtained with MG *in vitro*, MG (56 mg/kg, i.p.) produced modest leftward shifts in the dose-effect functions of 7-OH-MG (2.1-fold) and morphine (2.2-fold). At 52°C, buprenorphine (0.32 mg/kg, i.p.) reduced the effects morphine down to the effects produced by buprenorphine alone, and antagonized morphine 7.3-fold. These results show markedly (i.e., quantitatively and qualitatively) different effects of MG and 7-OH-MG evidenced by antagonistic activity *in vitro*, and apparent synergistic activity *in vivo*. Apparent rank order efficacy is 7-OH-MG>buprenorphine>MG; this unique pharmacological profile could render MG and its 7-OH metabolite effective replacements for other clinically used opioids.

Disclosures: **A. Patel:** None. **J.D. Zuarth Gonzalez:** None. **S. Obeng:** None. **V.L.C. Pallares:** None. **R. Patel:** None. **M. Patel:** None. **L.R. Gamez-Jimenez:** None. **N.P. Ho:** None. **F. León:** None. **M. Mottinelli:** None. **C.R. McCurdy:** None. **L.R. McMahon:** None. **T. Hiranita:** None.

Digital Abstract Session

P048. Neuromodulator and Neuropeptide Receptor Signaling

Program #/Poster #: P048.07

Topic: B.02. Ligand-Gated Ion Channels

Title: Varenicline increases δ and θ band power and attenuates the 5-HT_{2A}R induced head-twitch response

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Abstract: Varenicline increases δ and θ band power and attenuates the 5-HT_{2A}R induced head-twitch response

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Pre-Columbian Mesoamerican practitioners expertly utilized hallucinogens in combination with *Nicotiana rustica* (tobacco) during ritualistic practices to treat ailments in their communities.

Modern Westernized medicinal practices are beginning to understand the potential of hallucinogens in treating psychiatric and mood disorders, but less is understood about the synergistic effects that activation of serotonin and nicotinic acetylcholine receptors have on hallucinogenic-induced changes in arousal. Utilizing both the open field and EEG, we characterized the head-twitch response (HTR), a behavioral assay for 5-HT_{2A}R activation and its hallucinogenic effects in rodents following combined administration of the α7 nAChR agonist varenicline and the highly selective 5-HT_{2A}R agonist 25I-NBOH. We identified an increase in time stopped and a robust attenuation of the HTR in the open field, as well as a sustained enhancement of δ and θ power in EEG recordings which became pronounced at least 30 minutes after dual administration of varenicline and 25I-NBOH. These findings contribute to our understanding of specific α7 nAChR agonism in modulating the effects of 5-HT2AR activation. Taken together, these findings have major implications for clarifying the therapeutic potential of hallucinogens.

Disclosures: A. Contreras: None. **R.M. Hines:** None. **D.J. Hines:** None.

Digital Abstract Session

P049. Sodium Channels

Program #/Poster #: P049.01

Topic: B.04. Ion Channels

Support: U.S. Department of Veterans Affairs (BX002547)

Title: Strong G-protein-mediated, voltage-dependent, inhibition of voltage-gated sodium channels by endocannabinoids

Authors: *L. J. STEIGER, *G. MATTHEISEN, B. MARTISZUS, M. FELDTHOUSE, *S. M. SMITH;
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Abstract: Voltage gated sodium channels (VGSC) generate action potentials and thereby mediate intra and inter cellular communication in excitable cells. VGSC current inhibition is an important mechanism for a wide range of therapeutics. A number of endogenous ligands block VGSC currents indicating that VGSC modulation may impact excitability physiologically. The endogenous cannabinoid, anandamide (AEA), has been proposed to directly inhibit a number of isoforms of the VGSC in expression systems. Here we report that anandamide almost completely blocks VGSC currents in voltage-clamped mouse, cultured, neocortical neurons that express VGSC α-subunits Nav1.1, 1.2, 1.3 and 1.6. The effect is widespread, being present in >99% of neurons tested. We tested if AEA blocked VGSC currents via cannabinoid CB_1 receptors. The CB_1 antagonist, AM 251, did not alter AEA inhibition of VGSC current (93 ± 1%) suggesting that this inhibition is independent of CB_1 receptors. To determine if the AEA-induced block of VGSC current relied on G-protein signaling, we tested the effect of GDPβS which inhibits G-protein cycling. AEA (10 μM) inhibited VGSC current by 71 ± 7% (n = 8), measured 250 s after

onset of application. In contrast, AEA reduced VGSC current by only 3 ± 3% (n = 6) following inclusion of GDPβS (2 mM) in the pipette solution ($P = 5 \times 10^{-6}$). We next tested if AEA block of VGSC currents was voltage-dependent. Peak VGSC currents were elicited by a 30 ms step to -10 mV (5 s duty cycle) at holding potential (V_h) of -60, -80, or -100 mV (N = 21). The fractional inhibition by AEA, 200 s after application, was increased 20-fold as V_h was depolarized from -100 to -60 mV ($P=4x10^{-7}$). Like some other indirect VGSC blockers, the time course of inhibition by AEA was described well by f(t) $=A+Be-(t/\tau)2$ where t, A and B, and T represent time, constants, and the time constant respectively. Hyperpolarization of V_h over the voltage range increased T 14-fold ($P= 6 \times 10^{-8}$). These data indicate AEA block of VGSCs is voltage-dependent. AEA may act to preferentially stabilize the inactivated VGSC state. To examine this possibility we tested if hyperpolarization reversed AEA inhibition of VGSC currents. VGSC currents were elicited by two 10 ms steps (S1, S2) to -10 mV separated by a variable length recovery period at -120 mV. Before AEA application, S2 was 50% recovered by 0.8 ms In contrast, after full block by AEA, S2 recovered to 50% in 130 ms. The recovery of VGSC current with strong hyperpolarization following AEA exposure suggests that AEA acts indirectly to stabilize the inactivated state. The abundance and strength of this pathway highlights a voltage-dependent mechanism by which AEA influences cortical excitability.

Disclosures: L.J. Steiger: None. **G. Mattheisen:** None. **M. Feldthouse:** None. **S.M. Smith:** None. **B. Martiszus:** None.

Digital Abstract Session

P049. Sodium Channels

Program #/Poster #: P049.02

Topic: B.04. Ion Channels

Support: NIH Grant NS036855
NIH Grant NS110860
Charles R. Broderick III Phytocannabinoid Research Initiative
Bertarelli Foundation

Title: Cannabidiol inhibition of murine primary nociceptors: Tight binding to slow-inactivated states of Nav1.8 channels

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Abstract: The non-pychoactive phytocannabinoid cannabidiol (CBD) has been shown to have analgesic effects in animal studies but its molecular targets are not clear. We examined effects of CBD on intrinsic excitability of primary pain-sensing neurons. Studying acutely-dissociated capsaicin-sensitive mouse DRG neurons at 37°C, we found that CBD effectively inhibited action potentials evoked by current injection, with 2 μM CBD reducing the number of action potentials evoked by 80-pA 1-s current injections from 18 ± 2.6 in control to 2.2 ± 1.0 in 2 μM CBD (mean

± SEM, n = 14 pairs). Remarkably, CBD was more effective in inhibiting action potential firing than the local anesthetic bupivacaine. CBD reduction of firing was accompanied by a reduction in action potential peak, increased action potential width, reduced afterhyperpolarization, and increased propensity to enter depolarization block. In voltage clamp experiments, CBD inhibited both TTX-sensitive (TTX-S) and TTX-resistant (TTX-R) sodium currents, which had Nav1.8-like kinetics and voltage-dependence. CBD showed strong state-dependent inhibition of TTX-R channels with fast binding to inactivated channels during depolarizations and slow unbinding on repolarization. As for most Nav channels, maintained depolarization causes Nav1.8 channels to enter a "slow inactivated" state from which recovery is far slower than from regular fast inactivation. However, at least for native Nav1.8 channels in mouse nociceptors, slow inactivation of Nav1.8 channels is unusual in that it occurs at more hyperpolarized voltages than fast inactivation. By examining CBD alteration of channel availability following conditioning pulse of various durations, we found that CBD appears to bind especially tightly to slow inactivated Nav1.8 channels. The results could be fit by a model whereby CBD binds to the resting state of the channels with a Kd of 4.5 μM, to fast inactivated states with a Kd of 300 nM, and to slow inactivated states with a Kd of 120 nM. The tight binding to slow inactivated states likely contributes to CBD's efficacy in inhibiting repetitive firing of nociceptors. We conclude that CBD can likely produce analgesic effects by direct effects on neuronal excitability, with *in vivo* effects likely strongly dependent on mode of application and pharmacokinetics.

Disclosures: H.X.B. Zhang: None. **B.P. Bean:** None.

Digital Abstract Session

P049. Sodium Channels

Program #/Poster #: P049.03

Topic: B.04. Ion Channels

Support: NIGMS Grant R01 GM134110
U.S. Department of Veterans Affairs Grant BX002547

Title: Voltage-dependence of indirect sodium channel inhibition by cinacalcet

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Abstract: Voltage-gated sodium channel (VGSC) activation is essential for action potential generation in the brain. Allosteric calcium sensing receptor (CaSR) agonist, cinacalcet, strongly and ubiquitously inhibits VGSC current in neocortical neurons. This effect persists in CaSR-deficient neurons, indicating a CaSR-independent mechanism, and is blocked by the G-protein signaling blocker GDPβS, indicating an indirect pathway mediated by G-proteins. Here, using whole cell patch clamp, we investigated the voltage dependence of cinacalcet-mediated inhibition of VGSCs and their recovery from inactivation. The time course of inhibition was well described by the function f(t) ($f(t) = A + Be^{-(t/\tau)^2}$, where t is time, A and B are constants, and τ is

the time constant) consistent with indirect VGSC block. Similarly, in nucleated outside-out patch clamp recordings, the time course of inhibition of VGSC currents by cinacalcet was well described by the same function, eliminating concerns about voltage clamp errors distorting the time course of inhibition. The rate of inhibition, in both whole cell and outside-out patch recordings, was markedly faster at more depolarized voltages. Hyperpolarization of the holding potential over the range of -60 to -100 mV increased mean τ five-fold in whole cell recordings (n=6 at each voltage, P = 0.001), and 4-fold in outside-out recordings over the range of -80 to -100 mV (n=4 at each voltage, P = 0.04). As more VGSCs are in the inactivated state at depolarized holding potentials, the differences in blocking potency are explained if cinacalcet binds more tightly to the inactivated state. To evaluate whether cinacalcet inhibits sodium channel activity by affecting slow or fast inactivation, we implemented a voltage protocol that assays the voltage-dependence with which channels enter slow inactivation. In the presence of cinacalcet (10 μM), we observed a substantial increase in the fraction of channels recovering slowly after 5 s prepulses (96 ± 0.6%, n=6) compared to control (56 ± 4%, n=6). In addition, the voltage-dependence of slow inactivation shifted in the hyperpolarizing direction in the presence of cinacalcet, with a midpoint of -59 ± 2 mV in control (n=6) and -97 ± 1 mV (n=6; P = 0.004) in cinacalcet. Taken together, these results indicate enhancement by cinacalcet of slowly recovering channel states, though it is yet unclear whether this originates from binding to slow-inactivated channels or fast-inactivated channels from which recovery is slow. This dynamic and ubiquitous signaling pathway by which G-proteins regulate VGSC currents describes an important voltage-dependent mechanism for modulating central neuronal excitability.

Disclosures: S.M. Smith: None. **J. Lindner:** None.

Digital Abstract Session

P049. Sodium Channels

Program #/Poster #: P049.04

Topic: B.04. Ion Channels

Support: NIH/NINDS Grant R25NS107173
NIH/NINDS Grant U54NS108874

Title: Use of database-obtained cryo-EM data of voltage-gated sodium channels to explain voltage electrophysiology in SCN2A mutations

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Abstract: Mutations in brain voltage-gated sodium channels can cause epilepsy, which can be a severe condition in childhood and early development. The SCN2A gene is one of these protein-coding genes that contains many point mutations causing epilepsy. We have utilized cryo-EM data of the Nav1.2-beta2-KIIIA ternary complex from the Worldwide Protein Data Bank (PDB entry: 6J8E; Pan, X., Li, Z., Huang, X., Huang, G., Yan, N., 2019). The UCSF Chimera program

was then used to explore how point mutations affected the Nav1.2 structure. We were able to document changes in channel structure with four mutations, which we believe to have affected mobility of nearby channel segments and pore morphology. We also noted one mutation that did not significantly affect channel structure, in which the mutation had similar electrophysiology data to the control. We believe that this work suggests that analysis of cryo-EM structures can predict pathogenicity, or lack thereof, better than proximity in a primary protein sequence, which might be cited in online databases for the likelihood of pathogenicity. ClinVar marks Ala215Pro as likely pathogenic; however, it's criteria for this is based on other mutations in the same primary region of the protein. Electrophysiology data collected on this mutation was unremarkable, and our structural analysis concluded that it was likely nonpathogenic since there were no major interactions in the pore, other channel segments, or the inactivation gate. Gly899Ser is marked as likely pathogenic; we agree with the conclusion that its position in a transmembrane segment and that a change from a non-polar to a polar amino acid residue is likely to cause disease. Electrophysiology data confirms that it is likely pathogenic; however, our structural analysis furthers an explanation that interactions affecting pore morphology might better explain the data collected. Arg937Cys is reported as pathogenic and that this pathogenicity is believed to be caused by pore morphology. We were able to confirm this in our structural analysis through a predicted disulfide linkage of a nearby cysteine. Ala1333Thr is reported as likely pathogenic; we agree with the conclusion that its position in a transmembrane segment and that a change from a non-polar to a polar amino acid residue is likely to cause disease. Electrophysiology data confirms that it is likely pathogenic; however, our structural analysis furthers an explanation that interactions would occur between a nearby segment affecting its mobility in voltage sensing. In conclusion, we believe that modeling putative disease variants in cryo-EM structures may enhance predictions of pathogenicity in voltage-gated sodium channels.

Disclosures: L.A. Montgomery: None. **Z. Pei:** None. **T.R. Cummins:** None.

Digital Abstract Session

P050. Calcium Channels

Program #/Poster #: P050.01

Topic: B.04. Ion Channels

Support: CIHR (RWT)
NSERC (RWT)
NSERC (WN)
Harley N. Hotchkiss Doctoral Scholarship (CS-A)

Title: Cav3.1 and CaM colocalize in internal cellular structures

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Abstract: It is well established that calmodulin (CaM) exhibits a calcium-dependent association with the C-terminal region of high-voltage activated voltage-gated calcium channels. We showed that the low voltage-gated Cav3.1 (T-type) channels exhibit a constitutive association with CaM at rest that is close enough to support FRET across a wide region of tsA-201 cells. However, FRET was lost upon membrane depolarization in a manner that was dependent on Cav3 calcium conductance, followed by aggregation of CaMKII in the cytoplasm. Initial studies for FRET used GFP tagged to the Cav3.1 N-terminus as the donor molecule and CaM-mKate as acceptor. A loss of FRET thus suggested a dissociation of CaM from an IQ-like domain on the C-terminus of Cav3.1. However, the means by which CaM associated with Cav3.1 at the membrane could so quickly promote CaMKII aggregation was unsolved. An alternative explanation would be that a portion of the C-terminus bound to CaM-mKate translocates with CaM into the interior of a cell in a manner analogous to Cav1 calcium channels. To test this we first verified that FRET could be recorded between Cav3.1 tagged with GFP at the channel's C-terminus and CaM-mKate. Upon depolarization with high K+ medium, FRET between the Cav3.1 C-terminus and CaM was still lost, suggesting that the Cav3.1 C-terminus does not translocate with CaM. Given the strength of fluorescent signal supporting FRET in the cytoplasmic region we then sought to determine where Cav3.1-CaM FRET was occurring using TIRF imaging combined with Super-Resolution Radial Fluctuations (SRRF) reconstructions. We found that a large proportion of Cav3.1 and CaM colocalization was found internally with a few discrete points on the cell plasma membrane. TIRF-SRRF imaging revealed that Cav3.1 labeling had an organization highly comparable to what would be expected of the smooth endoplasmic reticular syncytium. Given these results, we will explore the relationship between Cav3.1 and CaM with endoplasmic reticular membranes using TIRF-SRRF or STORM-TIRF on images obtained during FRET and after membrane depolarizations.

Disclosures: C. Szalay-Anderson: None. **H. Asmara:** None. **W. Nicola:** None. **C. Drummond-Main:** None. **G. Sahu:** None. **R.W. Turner:** None.

Digital Abstract Session

P050. Calcium Channels

Program #/Poster #: P050.02

Topic: B.04. Ion Channels

Title: Effect of the geometry of the endoplasmic reticulum on astrocytic calcium signals at tripartite synapses: insights from simulations in realistic 3D geometries

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Abstract: Astroglial cells have been shown to actively modulate neuronal activity at tripartite synapses. Astrocytes respond to stimuli with calcium signals of various spatio-temporal characteristics. Most of those signals occur in close proximity to synapses, in branchlets that are

< 200nm in diameter, which appear blurry with conventional light microscopy [1]. Most astrocytic calcium signals are downstream the activation of IP3R channels on the membrane of the endosplamic reticulum (ER). Because of their small size, branchlets have long been considered devoid of ER [3]. However, IP3R2-/- mice are characterized by a 50% decrease of calcium activity in branchlets [2]. A recent immuno-labelling study resolved this controversy by suggesting that ~30% of synapses in the hippocampus are < 1 µm away from astrocytic ER [4]. Here, we use computational tools to investigate the causal relationship between ER geometry in astrocyte branchlets and the spatio-temporal characteristics of the resulting calcium signals. Reaction-diffusion simulations are performed in simplified geometries and reconstructions from electron microscopy in 3 spatial dimensions characterized by various ER geometries. We propose key geometrical features that regulate signal probability and size in astrocyte branchlets, contributing to a better understanding of astrocyte function and neuromodulation at tripartite synapses.

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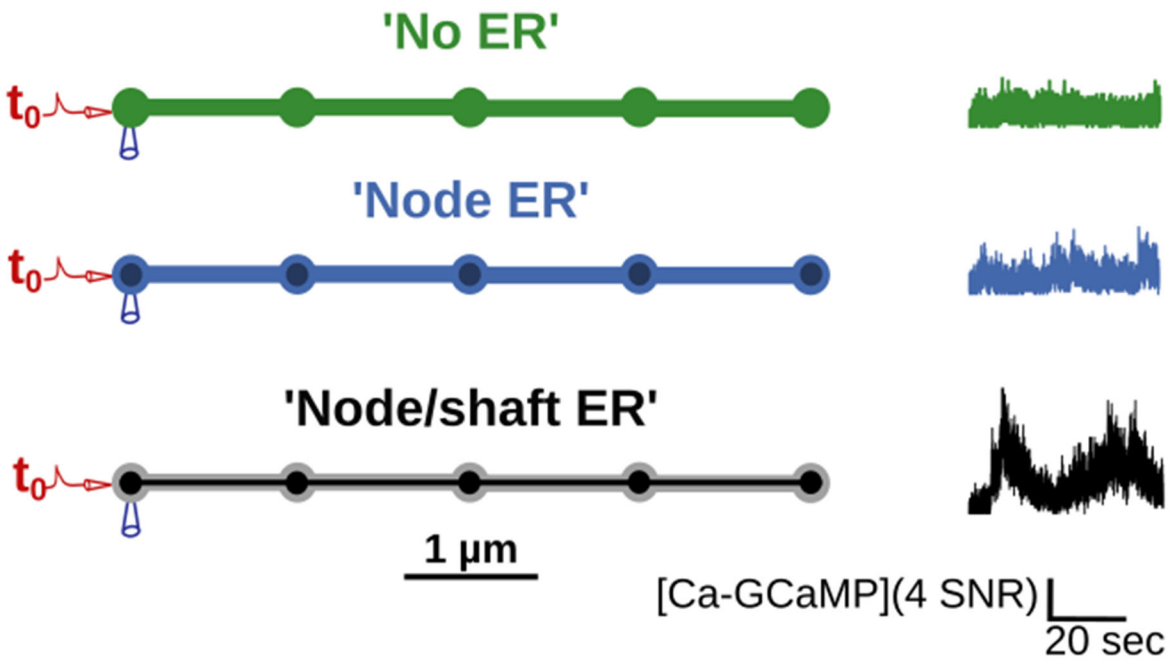

Disclosures: **A. Denizot:** None. **C. Calì:** None. **H. Berry:** None. **E. De Schutter:** None.

Digital Abstract Session

P050. Calcium Channels

Program #/Poster #: P050.03

Topic: B.04. Ion Channels

Support: Natural Sciences and Engineering Research Council of Canada Discovery Grant 2018-04401 to QY
ARC-NL

Title: Age-dependent changes of synaptic and extrasynaptic L-type calcium channels and NMDA receptors in rats

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Abstract: Age-associated impairments in learning and memory have been linked to abnormally elevated Ca^{2+} levels in hippocampal neurons. Both L-type calcium channels (LTCCs) and N-methyl-D-aspartate receptors (NMDARs) permit the entry of Ca^{2+} into cells. It is not well understood whether both channels contribute to age-related calcium overload. This study aims to investigate the intracellular distribution of LTCCs and NMDARs in the hippocampus at various age groups. Neonatal (10-14 days), adult (3-6 months), and aged (18-24 months) rats of both sexes were used. Expression of LTCCs (Cav1.2) and various subtypes of NMDARs (GluN1, GluN2A, and GluN2B) in both synaptic and extrasynaptic membranes were compared by western blotting. Immunohistochemistry and confocal imaging were employed to compare synaptic (co-labeling with PSD95) and non-synaptic Cav1.2 and GluN2B expression in dorsal hippocampal subdivisions (CA1, CA3, and DG). Preliminary western blotting results showed that GluN1, GluN2A, and GluN2B were higher in the synaptic fraction of the adult and aged hippocampus than in neonatal brains, although no difference was observed between adult and aged. In contrast, GluN1 extrasynaptic expression was higher in neonates. GluN2A/2B ratios were similar among different age groups. Western Blotting data shows that the synaptic expression of Cav1.2 appeared higher in the adult hippocampus but did not reach statistical significance. With western blotting, the specific location of this upregulation could not be determined; therefore we performed immunohistochemical analysis on hippocampal subregions. Confocal analysis of Cav1.2 in the CA1 region revealed lower synaptic expression and higher non-synaptic expression in aged brains compared to adult ones, whereas GluN2B exhibited similar levels of expression in the two age groups. These preliminary results suggest that age-related excess Ca^{2+} entry through non-synaptic LTCCs in CA1 may be a factor to explain the detrimental effect of age in learning and memory.

Disclosures: **A. Maziar:** None. **S. Carew:** None. **C. Reinhardt:** None. **Q. Yuan:** None.

Digital Abstract Session

P050. Calcium Channels

Program #/Poster #: P050.04

Topic: B.04. Ion Channels

Support: NIH Grant GM102525

Title: Different roles of T-type calcium channel isoforms in hypnosis induced by an endogenous neurosteroid epipregnanolone

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Abstract: Many neuroactive steroids induce sedation/hypnosis by potentiating gamma-aminobutyric acid (GABAA) currents. However, we previously demonstrated that an endogenous neuroactive steroid epipregnanolone [(3β,5β)-3-hydroxypregnan-20-one] exerts potent peripheral analgesia by blocking T-type calcium channels (T-channels) while sparing GABAA currents in rat sensory neurons (Ayoola et al., 2014). Here, we utilized mouse genetics to investigate potential sedative/hypnotic and immobilizing properties of epipregnanolone by assessing Loss of Righting Reflex (LORR) and Loss of Withdrawal reflex (LOWR), respectively. We found that epipregnanolone is an efficacious dose-dependent hypnotic agent when injected alone intra-peritoneally (i.p.) and also that it significantly lowered LORR and LOWR concentrations of a common volatile anesthetic isoflurane in wild type (WT) mice. To better understand the hypnotic effect on WT mice we measured EEG changes after intraperitoneal injection of epipregnanolone. We noted a rise in the total power of all frequencies except the γ frequencies. When analyzing relative power, it was discovered that there was a rise in β frequency 15 minutes after injection coinciding with the measured onset time of LORR yielded by our behavioral experiments. We also investigated hypnotic effects of epipregnanolone between different knockout (KO) models of T-channel isoforms. The CaV3.1 (CACNA1G) KO mice demonstrated decreased sensitivity to epipregnanolone-induced hypnosis when compared to WT mice, whereas no significant difference was noted between CaV3.2 (CACNA1H) and WT mice. However, when compared to WT mice, onset of epipregnanolone-induced hypnosis was delayed in CaV3.2 KO mice but not in CaV3.1 KO mice. We also found that across all genotypes, female mice were significantly more sensitive to epipregnanolone-induced hypnosis when compared to age-matched males. To our knowledge, this work is the first to report on the hypnotic properties of epipregnanolone in rodents. We speculate that distinct hypnotic effect of epipregnanolone across all T-channel isoforms is due to their differential expression in thalamocortical circuitry. Further research will be necessary to determine the exact mechanism underlying this sex difference and to what degree it will impact potential future clinical use. We posit that endogenous neuroactive steroids that target neuronal T-channels may have an important role as novel hypnotics and/or adjuvants to anesthetic agents.

Disclosures: I. Coulter: None. **T. Timic Stamenic:** None. **S.M. Todorovic:** None.

Digital Abstract Session

P050. Calcium Channels

Program #/Poster #: P050.05

Topic: B.04. Ion Channels

Support: NS055251 (DL)
F31NS093818 (DD)
T32MH020068 (DD)
NS062443 (DD)
Warren Alpert Fellowship Award (EJLS)
K99NS116123 (EJLS)
Robert J and Nancy D Carney Institute for Brain Science (AF)

Title: Voltage-gated calcium channels in skin are critical for transient hyperalgesia in mice

Authors: *R. Y. MEIR[1], E. J. LOPEZ SOTO[1], D. M. DUBREUIL[2], S. DASTE[1], A. FLEISCHMANN[1], D. LIPSCOMBE[1];
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Abstract: Voltage-gated calcium channels (Cav) are essential for transmitting information about noxious stimuli from detection in the periphery to perception centrally. Cav2.1 and Cav2.2 channels control excitation-dependent calcium entry at nociceptor presynaptic termini in spinal cord dorsal horn. Intrathecal Cav2.2 channel blockade relieves chronic pain in humans and rodents, but here we explore the role of peripheral Cav2.1 and Cav2.2 channels, at the point of stimulus detection in skin, in short-term sensitization of nociceptors. Nociceptors of dorsal root ganglia are pseudo-unipolar with a single axon that bifurcates, sending a branch centrally to spinal cord dorsal horn and another peripherally to innervate target organs including skin. With few exceptions, studies of Cav channels in nociceptors focus on their role controlling transmitter release at central presynaptic termini, while overlooking their potential importance at peripheral nerve endings. Here we present evidence that Cav2.1 and Cav2.2 channels are at axonal nerve endings in skin where they regulate capsaicin-induced changes in neuronal excitability but in distinctly different ways. To visualize functional Cav2.2 channels in peripheral nerve endings in skin, we used 2-photon microscopy live imaging. We imaged GCAMP6f in hind paw skin 5 and 15 mins after intraplantar capsaicin or capsaicin plus conotoxin in anesthetized GCAMP6f^{lox}/Trpv1^{Cre} mice. Capsaicin-induced Ca^{2+} signals in nociceptor projections were reduced by w-conotoxin MVIIA to ~40% of control levels indicating the presence of functional Cav2.2 channels. We next assessed the contribution of Cav channels to the robust, transient hypersensitivity to mechanical and heat stimuli that develops following prolonged activation of Trpv1 nociceptors. We used intraplantar 0.1% capsaicin, without or co-injected with w-conotoxin MVIIA or w-agatoxin IVA to inhibit peripheral Cav2.2 or Cav2.1 channels, respectively. Local inhibition of Cav2.2 channels reduced capsaicin-induced hyperalgesia selectively (not mechanical sensitivity) by 50% compared to capsaicin alone, without affecting

baseline behavioral responses to local thermal stimuli (n = 17 to 33). By contrast, local inhibition of $Ca_V2.1$ channels reduced capsaicin-induced mechanical hypersensitivity selectively (not heat) by 50% compared to capsaicin alone, without affecting baseline mechanical hypersensitivity (n = 4 to 6). Our results reveal that capsaicin-induced hypersensitivity to thermal and mechanical stimuli use two different local signaling pathways that depend on activation of two different Ca_V2 channels expressed in peripheral nerve endings in skin.

Disclosures: R.Y. Meir: None. **E.J. Lopez Soto:** None. **D.M. Dubreuil:** None. **S. Daste:** None. **A. Fleischmann:** None. **D. Lipscombe:** None.

Digital Abstract Session

P050. Calcium Channels

Program #/Poster #: P050.06

Topic: B.04. Ion Channels

Support: NS055251
R25GM083270
K99NS116123
R01HL126887

Title: A role for peripheral $Ca_V2.2$ channels in neurogenic inflammatory hyperalgesia

Authors: *A.-M. N. SALIB[1], M. J. CRANE[2], D. M. DUBREUIL[3], E. J. LOPEZ SOTO[1], A. M. JAMIESON[2], D. LIPSCOMBE[1];
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Abstract: Voltage-gated $Ca_V2.2$ channels are essential gatekeepers between detection of noxious stimuli at peripheral nociceptive nerve endings and central perception of pain. $Ca_V2.2$ channels in nociceptors control release of glutamate, neuropeptides and ATP from presynaptic termini and soma. Neuropeptides and ATP are pro-inflammatory mediators in the spinal cord that trigger the central sensitization that accompanies neurogenic inflammation. In an accompanying abstract (Meir et al.), we show $Ca_V2.2$ channels mediate voltage-dependent calcium entry in Trpv1 nociceptor nerve endings in skin, and are essential for transient hyperalgesia induced by prolonged Trpv1 nociceptor activation. We set out to test the hypothesis that activation of $Ca_V2.2$ channels is a critical step in triggering proinflammatory signaling cascades in models of hyperalgesia. We compared behavioral responses in wild type and $Ca_V2.2$ global knockout (KO) mice following intraplantar CFA and observed reduced hyperalgesia in $Ca_V2.2$ KO mice within 1 day and up to 5 days following CFA treatment. The interleukin 1 (IL-1) family of cytokines are early proinflammatory mediators which initiate secondary inflammatory responses. Interestingly, in hindpaw skin extracts from wild type and $Ca_V2.2$ KO mice we observed 60% lower IL-1β levels in $Ca_V2.2$ KO animals compared to WT controls. These findings suggest a role for $Ca_V2.2$ channels in chronic inflammation and we therefore established methods to address this

hypothesis. We used a post-operative injury model, the dorsal subcutaneous implantation of polyvinyl alcohol (PVA) sponges, in Cav2.2KO and WT animals to quantify local cytokine levels and leukocyte populations 24 hours and 7 days post injury. Leukocyte populations were identified by flow cytometry, and 13 cytokines assessed by a multiplex bead-based immunoassay. Our analyses were similar in $Ca_v2.2$ KO and WT mice with expected early increases in neutrophils on day 1, invasion of additional innate leukocytes at day 7, and early and late cytokines released in both genotypes. Having established conditions to analyze essential components of the inflammasome, we are analyzing the interstitial fluid in mouse hindpaw to link these analyses directly to behavioral assays. We compared cytokine levels in hindpaw interstitial fluid of WT and $Ca_v2.2$ KO mice 15 mins after capsaicin injection, the peak of capsaicin-induced hyperalgesia. Our preliminary data show 70% lower IL-1α levels in Cav2.2 knockout mice as compared to wild type (6-8 animals per group). These preliminary data suggest a critical role for $Ca_v2.2$ channels in the initiation of early signaling cascades that underlie heat hyperalgesia.

Disclosures: **A.N. Salib:** None. **M.J. Crane:** None. **D.M. Dubreuil:** None. **E.J. Lopez Soto:** None. **D. Lipscombe:** None. **A.M. Jamieson:** None.

Digital Abstract Session

P051. Potassium Channels

Program #/Poster #: P051.01

Topic: B.04. Ion Channels

Support: NIH Grant NS118262
NIH Grant NS101596

Title: Identifying protein interactions of KCNQ2 channels using epitope-tagged Kcnq2 targeted mice

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Abstract: Recent human genetic studies have shown that loss or gain of function variants of the voltage-gated potassium channel KCNQ2 causes neonatal epileptic encephalopathy. It is currently assumed that KCNQ2 channels primarily associate with KCNQ3 channels in the brain; however, recent published work has suggested that KCNQ2 channels may also interact with additional transmembrane proteins. Identifying the KCNQ2 membrane complex is necessary in order to understand how KCNQ2 channels dysfunction could lead to epilepsy and to also design better therapeutics. To address this question, we have developed a new epitope FLAG-tagged mouse line allowing us to analyze KCNQ2 affinity purified complexes from the neocortex using mass spectrometry. We have now validated this mouse line using electrophysiology and immunohistochemistry and show that inclusion of a FLAG tag to KCNQ2 channels does not

interfere with KCNQ2 function in the brain. Analysis of affinity purified KCNQ2 complexes from the neocortex by mass spectrometry demonstrated that a subset of KCNQ2 channels are in a complex with not only KCNQ3 channels but also KCNQ5 channels. Our data suggest that KCNQ2 channels could form tri-heteromeric KCNQ2/KCNQ3/KCNQ5 channels in the brain.

Disclosures: H. Soh: None. **K. Springer:** None. **K. Doci:** None. **A. Tzingounis:** None.

Digital Abstract Session

P051. Potassium Channels

Program #/Poster #: P051.02

Topic: B.04. Ion Channels

Support: NIH Grant NS036855

Title: Therapeutic concentrations of 4-AP inhibit Kv3.3 channels in Purkinje neurons

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Abstract: 4-aminopyridine (4-AP) is a small molecule known to inhibit a number of different voltage-gated potassium channels with different concentration-dependence. 4-AP is also a therapeutic treatment for various neurological disorders, including the cerebellar ataxia episodic ataxia type 2. The mechanism of action of 4-AP on cerebellar ataxia has been proposed to center on the improvement of firing regularity of cerebellar Purkinje neurons (Alviña and Khodakhah, 2010). However, the major molecular target remains unclear. We report that the main target of 4-AP in cerebellar Purkinje neurons is Kv3.3. Performing *in vitro* electrophysiological recording from acutely dissociated Purkinje neurons at 37 °C (mice aged P13-P18), we found that 4-AP dose-dependently reduced the coefficient of variance of spontaneous firing, as previously reported for Purkinje neurons in cerebellar slice recordings. This effect was accompanied by a dose-dependent increase in action potential width. There was little effect on the frequency of spontaneous firing, but high-frequency firing driven by current injections was slowed by 4-AP. These effects of 4-AP were absent or severely diminished in Kv3.3-null mice. To define the profile of 4-AP sensitive current in Purkinje neurons, we recorded currents in voltage clamp using action potential waveforms as the voltage command (action potential clamp). We found that current blocked by 4-AP flows primarily during the repolarization phase of the spike, consistent with the fast kinetics characteristic of Kv3 channels and their prominent role in action potential repolarization in Purkinje neurons, as previously reported. Previous work showed that the Kv1.5 inhibitor diphenyl phosphine oxide-1 (DPO-1) mimicked the effects of 10 μM 4-AP on Purkinje neuron firing and that combination of the two drugs had a non-additive effect, suggesting Kv1.5 as the molecular target of low concentrations of 4-AP. However, we found that DPO-1 in the low micromolar range effectively blocked current produced by mouse Kv3.3 expressed in HEK293 cells. Taken together, our data demonstrate that Kv3.3 is the likely major molecular target of low-concentrations of 4-AP in cerebellar Purkinje neurons.

Disclosures: **Z. Niday:** None. **B.C. Carter:** None. **B.P. Bean:** None.

Digital Abstract Session

P051. Potassium Channels

Program #/Poster #: P051.03

Topic: B.04. Ion Channels

Support: DOD/Army/CDMRP/W81XWH-19-1-0657

Title: Combining Kv7 openers amplifies outward current and blocks deep tissue nociceptor discharge

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Abstract: Background: In order to repurpose analgesics for use in GWI (Gulf War Illness), we examined whether combinations of classic (Retigabine, RET) and secondary (Diclofenac, DIC; Meclofenamate, MEC) K_v7 channel openers (KVO) could retard deep tissue nociceptor activity.
Methods: Cells were harvested from the dorsal root ganglion of male rats (n=106). Delayed rectifier K^+ currents were isolated from other voltage sensitive currents using a K^+ isolation solution. A stepped voltage protocol was applied (-55 to -40 mV; V_h=-60 mV; 1500 msec) before and after KVO or vehicle (2 min; RET, DIC, MEC). Outward currents were assessed. Linopirdine (20 uM), was then applied for 3 minutes. The Linopirdine sensitive currents were determined by subtraction. The influence of KVOs was also assessed in current clamp studies where nociceptors were challenged by a muscarinic agonist known to evoke burst discharges. (Oxotremorine-M, OXO; 10 uM, 1 min). This is the first attempt to conduct such studies in confirmed nociceptors.
Results: KVOs differed widely with respect to their influence on nociceptors. MEC produced the highest maximal currents and broadest voltage sensitivity shifts (I_{max} = 6.3 pA/pF; p<.002 and p<.03, respectively; ED_{50}=60.7 uM; n=55). DIC and RET produced weaker maximal currents (I_{max} = 1.0 pA/pF DIC and 1.24 pA/pF RET; ED_{50}: 96.1 uM DIC and 62.0 uM RET; n=46 and 42, respectively), but voltage sensitivity shifts were observed only near current activation thresholds. Using subthreshold combinations of DIC (50 uM) and RET (5 uM), we observed that evoked currents were significantly amplified. The combination of DIC (50 uM) and RET (5 uM; n=8) outperformed individual applications of 90 uM DIC (p<.004) or 40 υM RET (p<.05), but there was little influence on voltage sensitivity. In contrast, the combination of DIC 70 uM and RET 2.5 uM (n=5) produced no significant current amplification in voltage clamp studies. In current clamp studies, both of the above combinations produced hyperpolarization (-3.5.48 +/-1.58 mV and -6.44 +/-1.86 mV; p<.04 and .005; n=10 and 9) and significantly blocked OXO evoked action potential bursts (p<.03 and .001, respectively, n=8, 10 and 9).
Conclusions: When used in combinations, KVOs that bind to different K_v7 subunits ($K_v7.2$ vs $K_v7.3$) can amplify evoked currents through nociceptor K_v7 channels and block action potentials evoked by a cholinergic challenge. However, current clamp studies indicated that factors other

than K_V7 opening might also be involved. Combinations of KVOs could be an effective strategy for treatment of deep tissue pain as experienced by veterans suffering from GWI.

Disclosures: B.Y. Cooper: None. **T.J. Nutter:** None.

Digital Abstract Session

P051. Potassium Channels

Program #/Poster #: P051.04

Topic: B.04. Ion Channels

Title: Suppression of macro- and micro-scopic Kv1.1 channel activities by beta-amyloid peptides: Involvement of both intracellular calcium-signaling pathways and direct peptide-peptide interactions

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Abstract: Past studies indicate that Aβ's role(s) in Alzheimer's disease (AD) pathogenesis involves disruption of Ca^{2+} homeostasis, synaptic communication, impairment of learning- and memory-related synaptic plasticity [e.g., hippocampal LTP], and eventual cell death. The mechanism(s) underlying these effects are still unclear. Because Kv1.1 and related channels are activated during an action potential, regulate depolarization-produced Ca^{2+} influx, and inhibition of Kv1 channels can produce hyperexcitability and neurotoxicity, we have speculated that Aβ-suppression of Kv1 channels may contribute to AD pathology. Here we describe the effects of Aβ peptides on macro- and micro-scopic Kv1.1 currents using two electrode voltage-clamp (TEVC) and patch clamp techniques, respectively. Bath application of either Aβ(1-42) or "core" peptide Aβ(25-35) produced 40-50% suppression of macroscopic Kv1.1 current within 30 m (murine homomeric Kv1.1 channels expressed in *Xenopus* oocytes). Little or no suppression was produced by reverse peptide Aβ(40-1) or solvent control. Clear suppression of Kv1.2 channels by Aβ(1-42) also occurred. Suppression of Kv1.1 by Aβ(1-42) was partially dependent on intracellular Ca^{2+} and PP2B. ~25% reduction occurred when cells were loaded with BAPTA-AM or exposed to the PP2B-inhibitor cyclosporine A. Membrane capacitance measurements and Western Blot analyses failed to detect any Aβ(1-42)-produced endocytosis of Kv1.1 in these acute experiments. In contrast, clear endocytosis was observed when large increases in intracellular Ca^{2+} were produced by A23187, or by PMA-activation of PKC. Aβ-suppression of Kv1.1 involved both PP2B-dephosphorylation and direct protein-protein interaction of Aβ with Kv1.1 subunits. Exposure of the intracellular face of Kv1.1 channels in ripped-off oocyte patches to either purified catalytically-active PP2B, or Aβ(1-42), produced gradual reductions in *p*(open), followed by abrupt disappearance of Kv1.1 activity. Additional single-channel results [involving "tip-dip" and black lipid membranes (BLMs) studies] found that direct application of Aβ peptides to individual Kv1.1 channels reduced their activity, whether applied intra- or extra-cellularly. We conclude that Aβ peptides (1-42) and (25-35) suppress Kv1.1 and 1.2 channels. Suppression of these and related K^+ channels presynaptically may lead to larger and longer

spikes, greater Ca^{2+}influx, and increased release of glutamate. Postsynaptically, increased glutamate release and suppression of Kv1 channels, may lead to enhanced activation of AMPA and NMDA receptors, and thus contribute to AD hyperexcitability and excitotoxicity.

Disclosures: K. DeBoeuf: None. **M. Islam:** None. **N. Thelen:** None. **J. Watkins:** None. **J. Farley:** None.

Digital Abstract Session

P052. Other Ion Channels

Program #/Poster #: P052.01

Topic: B.04. Ion Channels

Title: Pharmacology of transient receptor potential cation (TRP) channels using different activation stimuli

Authors: A. HORVÁTH[1], T. A. GOETZE[1], I. RINKE-WEIß[1], N. BECKER[1], L. FOCKE[2], O. WEHMEIER[2], R. SCHWANDNER[3], G. OKEYO[4], T. STRASSMAIER[4], R. HAEDO[4], N. FERTIG[1], ***A. R. OBERGRUSSBERGER**[1];
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Abstract: Transient Receptor Potential (TRP) channels are widely distributed throughout the mammalian central and peripheral nervous systems. They can be directly activated by ligands, heat or cold and mechano-stimulation, and are important targets in drug discovery for the treatment of pain, respiratory diseases, cancer, immune disorders and others. Here, we studied the responses of TRPA1, TRPV1, TRPV3, TRPV4 and TRPM8 assay-ready and cultured cells activated using a variety of stimuli on automated patch clamp (APC) systems.
TRPA1 and TRPM8 are crucial in sensing noxious cold and inflammatory pain, responding to irritant environmental and food compounds, and metabolites produced during oxidative stress. TRPA1 is expressed in sensory neurons of the dorsal root ganglion (DRG) and trigeminal ganglion. Thus, TRPA1, and possibly TRPM8, antagonists are considered a promising approach for the treatment of acute and chronic pain. Desensitization is one of the biggest challenges for drug screening of TRPA1 channels. Here, we compare IC_{50}s obtained with long vs short ligand exposure using a high throughput device, the SyncroPatch 384i. TRPM8 was activated repetitively activated using solution at 10°C on the Port-a-Patch using temperature-controlled perfusion system at 10 °C. Capsazepine (10 μM) was used to block the activated current.
The TRPV1, 3 and 4 channels are ligand-gated, non-selective cation channels widely expressed in the peripheral and central nervous system and, like TRPA1, are involved in nociception. In addition, TRPV3 is robustly expressed in keratinocytes, and TRPV4 in various tissues, including human T cells and a range of epithelial (cornea, retina, trachea, lung) and endothelial (eye, liver, heart, kidneys) cells. The TRPVs respond to elevated temperatures as well as various compounds, and identifying compounds with differing effects on ligand- vs heat activation may be crucial in the discovery new treatments for pain with fewer side effects. Here, we developed

robust methods for temperature and pharmacological activation to study differential effects of blocker using APC systems (Patchliner and Port-a-Patch). Heat activation of TRPV1, 3, 4 channels was performed repeatedly by the heated pipetted (37-45°C) of the Patchliner. Ligand activation could be also performed on the Patchliner (10 µM Capsaicin - TRPV1, 200 µM 2-APB - TRPV3, 100 nM GSK1016790 - TRPV4). Various blockers were used to inhibit the response. Experiments involving TRPV4 were also performed on the Port-a-Patch. The channel was by heat (38°C) and partially blocked by ruthenium red.

Disclosures: **A. Horváth:** A. Employment/Salary (full or part-time):; Nanion Technologies. **T.A. Goetze:** A. Employment/Salary (full or part-time):; Nanion Technologies. **I. Rinke-Weiß:** A. Employment/Salary (full or part-time):; Nanion Technologies. **N. Becker:** A. Employment/Salary (full or part-time):; Nanion Technologies. **L. Focke:** A. Employment/Salary (full or part-time):; acCELLerate GmbH. **O. Wehmeier:** A. Employment/Salary (full or part-time):; acCELLerate GmbH. **R. Schwandner:** A. Employment/Salary (full or part-time):; Assay.Works Gmbh. **G. Okeyo:** A. Employment/Salary (full or part-time):; Nanion Technologies. **T. Strassmaier:** A. Employment/Salary (full or part-time):; Nanion Technologies. **R. Haedo:** A. Employment/Salary (full or part-time):; Nanion Technologies. **N. Fertig:** A. Employment/Salary (full or part-time):; Nanion Technologies. **A.R. Obergrussberger:** A. Employment/Salary (full or part-time):; Nanion Technologies.

Digital Abstract Session

P052. Other Ion Channels

Program #/Poster #: P052.02

Topic: B.04. Ion Channels

Support: NSERC
CIHR

Title: Structure-function analysis of Pannexin-1 permeability to anandamide

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Abstract: Pannexin-1 (Panx1) is an ion and metabolite channel with broad cell/tissue expression, including neurons and glia of the central nervous system. Panx1 is best known for its efflux of ATP, contributing towards a multitude of pathological and physiological mechanisms. With the Panx1 cryo-EM structures recently reported, unique gating mechanisms of multiple small-molecule conduction pathways have been reported. These could explain the size- and charge- exclusion of certain molecules depending on channel conformation and pore access. We recently described a novel role for Panx1-dependent regulation of the extracellular concentration of the endocannabinoid, anandamide (AEA). Here, we hypothesize that Panx1 facilitates AEA permeation across the neuronal membrane to regulate fast synaptic uptake of AEA for extracellular clearance and degradation. Using combinations of electrophysiology and

fluorescent dye flux in HEK293T cells over-expressing Panx1, we are investigating site-specific interactions within Panx1 that may govern AEA transport. Our data show that AEA attenuates wild-type Panx1 currents in a concentration-dependant manner, in addition to altering charge-specific fluorescent dye uptake. This suggests these small molecules may 'compete' for channel permeation. We predict that mutating single or select groupings of residues will alter this putative interaction between Panx1 and AEA and decrease uptake.

Disclosures: C.L. Anderson: None. **R.J. Thompson:** None.

Digital Abstract Session

P053. Presynaptic Structure and Function

Program #/Poster #: P053.01

Topic: B.05. Neurotransmitter Release

Title: Gaba co-release from midbrain da neurons requires the membrane transporter gat1

Authors: *R. MELANI[1], N. X. TRITSCH[2];
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Abstract: There is a growing appreciation that many neurons throughout the central nervous system synaptically release multiple neurotransmitters. Amongst them, midbrain dopaminergic (DA) neurons in the Substantia Nigra pars compacta (SNc) have been shown to release GABA and glutamate along with dopamine onto target neurons in dorsal striatum. We previously demonstrated that GABA co-release does not rely on a canonical GABA synthetic machinery, as DA neurons do not express GAD65 and GAD67. Instead, we showed that DA neurons express the plasma membrane GABA transporter GAT1 (Slc6a1), and suggested that GABA uptake by GAT1 is necessary to sustain GABAergic transmission from DA neurons. However, it has been reported that DA neurons synthesize GABA by a non-canonical synthesis pathway involving Aldh1a1. To more directly test the role of GAT1 in GABA co-release, we generated a conditional knocked out (cKO) mouse in which GAT1 is selectively ablated from DA neurons. Using whole-cell voltage-clamp recordings from striatal projection neurons, we find that GABA co-release from midbrain DA neurons is entirely abolished, but that glutamatergic co-transmission is unimpaired. Tonic GABA is not affected in GAT1 cKO mice, indicating that the loss of GABA co-release is not secondary to a decreased GABA availability or increased tonic inhibition. In adult GAT1 cKO mice, viral expression of GAD65 and GAD67, as well as GAT1, fully restore GABA co-release, while Aldh1a1 overexpression is not sufficient. These results indicate that GAT1 deletion does not impair DA neuron survival or vesicular exocytosis, confirm that GABA co-release from midbrain DA neurons relies on GABA uptake from the extracellular milieu, and pave the way for future studies investigating the contribution of GABA co-release in modulating striatal function and behavior.

Disclosures: R. Melani: None. **N.X. Tritsch:** None.

Digital Abstract Session

P053. Presynaptic Structure and Function

Program #/Poster #: P053.02

Topic: B.05. Neurotransmitter Release

Title: Metformin: possible potentiation of glutamate-stimulated norepinephrine release in rat cerebral cortical slices

Authors: *Y. M. ALJOHANI, M. ROSS, F. WU, K. KELLAR, G. DEZFULI;
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Abstract: Metformin: possible potentiation of glutamate-stimulated norepinephrine release in rat cerebral cortical slices.
Yousef M. Aljohani, Madison Ross, Fan Wu, Kenneth J. Kellar and Ghazaul Dezfuli Department of Pharmacology & Physiology, Georgetown University, Washington DC 20057 Disclosures: Y.M. Aljohani: None. M. Ross: None. F. Wu: None. K.J. Kellar: None. G. Dezfuli: None.
Metformin is the most widely prescribed medication for type-2 diabetes. Studies on metformin's actions in the CNS (unrelated to glucose metabolism) reveal several kinds of effects, but few, if any, of these mechanisms reveal insight into metformin's potential effects on neurotransmission in the brain. The purpose of the present study was to assess the effects of metformin on glutamate-stimulated norepinephrine (NE) release using a highly reliable *in vitro* assay. To this end, experiments were performed in both young (2-3 months old) and aged (18-24 months old) Fischer 344 rats, and the glutamate-stimulated NE release was measured in the slices from the cerebral cortex. Our results show that (a) glutamate-stimulated NE release is mediated by n-methyl-d-aspartate (NMDA) receptors and (b) that metformin increases glutamate-stimulated NE release in the cerebral cortex of young Fischer 344 rats. Furthermore, in aged Fischer 344 rats, which demonstrate a significant decline in cortical glutamate-stimulated NE release (Dezfuli *et al.,* 2019), we found that metformin rescues the deficit in the release, restoring it to the levels seen in young rats. Our preliminary results indicate a novel mechanism of action for metformin in the brain, namely, to potentiate glutamate-stimulated NE release. These data have possible implications for the effects of aging on CNS functions, including on cognition and conditions stemming from dysregulated monoamine signaling.No authors have a conflict of interest.

Disclosures: Y.M. Aljohani: None. **M. Ross:** None. **F. Wu:** None. **K. Kellar:** None. **G. Dezfuli:** None.

Digital Abstract Session

P053. Presynaptic Structure and Function

Program #/Poster #: P053.03

Topic: B.05. Neurotransmitter Release

Support: Open Fund - Individual Research Grant (MOH-000225)
2017 Academic Research Fund (AcRF) Tier 3 Grant (MOE2017-T3-1-002)

Title: A role for synapsin IIa oligomerization in maintaining a reserve pool of synaptic vesicles

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Abstract: Although it is known that synapsins are involved in maintaining a reserve pool (RP) of synaptic vesicles (SVs) within presynaptic terminals, the precise mechanism involved in this action remains elusive. We have tested the role of synapsin oligomerization in this process by examining the ability of an oligomerization-deficient mutant of synapsin IIa to rescue the RP defect of cultured glutamatergic neurons from synapsin triple knock-out (TKO) mice. First, photobleaching of labelled SVs in individual presynaptic boutons was used to monitor how synapsins limit movement of SVs between boutons: the level of fluorescence recovery after bleaching SVs within one bouton reflects the mobility of SVs coming from neighboring boutons. In TKO neurons, a high level of fluorescence recovery showed that SVs were highly mobile. Expression of wild-type synapsin IIa could immobilize SVs in TKO neurons, indicating that synapsin IIa traps SVs within a bouton (*J. Neurosci.* 32:3969). However, expression of the oligomerization-deficient synapsin IIa mutant did not immobilize SVs. This reveals that the mutant is not able to confine SVs locally and therefore cannot retain SVs within the RP. Next, mobilization of SVs from the RP to the readily-releasable pool was monitored by examining the kinetics of short-term synaptic depression. Whole-cell patch clamp recordings were used to measure the amplitude of excitatory postsynaptic currents (EPSCs) during trains of stimuli (10 Hz). In wild-type neurons, mobilization of SVs from the RP slows the activity-dependent decrease in EPSC amplitude during such stimulus trains. Synaptic depression was very rapid in TKO neurons, indicating very little RP mobilization. Expression of wild-type synapsin IIa in TKO neurons slowed depression, reflecting rescue of RP mobilization (*J. Neurosci.* 28:10835). However, such rescue was minimal in neurons expressing the mutant synapsin IIa. Thus, oligomerization is important for mobilization of SVs from the RP. In summary, our results support the idea that oligomerization of synapsins is important for maintaining glutamatergic SVs within the RP, thereby providing a supply of SVs during sustained synaptic activity.

Disclosures: M. Zhang: None. **G.J. Augustine:** None.

Digital Abstract Session

P053. Presynaptic Structure and Function

Program #/Poster #: P053.04

Topic: B.05. Neurotransmitter Release

Support: Pilot project grant from Albert Einstein College of Medicine IDDRC

Title: Rare patient mutation in presynaptic calcium channel affects localization and synaptic function

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Abstract: Channelopathies cause a wide variety of disorders, but understanding how specific mutations lead to disease has been complicated by gene redundancy and compensatory effects in a variety of classical and complex vertebrate models. We use C. elegans to evaluate the functional and behavioral effect of a rare and novel human patient-derived point mutation (D1634N) in the P/Q-type voltage-gated calcium channel (CACNA1). Mutations in this channel are typically associated with autosomal dominant neurological disorders like familial hemiplegic migraine type 1 (FHM1), episodic ataxia type 2 (EA-2), and Spinocerebellar ataxia type 6 (SCA6), however patients with the D1634N mutation cannot be classified into any of these disorders, suggesting a novel mechanism of action. The worm has only one ortholog of the channel (UNC-2) expressed in its nervous system and shares high homology in the region of the single point mutation D1634N. Our CRISPR-generated D1634N worms show decreased channel expression at synapses and defects in locomotion resembling the human patients' ataxic phenotype. Additional interactions with presynaptic proteins are being evaluated to address the impact of this mutation on presynaptic morphology and synaptic function.

Disclosures: M. Zuluaga Forero: None. **P.T. Kurshan:** None. **H. Snell:** None. **K. Khodakhah:** None.

Digital Abstract Session

P053. Presynaptic Structure and Function

Program #/Poster #: P053.05

Topic: B.05. Neurotransmitter Release

Support: NIH S10 RR026445

Title: The effect of acute ATP depletion on synaptic vesicle endocytosis at the ultrastructural level

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Abstract: Following synaptic vesicle fusion at the presynaptic terminal, vesicular membrane and proteins must be recycled via endocytosis to replenish the vesicle pool. A previous study revealed using the pHluorin assay that local depletion of ATP at presynaptic terminals impairs the rate of vesicle cycling during sustained synaptic activity, possibly by blocking endocytosis. However, due to limitations inherent to the pHluorin approach, it is unknown which exact step in the vesicle cycling process is most sensitive to the availability of ATP. Moreover, it is unclear whether glycolysis or oxidative phosphorylation (OxPhos) is more important for supplying energy for the synaptic vesicle cycle. In the present study, we used time-resolved electron microscopy to capture rapid changes to the presynaptic membrane ultrastructure in cultured

hippocampal neurons at various time points following stimulation. We found that acute ATP depletion by blocking both glycolysis and OxPhos stalls synaptic vesicle endocytosis at a stage prior to membrane fission. In contrast, blocking OxPhos alone does not stall synaptic vesicle endocytosis. In addition, using pHluorin assay, we also found that blocking glycolysis alone slows down synaptic vesicle recycling, while blocking OxPhos has no effect. Taken together, these results suggest that membrane fission during endocytosis is the most energy-sensitive step in the synaptic vesicle cycle, and that glycolysis, but not OxPhos is the preferred energy source driving synaptic vesicle endocytosis.

Disclosures: Q. Gan: None. **S. Watanabe:** None.

Digital Abstract Session

P053. Presynaptic Structure and Function

Program #/Poster #: P053.06

Topic: B.05. Neurotransmitter Release

Support: This work was supported by a grant awarded by U.S. Department of Veterans Affairs (BX002547) to SMS.
Dr. Hee-sup Shin, S. Korea, kindly provided null mutant mice.

Title: Plc mediates spontaneous glutamate release triggered by extracellular calcium

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Abstract: Evoked and spontaneous glutamate release pathways are different in many ways. The underlying vesicle pools are distinct, are modulated by different receptors, and the two mechanisms are differentially affected by extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_o$). Physiological decreases in $[Ca^{2+}]_o$ occur with increased neuronal excitability and these changes in the microenvironment reduce evoked and spontaneous glutamate release. While Ca^{2+} entry via voltage-gated calcium channels transduces the action of $[Ca^{2+}]_o$ for evoked release, the same pathway does not contribute to spontaneous release. A number of G-protein coupled receptors appear to link $[Ca^{2+}]_o$ and spontaneous glutamate release and we have tested if phospholipase C (PLC), which is activated by G protein subunits and hydrolyzes the phospholipid phosphatidylinositol 4,5-biphosphate (PIP_2), contributes to a common downstream pathway. PLC is well positioned for this role as it increases inositol triphosphate (IP_3) and diacylglycerol (DAG), both of which are known modulators of synaptic transmission. Using whole cell patch clamp in primary neocortical neuronal cultures, we confirmed that frequency of spontaneous release, measured as miniature excitatory post-synaptic currents (mEPSCs), was substantially increased (Median (Mdn) relative to baseline to 302%, n=13) by elevation of $[Ca^{2+}]_o$ from basal (1.1 mM) to high (6.0 mM), however inhibition of PLC activity with U73122 (5 μM) completely blocked this effect (Mdn=87% baseline, n=8; Dunn's multiple comparisons, $P<0.001$). The response to $[Ca^{2+}]_o$ was

intact in vehicle and inactive analog (U73343) controls. PLC β1 is the most abundant isoform in the neocortex, but interestingly the $[Ca^{2+}]_o$-dependence of mEPSC frequency was the same in neurons from PLCβ1 null mutant and litter mate controls. U73122 substantially suppressed $[Ca^{2+}]_o$-mediated increase in mEPSC frequency in the PLCβ1 null mutants (Mdn=80% baseline, n=8, Dunn's, $P<0.01$), indicating that $[Ca^{2+}]_o$-sensitivity may be mediated by other PLC isoforms. We tested if vesicles that mediate evoked release were also sensitive to PLC. Using hypertonic sucrose to probe the readily releasable pool (RRP), we determined that the RRP was depleted more quickly after PLC blockade by U73122, resulting in a 73% reduction in sucrose-induced charge transfer (SICT) compared to a pre-incubation baseline (t-test, $p=0.048$). SICT was not reduced by U73343. Addition of the DAG analogue PDBu attenuated this effect. Together these data point to a strong role for PLC in mediating changes in mEPSC frequency in response to extracellular cues and in maintaining the vesicles mediating evoked glutamate release.

Disclosures: M.G. Feldthouse: None. **N.P. Vyleta:** None. **S.M. Smith:** None.

Digital Abstract Session

P054. Regulation of Synaptic Transmission By Neurotransmitters and Neuromodulators

Program #/Poster #: P054.01

Topic: B.06. Synaptic Transmission

Support: NIH Grant DA011289-19

Title: Somatodendritic release of cholecystokinin potentiates ventral tegmental area GABAergic synapses

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Abstract: Cholecystokinin (CCK) is a neuropeptide found in most ventral tegmental area (VTA) dopamine (DA) neurons. Intra-VTA CCK injection reportedly modulates reward-related behaviors, but its role in VTA circuitry has remained unclear. Inhibitory inputs are essential for controlling the firing pattern of DA VTA neurons, and plasticity at GABAergic synapses can powerfully shape dopaminergic output. CCK has been shown to potentiate GABAergic synapses in other brain areas. Here we explored the hypothesis that CCK can control GABAergic synaptic strength in the VTA.Previously, we used whole-cell recordings from VTA DA cells in mouse brain slices to examine synaptic plasticity with the stimulating electrode placed caudal to the VTA to elicit inhibitory postsynaptic currents (caudal IPSCs). We reported that low frequency stimulation (LFS) paired with modest depolarization (dep) induces input-specific long-term potentiation (LTP) of GABAergic synapses (131.9 ± 6.5 % of baseline, $p=0.0012$, n=9). This form of LTP (dep+LFS LTP) is NMDAR-independent, requires the depolarization of the postsynaptic dopamine cell and is blocked by a BAPTA-containing intracellular pipette solution *(St. Laurent et al., 2020; Neuron 106:624)*. We have now found that depolarization of the

postsynaptic DA cell by itself potentiates caudal IPSCs, even without LFS (145.9 ± 18.1 % of baseline, p=0.0264, n = 13). Together, these properties are consistent with the somatodendritic release of a signaling molecule as the underlying mechanism supporting dep+LFS. Somatodendritic release can occur with both classical neurotransmitters and neuropeptides, and it has been proven to be an intrinsic feature of DA cells (Geffen et al., 1976; Nature 260:258; Rice et al., 1997; J. Neurophysiol. 77:853; Beckstead et al., 2004; Neuron 42:939).We now report that bath application of CCK (0.1 μM), potentiates GABAergic synapses onto VTA DA neurons (123.9 ±7.3, p=0.0135, n = 8). CCK bath application occludes dep+LFS LTP, since after potentiation by CCK, further LTP cannot be elicited with dep+LFS (97.5 ± 11.6 % of baseline, p=0.8385, n = 7). Furthermore, incubation with the CCK_2 receptor antagonist, LY225910 (1 μM), blocks dep+LFS LTP (87.9 ± 9.3 of baseline, p=0.2407, n = 7) as well as depolarization-induced LTP (104.3 ± 5.2 % of baseline, p=0.4410, n = 6). In future experiments we will explore the mechanisms that underlie CCK-induced potentiation, and which presynaptic afferents can be potentiated by this mechanism.Our work shows for the first time that CCK promotes LTP at GABAergic afferents that regulate DA neuron firing, suggesting that somatodendritic CCK release is expected to decrease VTA DA cell excitability.

Disclosures: V. Martinez Damonte: None. **R. St. Laurent:** None. **J.A. Kauer:** None.

Digital Abstract Session

P054. Regulation of Synaptic Transmission By Neurotransmitters and Neuromodulators

Program #/Poster #: P054.02

Topic: B.06. Synaptic Transmission

Support: KAKENHI Grant 18J21665

Title: Identification of factors regulating activity of corticotropin-releasing factor-producing neurons in the paraventricular nucleus of the hypothalamus

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Abstract: To know the nature of certain neuron is to reveal what factors regulate its activity. Corticotropin-releasing factor-producing neurons in the paraventricular nucleus of the hypothalamus (PVN-CRF neurons) are known to play a key role in the stress response as a commander of hypothalamic-pituitary-adrenal axis. To consider how the activity of PVN-CRF neurons is regulated to generate appropriate response to stress, it is important to identify factors which regulate the activity of these neurons. However, there have been few reports about regulators of PVN-CRF neurons. Since there are various type of neurons in the PVN, it has been difficult to record the activity of PVN-CRF neurons exclusively. Therefore, we performed

calcium (Ca^{2+}) imaging in PVN-CRF neurons of mouse acute brain slice to monitor neuronal activity, and screened change of the activity induced by various physiologically active substances. We created adeno-associated viral vector (AAV9-CMV-FLEX-YC) to express a calcium indicator, yellow cameleon-Nano50 (YC), in the cre recombinase expressing cells. We injected the AAV into the PVN of *CRF-iCre* knock-in mice (Itoi et al. 2014 *Endocrinology*; over 6-week-old, both male and female). Four weeks after infection, we made acute brain slices from the animal and subjected the slice to Ca^{2+} imaging. We individually applied 63 substances including neurotransmitters, neuropeptides and lipids through perfusion. As a result, we found that 12 substances increased Ca^{2+}, while 3 substances decreased Ca^{2+}. Among these substances, angiotensin II and histamine mainly increased calcium in the ventral portion of the PVN-CRF neurons via AT_1 and H_1 receptors, respectively. Conversely, carbachol mainly increased calcium in the dorsal portion of the PVN-CRF neurons via both nicotinic and muscarinic acetylcholine receptors. In this study, we established a novel universal system for screening of factors modulating Ca^{2+} signal in any cre-expressing cells by AAV infection and calcium imaging. In addition, we also got a clue to investigate how the activity of PVN-CRF neurons are regulated in terms of neurotransmitters and neuromodulators. The factors found in this study included those implicated in variety of physiological roles such as regulation of sleep-wakefulness, water intake and blood pressure. Our finding will contribute to understand how appropriate response to stress is generated through PVN-CRF neurons.

Disclosures: Y. Mukai: None. **A. Nagayama:** None. **K. Itoi:** None. **A. Yamanaka:** None.

Digital Abstract Session

P054. Regulation of Synaptic Transmission By Neurotransmitters and Neuromodulators

Program #/Poster #: P054.03

Topic: B.06. Synaptic Transmission

Support: NSERC of Canada 05407-2014
Fonds de Récherche du Québec- Santé

Title: Estrogen facilitates excitatory synaptic transmission in layer II of the entorhinal cortex via activation of GPER1

Authors: *A. A. BATALLÁN BURROWES, A. SUNDARAKRISHNAN, C. BOUHOUR, C. CHAPMAN;
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Abstract: Ovarian hormones, estrogens and progestogens, are thought to influence cognitive function via rapid synaptic changes associated with activation of their receptors and modulation of intracellular signaling. Hippocampal application of 17-β estradiol (E2) enhances excitatory synaptic transmission and improves working memory performance. Activation of estrogen receptors (ERs) can inhibit GABAergic neurons, modulate excitatory NMDA and AMPA currents, and reversibly increase CA1 pyramidal neuron dendritic spine densities.

Comparatively, progesterone (P4) can facilitate GABAergic function via its metabolite allopregnanolone. The entorhinal cortex is a significant source of cortical sensory and associational inputs to the hippocampus. However, it is unclear how ovarian hormones may impact synaptic transmission in the entorhinal cortex. The present study assessed the effects of the acute application of E2, P4, and allopregnanolone on excitatory glutamatergic synaptic transmission in layer II of the rat entorhinal cortex in vitro. On PD63, rats were ovariectomized and implanted with a subdermal E2 capsule to maintain constant low levels of circulating E2. Electrophysiological recordings were collected between PD70 and PD91. Acute horizontal brain slices were obtained and evoked field excitatory postsynaptic potentials (fEPSP) were recorded in layer II following stimulation of layer I afferents. After baseline recordings, a 20-min application of E2 (100 nM) resulted in an increase in fEPSP amplitude that reversed during the 30-min washout period. Neither application of the ERα agonist PPT (100 nM) nor the ERβ agonist DPN (1 μM) significantly altered synaptic responses compared to baseline. However, the GPER1 agonist G1 (100 nM) induced a reversible increase in fEPSP amplitudes similar to that induced by E2. Further, the GPER1 antagonist G15 (1 μM), prevented the facilitation induced by E2. The application of P4 (100 nM) or allopregnanolone (1μM) did not result in significant synaptic changes. This indicates that the rapid enhancement of excitatory synaptic transmission induced by estrogen in the entorhinal cortex is likely mediated by activation of GPER1 receptors. This enhancement may support the contribution of estrogen to cognitive function in the hippocampal region.

Disclosures: A.A. Batallán Burrowes: None. **C. Chapman:** None. **C. Bouhour:** None. **A. Sundarakrishnan:** None.

Digital Abstract Session

P054. Regulation of Synaptic Transmission By Neurotransmitters and Neuromodulators

Program #/Poster #: P054.04

Topic: B.06. Synaptic Transmission

Support: MOE2015-T2-2-095
MOE2017-T3-1-002

Title: Serotonin reduces neuronal gain in the claustrum

Authors: *K. L. L. WONG, G. J. AUGUSTINE;
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Abstract: The claustrum is a thin brain structure that is connected to various cortical regions. While it receives serotonergic innervation (J Comp Neurol. 526:2428), little is known about what serotonin (5-HT) does to the claustrum. Whole-cell patch clamp recordings in brain slices were used to determine the response of claustral neurons to pressure application of 5-HT. Projection neurons were identified by a classification scheme based on their intrinsic electrical properties (eNeuro 0216-20.2020). About 65% of projection neurons responded to 5-HT application, even

when polysynaptic effects were eliminated by blockade of receptors for glutamate (kynurenic acid; 100 μM) and GABA (GABAzine; 10 μM). In all five projection neuron types, 5-HT evoked a long-lasting outward current at a holding potential of -60 mV. This response was caused by a potassium conductance increase and was largely mediated by the 5-HTR-1A receptor, because it was eliminated by a specific antagonist of this receptor (WAY100635; 1 μM). To understand the functional consequences of this 5-HT response, we determined the input-output (I-O) relationship between current amplitude and action potential frequency. For this purpose, a ramp current (5 s, 0 - 400 pA) was injected into neurons while applying 5-HT. Compared to control conditions, 5-HT caused a rightward shift in the I-O curve, without changing the slope or maximal level of the I-O curve. This indicates that the 5-HT effect is subtractive. Further, the time course of the 5-HT induced change in neuronal excitability tracked the kinetics of the 5-HT induced outward current, indicating that the current reduces input gain by simply hyperpolarizing claustrum neurons. In conclusion, 5-HT reduces the response of claustrum neurons to excitatory input. Hence, 5-HT likely gates claustral function, thereby relieving the feedforward inhibition that the claustrum provides to its downstream cortical targets.

Disclosures: K.L.L. Wong: None. **G.J. Augustine:** None.

Digital Abstract Session

P054. Regulation of Synaptic Transmission By Neurotransmitters and Neuromodulators

Program #/Poster #: P054.05

Topic: B.06. Synaptic Transmission

Support: Beijing Municipal Science & Technology Commission (grant no. Z181100001318002)
Beijing Brain Initiative of Beijing Municipal Science & Technology Commission (grant no. Z181100001518004)
Guangdong Grant 'Key Technologies for Treatment of Brain Disorders' (grant no. 2018B030332001)
General Program of the National Natural Science Foundation of China (project nos. 31671118, 31871087 and 31925017)
NIH BRAIN Initiative (grant no. NS103558)
Peking-Tsinghua Center for Life Sciences
State Key Laboratory of Membrane Biology at Peking University School of Life Sciences

Title: Local 5-HT dynamics in a high brain learning center that critically modulate time dependent synaptic integration revealed by a GRAB sensor

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Abstract: Serotonin (5-HT), an important biogenic monoamine neuromodulator across animal phyla, has been implicated to participate in diverse physiological processes including learning and memory. In *Drosophila*, a single serotonergic DPM neuron innervates the olfactory learning center mushroom body (MB) of each hemisphere. Previous work established that perturbation of 5-HT metabolism or signaling by genetically or pharmacological means could lead to impaired olfactory learning, however, the dynamics of 5-HT *in vivo* and how it is regulated remains largely unknown, nor is clear how 5-HT may affect learning circuit in the MB. Here, capitalizing on transgenic flies that expressed our newly developed GRAB-5-HT sensor, we found physiological relevant stimuli, such as odor or aversive electrical body shock, readily triggered compartmental 5-HT dynamics *in vivo*. This compartmental 5-HT release relies on Ca2+ influx through DPM presynaptic nicotinic receptors. The DPM nicotinic receptors are in turn activated by local ACh release from upstream MB Kenyon cells (KCs). Next, we found locally released 5-HT from DPM could reciprocally act on KC terminals to turn down cAMP level as well as ACh release. Finally, by perturbation of 5-HT release from DPM neuron, we found 5-HT signaling is critical to control the length of time window where the plasticity of KC to MB output neuron may occur during odor-shock paring in γ1 compartment of MB. Thus, our work reveals *in vivo* 5-HT dynamics that may be important for optimal time window of synaptic plasticity during olfactory learning in *Drosophila*.

Disclosures: J. Zeng: None. **X. Li:** None. **Z. Zhangren:** None. **Y. Wang:** None. **X. Xia:** None. **M. Lv:** None. **K. Tan:** None. **J. Wan:** None. **Y. Li:** None.

Digital Abstract Session

P054. Regulation of Synaptic Transmission By Neurotransmitters and Neuromodulators

Program #/Poster #: P054.06

Topic: B.06. Synaptic Transmission

Support: AA02660

Title: Prenatal Ethanol Exposure Increases the Activity of Serotonin Neurons via Alterations of Endocannabinoid and Nitric Oxide Signaling in the Dorsal Raphe Nucleus

Authors: *S. OUBRAIM[1], R. WANG[1], K. HAUSKNECHT[1], M. KACZOCHA[2], R.-Y. SHEN[1], S. HAJ-DAHMANE[1];
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Abstract: Prenatal ethanol exposure (PE) leads to several neurodevelopmental and behavioral disorders referred to as fetal alcohol spectrum disorders (FASD) by affecting the function of numerous neurotransmitter systems, including the serotonergic (5-HT). Results from early

studies have shown that the altered stress homeostasis and increased anxiety-like behaviors induced by PE are mediated by alterations in the development and function of the 5-HT system. Yet, the cellular mechanisms by which PE alter the function of dorsal raphe 5-HT neurons are not well understood. Here, we characterize the effect of PE on the excitability of dorsal raphe nucleus 5-HT neurons and on the excitatory synaptic transmission in the DRn. We found that compared to prenatal control (PC), PE leads to a persistent increase on electrical activity of DRn 5-HT neurons. This effect was associated with a potentiation of glutamatergic transmission caused by a persistent increase in glutamate release. Examination of the mechanisms underlying these effects revealed that PE-induced potentiation of glutamatergic synaptic transmission is mediated at least in part by an enhanced tonic nitric oxide (NO). Thus, pharmacological inhibition of nNOS or NO scavenging depressed glutamatergic synaptic transmission in the DRn of PE, but not in PC rats. In addition, PE also impaired tonic endocannabinoid (eCB) signaling by reducing cannabinoid type 1 receptors (CB1Rs). As such, the results of the present study unravel the impact PE nitrergic and eCB systems, which play key role in controlling the function of DRn 5-HT neurons, and impaired eCB signaling in the DRn, which may contribute to PE associated disorders. They also establish a novel interaction between NO and eCBs that tightly modulate synaptic transmission onto 5-HT neurons.

Disclosures: **S. Oubraim:** None. **R. Wang:** None. **K. Hausknecht:** None. **M. Kaczocha:** None. **R. Shen:** None. **S. Haj-Dahmane:** None.

Digital Abstract Session

P054. Regulation of Synaptic Transmission By Neurotransmitters and Neuromodulators

Program #/Poster #: P054.07

Topic: B.06. Synaptic Transmission

Title: Adolescent binge drinking and dysregulated stress reactivity in adulthood: interneuronal tonic inhibition in the hippocampal regulation of the HPA axis

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Abstract: Adolescent binge drinking increases vulnerability to Alcohol Use Disorder and causes aberrant stress reactivity in adulthood. Recent work shows that alcohol changes the expression of δ-$GABA_A$Rs on inhibitory PV interneurons (PV+INs) in the hippocampus potentially disrupting hippocampal signaling. The hippocampus is an important negative regulator of the HPA axis. We hypothesize that the effect adolescent binge drinking has on stress reactivity in adulthood involves alcohol's impact on δ-$GABA_A$ receptor mediated tonic inhibition in the ventral hippocampus. Socially housed adolescent (P28-42) male and female mice were given two weeks of access to 20%v/v ethanol or water for three separate hours during their dark cycle to induce binge drinking. All mice went through withdrawal until they reached adulthood (P60) before forced swim (FST) and social defeat stress (SDS) tests were performed. Mice were sacrificed

after SDS, and the δ-$GABA_AR$ expression on the hippocampal PV+INs was evaluated with IHC and confocal microscopy. All data were analyzed by two independent researchers who were blind to the groups. Adult females who were adolescent binge drinkers displayed higher basal CORT (M=34.1 ±6.4) compared to water controls (M=16.9 ±2.9) and exhibited significantly increased immobility in the FST. Adolescent binge drinking had no effect on the initial CORT response to FST for either sex, but caused a dysregulated return to baseline for females. Mice were exposed to an aggressor for 5 minutes to assess neuroendocrine and behavioral response to a social stressor. Males with a history of adolescent binge drinking showed a blunted CORT response (M= 377.6 ± 36.1) to social stress when compared to water controls (M= 567.2±85.9). These results suggest that adolescent binge drinking results in aberrant endocrine response to stress even in the absence of isolation. The discrepancies in the stress responses of males and females suggest sex differences in this effect. Although adult binge drinking in adult females is associated with an increase in δ-$GABA_AR$ expression on hippocampal PV+INs, adolescent binge drinkers do not show this effect. However, significant sex differences in the expression of δ-$GABA_AR$ on PN+INs was observed, with males showing lower δ-$GABA_AR$ expression than females in either group. These results propose a possible mechanism for the aberrant neuroendocrine response in adulthood following adolescent binge drinking. Currently, we are evaluating changes associated with other proteins implicated in δ mediated tonic inhibition (α4) and assessing the role that interneuronal tonic inhibition in the ventral hippocampus plays in the regulation of stress.

Disclosures: B. Büyükdemirtas;: None. **M.L. Benn:** None. **A. Zuniga-Aleman:** None. **T. Brunner:** None. **A. Finnerty-Haggerty:** None. **J. Kwon:** None. **I. Watkis:** None. **C. Glickman:** None. **L.C. Melón:** None.

Digital Abstract Session

P055. Synaptic Integration

Program #/Poster #: P055.01

Topic: B.06. Synaptic Transmission

Support: NIMH R01MH123686
NIDCD T32DC010775-10
Whitehall Foundation grant 2018-12-9

Title: Cell type-specific nonlinearities of multi-modal inputs to posterior parietal cortex

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Abstract: Top-down signals provide rich contextual and goal orientation cues to facilitate sensory processing. Nonetheless little is known about top-down and bottom-up integration at the cellular level, partially due to difficulty in manipulating input afferents with population specificity. We overcome this limitation using a dual-color optogenetic strategy to determine

spatiotemporal features of multi-modal synaptic integration in the mouse posterior parietal cortex (PPC). We expressed Chrimson, a red light-sensitive channelrhodopsin variant, in the primary auditory cortex (A1) and ChR2, a blue light-sensitive opsin, in the anterior cingulate cortex (ACC), a frontal region commonly associated with attention and working memory. This opsin combination allowed independent control of sensory and frontal inputs to the PPC. Using whole-cell patch clamp recordings in acute brain slices we show that the PPC receives direct, monosynaptic inputs from both A1 and ACC. Similarly to other cortical areas, layer 5 principal cells in the PPC show heterogeneity in their firing patterns. We found that two classes of pyramidal neurons, intrinsically bursting and regular spiking, have distinct patterns of frontal-sensory integration. Intrinsically bursting cells displayed a robust enhancement of coincident input from ACC and A1, an effect that diminished with increasing delays between inputs. Conversely, regular spiking cells showed maximal enhancement when afferents were activated with delay, and a suppression of coincident inputs. Notably, bursting cell coincidence detection is mediated by a sodium and low-threshold calcium conductance, whereas regular spiking cell delayed integration requires NMDA receptor activity. Our data suggest that frontal-sensory integration has cell type specificity in the PPC, and begins to unravel the circuit architecture of multi-modal integration.

Disclosures: D.J. Rindner: None. **G. Lur:** None.

Digital Abstract Session

P055. Synaptic Integration

Program #/Poster #: P055.02

Topic: B.06. Synaptic Transmission

Support: NIH/NINDS NS097185

Title: Local synaptic inhibition in mouse globus pallidus during autonomous firing

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Abstract: Neurons in globus pallidus pars externa (GPe) are autonomous oscillators, but their intrinsic rhythmic firing is perturbed by synaptic input, including local inhibition from axon collaterals of other GPe cells. To characterize the local inhibition during natural firing, we recorded spontaneous IPSCs in coronal slices of GPe from male and female mice. The spontaneous IPSCs were sensitive to gabazine, and many of them were blocked by tetrodotoxin, indicating $GABA_A$ receptor-mediated, action potential-dependent events. In slices, GPe neurons fire relatively regularly, and this allowed us to identify periodic components of the IPSC time series corresponding to unitary synaptic inputs. By analyzing the periodic IPSCs, we identified the firing frequency and inter-spike interval variability (CV_{ISI}) of the presynaptic neurons and estimated the unitary synaptic reliability and the unitary IPSC amplitudes. To determine what type(s) of GPe neurons received the spontaneous inhibition, we recorded from genetically

labeled parvalbumin (PV) and NPAS1 expressing neurons. Both cell types received periodic spontaneous IPSCs with similar frequencies, suggesting a common presynaptic cell type(s). Optogenetic inhibition of PV neurons using Archaerhodopsin reduced the spontaneous IPSC rate in almost all PV neurons with active unitary inputs, whereas optogenetic inhibition of NPAS neurons rarely affected the spontaneous IPSCs in other NPAS cells. These results suggest that PV neurons provide most of the active unitary inputs to both cell types. In cells with active unitary inputs, optogenetic stimulation of striatal indirect pathway axons caused a pause and temporal resetting of the periodic input, confirming that it arose from local neurons subject to striatal inhibition. Our results show that the local inhibitory network is a powerful source of inhibition to both PV and NPAS neurons, and they suggest that the local network may influence GPe cells' responses to external synaptic input.

Disclosures: J. Jones: None. **M. Higgs:** None. **C.J. Wilson:** None.

Digital Abstract Session

P056. Electrical Synapses and Gap Junctions

Program #/Poster #: P056.01

Topic: B.06. Synaptic Transmission

Support: NSF Grant IOS 1557474

Title: Dopaminergic modulation of excitability and electrical synapses in the thalamic reticular nucleus

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Abstract: The thalamic reticular nucleus (TRN) is a thin shell of electrically coupled inhibitory interneurons that regulates afferent sensory signaling in the thalamus. The TRN receives dopaminergic innervation from the midbrain, and it expresses high concentrations of D_1 and D_4 receptors. Dopamine release in the TRN could add a reward valence to thalamic processing of salient sensory surround. The direct effect of dopamine on TRN neurons and synapses is largely unknown and is key to understanding modulatory control of thalamocortical processing. Synaptic communication within the mature TRN occurs exclusively through its powerful and dense electrical synapses. Dopamine has been shown to modulate electrical synapse strength in other systems, including retina and the goldfish Mauthner synapse. To characterize how dopamine affects neuronal excitability and electrical synapses in the TRN, we utilized paired whole-cell patch clamping. Each cell of a patched pair was injected with 500-ms current pulses to measure resting membrane potential, input resistance, threshold, spiking frequency, spontaneous synaptic inputs, and coupling conductance. Measurements were taken before and after bath application of dopamine, a D_1 agonist, or a D_4 agonist. Our results show that bath application of dopamine results in heterogeneous alternations to tonic spiking frequency, input resistance, and electrical coupling in the TRN. Next, using specific receptor agonists, we determined that the bidirectional

effects of dopamine can be explained by contributions from different receptor subtypes. Agonization of D_4 receptors consistently decreased tonic spike frequency, while agonization of D_1 receptors consistently increased tonic spike frequency. Further, D_4 receptor agonization consistently depressed electrical synapses, while D_1 agonization had no consistent effect on electrical synapse strength. Taken together, our results show that dopamine release can regulate excitability and electrical connectivity in the TRN in a manner wherein valence of modulation is specific to the receptor subtype activated. We hypothesize that dopamine receptors recruit multiple intracellular signaling cascades with opposing effects on gap junction conductance, and the availability of each cascade determines whether dopamine receptors potentiate or depress electrical synapses. We further hypothesize that dopaminergic inputs modulate the intensity and coordination of inhibitory signals sent to thalamus by TRN neurons, and consequently affect cortical attention to the sensory surround.

Disclosures: M.J. Vaughn: None. **J.S. Haas:** None.

Digital Abstract Session

P056. Electrical Synapses and Gap Junctions

Program #/Poster #: P056.02

Topic: B.06. Synaptic Transmission

Support: Rady Innovation fund

Title: Characterization of connexin36 at mixed chemical/electrical synapses formed by mossy fiber terminals in rat ventral hippocampus

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Abstract: Granule cells in the hippocampus project axons to hippocampal CA3 pyramidal cells, where they form large mossy fiber terminals known for their higher transmission fidelity. We reported that these terminals contain the gap junction protein connexin36 (Cx36), specifically in the stratum lucidum of rat ventral hippocampus, thus creating morphologically mixed synapses that have the potential for dual chemical/electrical transmission. There is some electrophysiological evidence that these terminals have a gap junction-mediated electrical transmission component under some conditions. Here, we have used immunofluorescence approaches to examine distinguishing molecular features of Cx36-containing gap junctions at mossy fiber terminals and their postsynaptic elements in combination with electrophysiological measures to probe functional consequences of having mixed synapses in the ventral hippocampus. In the CA3b and CA3c hippocampal regions, the vast majority of these terminals, identified by their selective expression of vesicular zinc transporter-3 (ZnT3), displayed multiple, fine immunofluorescent Cx36-puncta representing gap junctions, which were absent at

mossy fiber terminals in the dorsal hippocampus. Among those ZnT3+ terminals displaying Cx36-puncta in the ventral hippocampus, analysis of numerous terminals showed that they contained an average of 2.4 ± 0.2 puncta per terminal that were 3-5 μm in diameter. These puncta were invariably found in close proximity to the protein constituents of adherens junctions (i.e., N-cadherin and nectin-1) that are a structural hallmark of mossy fiber terminals that contact dendritic shafts of CA3 pyramidal cells, thus indicating the loci of gap junctions at these contacts. Cx36-puncta were also associated with adherens junctions at mixed synapses in other regions of the CNS. Mossy fibers exhibit multiple types of terminals on their way to the stratum lucidum. In the hilus, where they form collaterals that synapse onto interneurons and mossy cells, Cx36-puncta were very sparsely distributed, and none of these Cx36-puncta were associated with ZnT3+ mossy fiber terminals, suggesting these collaterals do not form mixed synapses with their postsynaptic targets. Electrophysiologically induced long-term potentiation of field responses evoked by mossy fiber stimulation was greater in the ventral than dorsal hippocampus, and it remains to be determined whether the electrical component of transmission at mossy fiber terminals contributes to the enhancement of these responses.

Disclosures: D. Thomas: None. **J.I. Nagy:** None. **A. Beyer:** None. **M. Jackson:** None. **B. Lynn:** None. **J. Senecal:** None.

Digital Abstract Session

P057. Cholinergic and Paracrine Modulation

Program #/Poster #: P057.01

Topic: B.06. Synaptic Transmission

Support: Oak Ridge Institute for Science and Education
CounterACT Interagency Agreement (NIH/NINDS and USAMRICD)

Title: Baseline acetylcholinesterase activity of blood and tissue in pediatric and adult rats

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Abstract: Chemical warfare nerve agents cause profound peripheral and central nervous system effects. Nerve agents exert their toxic effects by inhibiting the cholinesterase (ChE) family of enzymes, including acetylcholinesterase (AChE). These agents bind to the active site of the AChE enzyme, preventing it from hydrolyzing the neurotransmitter acetylcholine (ACh) and causing hyper-stimulation of the cholinergic system. This leads to detrimental physiological effects including miosis, muscle fasciculations, tremors, loss of respiratory control, and, most devastating to the central nervous system, repetitive seizures (McDonough, 2002). In the event of civilian nerve agent exposure, children would likely be among the worst casualties as they are more susceptible to seizures than are adults (Haut et al., 2004; Rakhade & Jensen, 2009; Sidell et al. 2008). In 2016, Wright and colleagues illustrated that toxicity of nerve agents differs greatly

across developmental ages in rats, such that immature animals were 2.7-fold more sensitive to the lethal effects of G series agents than were older animals. Toxicity of nerve agents results from the inhibition of AChE; therefore, the present study determined differences in baseline AChE levels in blood and tissues (brain and peripheral) across developmental ages. Red blood cells, whole blood, brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, striatum, perirhinal, diaphragm, heart, and skeletal muscle tissue were collected from post-natal day (PND) 7, 14, 21, 28, and 70 male and female rats ($N = 6$/group) and were assayed using a modified Ellman assay (Shih et al., 2010) to determine baseline level of AChE activity across age groups. Significant differences in baseline AChE activity in both tissues and blood were found. Generally, results indicated a trend of increasing AChE activity in brain tissue as animals matured (i.e., brainstem, cortex, hippocampus, striatum, perirhinal, $p < .001$), while there was a trend of decreasing AChE activity in skeletal muscle ($p < .001$) and red blood cells ($p < .001$) as animals matured. There were no differences between sexes within age groups. Overall, pediatric animals had lower AChE activity, providing a biological basis supporting the findings of Wright et al. (2016). Because baseline AChE levels differ across age groups, we can hypothesize that nerve agent exposure in humans would differentially impact children and adults.

Disclosures: A.N. Santoro: None. **J.H. McDonough:** None.

Digital Abstract Session

P057. Cholinergic and Paracrine Modulation

Program #/Poster #: P057.02

Topic: B.06. Synaptic Transmission

Support: DST India INSPIRE fellowship IF160611

Title: Cholinergic signals optimize information transmission and learning in the hippocampus

Authors: *R. SHARMA, S. NADKARNI;
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Abstract: Neuromodulators are essential to regulate and fine-tune nonlinear membrane and synaptic properties. This provides neural circuits with flexibility in function and across behavioral states. We focus on acetylcholine, in particular, released during active waking in the cortex and hippocampus. In the hippocampus, acetylcholine modulates neuronal activity predominantly via its action of the M1 and M4 metabotropic receptors. Their distinct localizations and biophysical properties produce rich dynamics across multiple timescales. We have developed a detailed biophysical model for the presynaptic activation of M4 and postsynaptic activation M1 receptors in the hippocampus. Our model describes activation of M1 receptors leading to IP3 production from membrane PIP2 molecules. Binding of IP3 to IP3 receptors ultimately causes calcium release from the endoplasmic reticulum (ER). The additional calcium release from the ER enhances the action of potassium channels like the calcium activated SK channels and modifies other downstream synaptic signaling. Interestingly, an

opposing effect of M1 receptors is to directly suppress SK channels and the voltage activated KCNQ2/3 channels. We quantify the effect of enhanced calcium signaling from the ER and antagonistic effects on SK channels via M1 receptor activation on long term plasticity of the schaffer-collateral synapse in the hippocampus. Our model also describes presynaptic reduction of the voltage sensitivity of voltage gated calcium channels (VGCCs) and resulting suppression of the neurotransmitter release via M4 receptors. We characterize the influence of apparent dichotomy of suppression of synaptic transmission by M4 and enhanced neuronal excitability by M1 on synaptic signaling and information transmission in a range of physiologically relevant activity regimes.

Disclosures: R. Sharma: None. **S. Nadkarni:** None.

Digital Abstract Session

P057. Cholinergic and Paracrine Modulation

Program #/Poster #: P057.03

Topic: B.06. Synaptic Transmission

Title: Modeling the synaptic effects of anesthesia and cortical cholinergic reversal

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Abstract: General anesthetics work through varied molecular mechanisms while leading to similar end points of sedation and loss of consciousness. The effects of general anesthesia range from alteration of global brain functional connectivity to changes in synchronization at the scale of neural populations. It is poorly understood, however, how these global and local changes are caused and how this leads to different states of awareness. At the cortical level, anesthesia leads to changes in spontaneous spiking activity and can be characterized by changes in cortical complexity. At the receptor level, the administration of common inhalation anesthetics generally leads to decreases in synaptic excitation while promoting synaptic inhibition (Rudolph & Antkowiak, 2004). Because $GABA_A$ receptors are known targets of common anesthetic agents such as Propofol, variations in $GABA_A$ synaptic efficacy have been extensively modeled and have been shown to correctly model biphasic changes in EEG power spectra (Hutt & Longtin, 2010). Beyond the effects at the synapse, it is shown that region-specific changes in acetylcholine concentration (and its agonists) can lead to partial reversal in the level of sedation in rats (Pal et al., 2018). To study the effects of these synaptic changes and induced reversal we develop a computational model that tracks changes in synaptic inhibition and excitation as well as accounts for the neuromodulatory effects of acetylcholine and compare the resultant network dynamics with observed changes in visual cortex spiking activity in rats under the administration of Desflurane. Specifically, by potentiating the synaptic effects at $GABA_A$ receptors and inhibiting the effects at NMDA receptors we can recover changes in spiking dynamics including changes in firing rate and synchronization associated with anesthesia administration. Moreover,

by introducing the effect of acetylcholine in our model we can undo the changes associated with the synaptic effects. This provides a framework in which induced recovery from anesthesia can occur by redundant mechanisms that increase network excitability.

Disclosures: B.P. Eniwaye: None. **A.G. Hudetz:** None. **M. Zochowski:** None. **V. Booth:** None.

Digital Abstract Session

P057. Cholinergic and Paracrine Modulation

Program #/Poster #: P057.04

Topic: B.06. Synaptic Transmission

Title: Kv4.2 channel is required for the rapid actions of anti-Müllerian hormone in regulating synaptic transmission in the hippocampus

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Abstract: Kv4.2 channel is required for the rapid actions of anti-Müllerian hormone in regulating synaptic transmission in the hippocampus

Authors
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Abstract
Anti-Müllerian hormone (AMH) is a paracrine factor generated by the gonads to regulate gonadal function in adult mammals. We recently reported that AMH-specific receptor is expressed in the hippocampus, and exogenous AMH protein addition rapidly increased synaptic transmission and long-term synaptic plasticity at the CA3-CA1 synapses. Therefore, in this study, we aimed to investigate the mechanism of the boosting effect of AMH in regulating CA3-CA1 excitatory synaptic transmission in the hippocampus. Brain tissue was obtained from CD-1 mice (6-8 weeks old). The direct actions of AMH on synaptic transmission were examined by whole-cell recordings in hippocampal slices with and without 0.4 nM recombinant human AMH protein addition. Evoked excitatory postsynaptic currents (EPSCs) and excitatory postsynaptic potentials (EPSPs) were measured under different conditions. Data from whole-cell voltage-clamp recordings showed that AMH did not affect AMPA-mediated EPSCs at -70 mV (99.9 ± 12.0 %; $p = 0.72$) or isolated NMDA-mediated EPSCs at -30 mV (93.1 ± 19.6 %; $p = 0.50$). These results suggested that neither AMPA nor NMDA receptors contribute to the boosting effect of AMH on synaptic transmission. The small-conductance Ca^{2+}-activated K^+ channel SK2 and voltage-gated K^+ channel Kv4.2 modulate evoked synaptic responses in CA1 pyramidal neurons. To determine whether AMH modulates SK2 or Kv4.2 activity, EPSPs were recorded with either apamin or 4-aminopyridine (4-AP) to block SK2 or Kv4.2 channels, respectively.

Whole-cell current-clamp recordings showed that blocking SK2 with apamin did not alter the AMH effect on increasing EPSPs (162.5 % ± 19.1 % in apamin versus 163.1 ± 16.3 % in control; $p < 0.01$). However, AMH treatment no longer increased EPSPs in CA1 pyramidal neurons when 4-AP was present either in external bath (89.5 % ± 15.1 %; $p = 0.54$) or internal pipette solution (72.5 % ± 18.8 %; $p = 0.14$). These results show that Kv4.2, but not SK2, channels are required for the rapid action of AMH on boosting synaptic transmission. Our findings provide functional evidence that AMH directly enhances synaptic transmission through Kv4.2 channel in the hippocampus, suggesting a possible role of Kv4.2 channel in AMH-regulated neuronal process underlying learning and memory.

Disclosures: K. Wang: None. **J. Maylie:** None. **J. Xu:** None.

Digital Abstract Session

P058. Modulation: Pharmacology

Program #/Poster #: P058.01

Topic: B.06. Synaptic Transmission

Title: Using the motor units of Drosophila to screen pharmacological therapeutic agents- riluzole and levetiracetam

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Abstract: The attributes of the larval Drosophila neuromuscular junction (NMJ) allow one to assess effects of compounds on the motor unit of neurons as well as the postsynaptic attributes of synaptic transmission. The ability to follow through with genetic studies to examine potential mechanisms of action in pharmacological agents serves a screening ground for future targeted investigations. The larval NMJ is glutamatergic and produces graded evoked excitatory junction potentials. There is also a high frequency of spontaneous quantal events which are readily able to be indexed. To aid in understanding the effects of two compounds currently used as therapeutics, in which the mechanisms are not fully determined we tested their effects on synaptic transmission at the larval NMJ. Riluzole (Rilutek) and levetiracetam (Keppra) are used to alter neural function in humans; however, the various effects are still being investigated. It appears riluzole blocks neuronal excitability by inhibiting action potential propagation without altering glutamate receptivity or frequency of spontaneous quantal events. Thirty minute preincubation to levetiracetam (1 mM) had no effect on the amplitude of evoked junctional potentials with low frequency stimulation but enhanced synaptic depression with higher frequency stimulation. Comparisons with NMJs of crayfish reveal levetiracetam enhanced evoked transmission but similar results occurred for riluzole.

Disclosures: R.L. Cooper: None. **S. McCubbin:** None. **N. deCastro:** None.

Digital Abstract Session

P058. Modulation: Pharmacology

Program #/Poster #: P058.02

Topic: B.06. Synaptic Transmission

Support: NIH Grant P60 AA011605

Title: Adaptations in Excitatory Signaling Onto Deep-Layer Prelimbic Cortical Pyramidal Neurons After Chronic Alcohol Exposure

Authors: *B. HUGHES, T. K. O'BUCKLEY, G. BOERO, M. A. HERMAN, A. MORROW; Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Chronic alcohol use impairs cognition resulting in working memory deficits, anxiety, and excessive drinking that are associated with prefrontal cortical (PFC) dysfunction. Our laboratory have shown multiple impairments in GABAergic neurotransmission onto deep-layer principal cells (PCs) in PFC that indicate reduced inhibitory tone and suggest dependence-induced cortical dysfunction is the product of overexcitability in these cells. The present work sought to further determine whether deep-layer PCs displayed coincident adaptations in excitatory signaling that further promote hyperexcitability, using a chronic exposure model previously employed by our laboratory (e.g. 5.0 g/kg, i.g., 15 days) in male and female Sprague-Dawley rats. Whole-cell electrophysiological experiments were conducted 24 hours after final administration in deep-layer prelimbic PCs, and electrically-evoked AMPA-mediated excitatory post-synaptic currents emanating from Layer II/III PCs recorded across a range of stimulation frequencies. We observed a robust shift toward paired-pulse depression after alcohol exposure in sub-cortically projecting Type-A PCs with no apparent change in intra-cortically projecting Type-B cells in males, but not females. This shift toward paired-pulse depression is indicative of enhanced glutamate release probability from superficial PCs onto deep-layer PCs, as modulating extracellular calcium concentration elicited differential enhancements of paired-pulse ratio in Type-A cells consistent with this conclusion. Given reports that PFC afferents regulate anxiety-like behavior, we next evaluated elevated plus maze behavior as a proxy measure of changes in Type-A cell activity, and observed significantly elevated responses after ethanol exposure. Ethanol exposure did not affect working memory performance, consistent with no changes in Type-B cell signaling and activity. Interestingly, alcohol-exposed females displayed enhanced working memory performance with no effect on anxiety-like behavior, suggesting fundamental differences between sexes. In sum, these observations demonstrate that excitatory signaling onto Type-A cells is particularly sensitive to the effects of chronic alcohol exposure in male rats and appear crucial for the expression of alcohol-induced behavioral changes. Furthermore, these findings show that alcohol effects on cortical function cannot be assumed equivalent between sexes. Additional experiments will delineate the pre- and post-synaptic mechanisms governing altered signaling among PC subtypes, and discern the potential causal role of Type-A cells in dependence-like behaviors.

Disclosures: **B. Hughes:** None. **T.K. O'Buckley:** None. **G. Boero:** None. **M.A. Herman:** None. **A. Morrow:** None.

Digital Abstract Session

P058. Modulation: Pharmacology

Program #/Poster #: P058.03

Topic: B.06. Synaptic Transmission

Support: NIAAA Grant 1R01AA025652-01
NIAAA Grant 1P50AA022534-01
NIAAA Grant T32AA014127

Title: Sex specific effect of Prenatal Alcohol Exposure on NMDARs functionality in orbitofrontal cortex pyramidal neurons.

Authors: *V. LICHERI[1], J. CHANDRASEKARAN[1], C. W. BIRD[1], F. C. VALENZUELA[1,2], J. L. BRIGMAN[1,2];
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Abstract: Alcohol consumption during the pregnancy represents a major public health problem. It is estimated that 10% of pregnant women drink alcohol. The most disabling outcomes of drinking during pregnancy are indicated with the term Fetal Alcohol Spectrum Disorders (FASDs). FASDs include deficits in learning, working memory, social behavior and executive function during early life and persist into adulthood. Using a moderate prenatal alcohol exposure (PAE) combining with touch screen behavioral tasks and in vivo electro-physiology we found a significant impairments in orbitofrontal cortex (OFC)- mediated reversal learning (Marquardt et al., 2014b), and also an altered firing of OFC pyramidal neurons (Marquardt et al., 2020). N-Methyl-D-Aspartate receptors (NMDARs) play a key role in the control of synaptic plasticity, moreover previous studies have shown that NMDAR containing GluN2B-subunit resulted to be altered by moderate PAE. Given that the PAE effects on NMDARs in OFC are less well investigated, here we studied possible changes in NMDARs function integrating a well-established voluntary drinking paradigm for moderate PAE with ex-vivo electrophysiology. Whole-cell patch clamp recordings were performed in slices coming from saccharine controls (SAC) and PAE male and female mice sacrificed at postnatal day (PND) 90-100 days. NMDA receptor-mediated evoked excitatory post-synaptic currents (NMDA-eEPSCs) were acquired at +40 mV and in the presence of the AMPAR antagonist NBQX (10 μM). GluN2B mediated currents were isolated pharmacologically using Ro25-6981 (1 μM). At the end, the NMDA receptor component was verified applying NMDAR-antagonist (APV 50 μM). Comparison of SAC and PAE showed that PAE females have significantly larger NMDA -eEPSCs amplitude than PAE males. Moreover, PAE males showed a significant decrease in current density. While, pharmacological isolation of GluN2B subunit mediated currents revealed that PAE treatment may positively modulate GluN2B function in PAE males. These findings help to elucidate the

molecular mechanism of cognitive impairment in FASDs and give an important tool for developing pharmacological therapies for executive dysfunction in FADSs.

Disclosures: **V. Licheri:** None. **J. Chandrasekaran:** None. **C.W. Bird:** None. **F.C. Valenzuela:** None. **J.L. Brigman:** None.

Digital Abstract Session

P058. Modulation: Pharmacology

Program #/Poster #: P058.04

Topic: B.06. Synaptic Transmission

Support: NIH Grant R01 AA026531

Title: Sex differences in the acute ethanol modulation of GABAergic inputs to principal neurons of the basolateral amygdala

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Abstract: Alcohol use disorder (AUD) is a chronic relapsing brain disorder characterized by compulsive use of alcohol and a loss of control of alcohol intake. Recent studies suggest there are sex-dependent behavioral changes following alcohol administration, as evidenced by rapid acquisition of self-administration of alcohol, rapid escalation of alcohol intake with extended alcohol administration, and less withdrawal with more recovery in females compared to males. In addition, findings in humans suggest that women are at higher risk of developing alcohol-related health disorders compared to men. Therefore, it is imperative to understand the underlying neurobiological substrate of the sex differences in alcohol effects in order to develop sex-specific therapeutic targets. The basolateral amygdala (BLA), which consists of both glutamatergic projection neurons and GABAergic interneurons, is known to play an important role in the development of AUD. The overall excitatory activity of the BLA is tightly regulated by GABAergic interneurons. The alcohol regulation of excitatory and inhibitory circuits of the adult BLA is not well understood. Thus, determining the effect of alcohol on the activity of specific GABAergic interneuron circuits in the BLA is crucial to understanding the neuronal basis for the neurophysiological and behavioral effects of alcohol consumption. In the present study, we tested the hypothesis that ethanol modulates GABA inhibitory inputs to BLA principal neurons from specific interneuron subpopulations, thereby regulating BLA principal neuronal outputs, in a sex-specific manner. Using *ex vivo* whole-cell patch-clamp recordings from BLA pyramidal neurons in amygdala slices from adult male and female Wistar rats, we investigated the effects of acute ethanol application on GABAergic inhibitory postsynaptic currents (IPSCs). Ethanol (44 mM) increased the frequency of miniature IPSCs (mIPSCs) without affecting mIPSC amplitude or decay-time. This ethanol-induced presynaptic modulation of GABA release was found to be dependent on P/Q-type calcium channel activity, but not N-type calcium channels, suggesting that it is specific to parvalbumin neurons. In BLA principal neurons from

females, on the other hand, there was no effect of ethanol on inhibitory synaptic inputs. These data suggest that ethanol causes a spike-independent, presynaptic modulation of GABA inhibitory inputs from parvalbumin interneurons to BLA principal neurons in adult male, but not female, rats. This sex difference in ethanol modulation of specific inhibitory synaptic circuits in the BLA may play a role in the differential susceptibility of males and females to AUD.

Disclosures: S. Munshi: None. **J.G. Tasker:** None.

Digital Abstract Session

P059. Connectome and Structural Plasticity

Program #/Poster #: P059.01

Topic: B.07. Synaptic Plasticity

Support: NIH Grant R01NS036715
La Caixa Fellowship

Title: Neuronal response adaptation and AMPA receptor plasticity during visual stimulation

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Abstract: Repeated exposure to visual stimulation induces connectivity changes in layer 4 of the primary visual cortex (V1) that have been associated with Hebbian synaptic plasticity and perceptual learning. However, the effects of this stimulation on the activity pattern of supragranular V1 excitatory neurons as well as on their synaptic structure and composition remain elusive. Here, we use longitudinal 2-photon imaging to monitor neuronal activity and synaptic AMPA receptor (AMPAR) expression in V1 layer 2/3 (L2/3) of awake mice during repetitive visual stimulation. Visual stimulation consisting of phase-reversing gratings in 6 different orientations recruits a subset of neurons that present phase specificity and within-session habituation. Moreover, this pool of responsive neurons decreases with daily stimulation, leading to a sparser neuronal response profile across all orientation preferences. In addition, we observe an overall increase in synaptic AMPAR levels in the dendritic spines of a subset of V1 L2/3 neurons with repeated exposure to visual stimulation. Together, our results demonstrate the capacity of sensory stimulation to refine circuitry and induce synaptic potentiation in their corresponding primary cortical areas.

Disclosures: E. Lopez-Ortega: None. **I. Hong:** None. **R.H. Roth:** None. **R.H. Cudmore:** None. **R.L. Huganir:** None.

Digital Abstract Session

P059. Connectome and Structural Plasticity

Program #/Poster #: P059.02

Topic: B.07. Synaptic Plasticity

Support: Supported by NIH grant NS047718, the Craig H. Neilson Foundation, and generous donations from Cure Medical, Research for Cure
J. Yonan was the recipient of a predoctoral fellowship from NIH 5T32 NS045540 and a diversity supplemental under NIH R01 NS108189 (OS)

Title: Aav-mediated conditional pten deletion in adult dentate granule cells triggers robust de novo neuronal growth without disruption of connectional specificity

Authors: *J. M. YONAN[1], O. STEWARD[2];
[1]Univ. of California Irvine, Irvine, CA; [2]Univ. of California, Irvine, Irvine, CA

Abstract: Phosphatase and tensin homolog (PTEN) is an important negative regulator of the mechanistic target of rapamycin (mTOR) pathway responsible for cell growth and proliferation during development. Studies utilizing PTEN deletion in various developmental animal models have reported alterations to neuronal growth and cell morphology that result in the formation of aberrant circuitry, seizures, and early death. We have recently documented that PTEN deletion in adult cortical motoneurons triggers *de novo* growth of neuronal cell bodies and dendrites that continues for months, without any obvious adverse outcomes. Still, the consequences of PTEN deletion, persistent mTOR activation, and *de novo* growth of mature neurons in the adult brain are not fully understood. Here, we explore consequences of focal PTEN deletion in adult dentate granule cells on morphological features and laminar specificity of their local and distant inputs and outputs. We use the approach of deleting PTEN by injecting AAV-Cre into adult PTEN-floxed, Rosa reporter mice. This technique allows for targeted PTEN deletion in granule cells and preservation of PTEN expression in granule cell input neurons and their target cells. Immunostaining revealed complete loss of PTEN protein within dentate granule cells in the area of injection, with graded deletion spanning its septal/temporal axis. At the core of transfection, there were increases in the area of the dentate gyrus over time and dramatic increases in the size of granule cell bodies by 2 months post injection that progressed out to 6 months after deletion. Growth of cell bodies was accompanied by increases in the thickness of the molecular layer, the caliber of apical dendrites, and density of dendritic spines, implying growth and modification of granule cell dendritic arbors. Despite this growth, tract tracing of perforant path and commissural inputs to the dentate molecular layers revealed that laminar specificity of termination was largely maintained. Additionally, the axonal projections of dentate granule cells (mossy fibers) enlarged over time as evidenced by increases in the thickness of the mossy fiber tract and terminal field in stratum lucidum of the CA3. However, there was no evidence of extension of mossy fibers into CA1 or stratum radiatum of CA3. Supra-granular mossy fibers were seen in some mice at later time posts. Our present results together with previous findings indicate that deletion of PTEN in adult dentate granule cells initiates *de novo* growth in fully mature neurons, indicating

transformation to a growth phenotype regardless of neuron age or type, while maintaining overall gross input and output specificity.

Disclosures: J.M. Yonan: None. **O. Steward:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OS is a co-founder and holds economic interests in the company "Axonis", which holds a license on patents relating to PTEN deletion and axon regeneration..

Digital Abstract Session

P059. Connectome and Structural Plasticity

Program #/Poster #: P059.03

Topic: B.07. Synaptic Plasticity

Support: Grant-in-Aid for Scientific Research on Innovative Areas, 19H05430

Title: The transition of network topology and connection sustainability in cultured neurons

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Abstract: During development, neurons extend their neurites and establish synapse connections to form a functional neuronal network. In the process of the neuronal network formation, the coordinated activation of neuronal assemblies is found in most physiological brain functions and influences proper network wiring during development. In cultured neurons, this coordinated neuronal activity is only observed within one month from the onset of culture and it was regulated by a small number of specific neurons. These specific neurons, with dense functional connections to many other neurons, have been known as 'hub-neurons'. However, measurement of long-term neuronal responses and assessment of functional connectivity in single cell level has not been performed yet. Thus, it is unclear that how hub-neurons have been generated and whether their properties are maintained in developing neuronal networks. Furthermore, many intracellular signals related to neuronal maturation and connectivity generation have been reported; CREB, ERK and mTOR promote synaptic plasticity, maturation of neurons, and energy metabolism. Their direct involvement in the generation of functional connectivity and maintenance is also unknown. In this study, we extracted functional connectivity by Ca^{2+} imaging and investigated the characteristics of hub-neurons in developing cultured hippocampal network. We measured neuronal responses over several days to assess the change and sustainability of the functional connectivity by using a viral vector expressing the calcium indicator. The coordinated activations of neuronal assemblies were only observed during the around second postnatal week *in vitro*. These activities were mediated by activation of $GABA_A$ receptors because they were abolished by the $GABA_A$ receptor antagonist Gabazine. The number of hub-neurons was not changed, but half of hub-neurons does not maintain its property of high connectivity. Moreover, the functional connections between hub-neuron and hub-neuron were maintained. We also evaluated several kinase activities by immunostaining after neuronal

activity measurement. Phosphorylation levels of ERK were different in hub-neurons and others, but not in CREB and 4E-BP1, a downstream effector of mTOR. These results demonstrate that hub-neurons establish many functional connections to other neurons in the early developmental stage and unnecessary functional connections could be removed by decrease of ERK phosphorylation levels.

Disclosures: **R. Ide:** None. **Y. Shindo:** None. **K. Hotta:** None. **K. Oka:** None.

Digital Abstract Session

P059. Connectome and Structural Plasticity

Program #/Poster #: P059.04

Topic: B.07. Synaptic Plasticity

Support: NIH grant P60AA011605
T32 grant AA007573

Title: Perineuronal net (PNN) density is increased in the orbitofrontal cortex in adult rats exposed to AIE

Authors: ***C. A. DANNENHOFFER**[1], V. A. MACHT[2], H. MEEKS[2], E. SUTHERLAND[2], *A. GOMEZ-A[2], C. A. BOETTIGER[3], D. L. ROBINSON[4];
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Abstract: Alcohol exposure during adolescence is detrimental to proper brain development, particularly to the prefrontal cortex which is involved in decision-making and cognitive flexibility. Our lab previously found that adolescent intermittent ethanol (AIE) exposure in rats impairs cognitive flexibility in behavioral tests and typical frontostriatal connectivity via fMRI. One mechanism integral to the molecular underpinnings of cognitive flexibility is the expression of perineuronal nets. Perineuronal nets (PNNs) are extracellular matrix components which surround inhibitory interneurons in a variety of brain regions to mediate flexibility in synaptic remodeling. As such, an increase in PNNs within the medial prefrontal cortex (mPFC) and orbitofrontal cortex (OFC) could mediate loss of cognitive flexibility evidenced after AIE. To test this hypothesis, we used immunohistochemistry (IHC) to investigate expression of the markers for the inhibitory interneuron parvalbumin (PV) as well as PNNs using the lectin *wisteria floribunda agglutinin* (WFA) after AIE or control treatment. Male rats were exposed to alcohol (5 g/kg ethanol) or water via intragastric intubation throughout adolescence (postnatal days (P)25-54) in a 2-days-on/2-days-off regimen. In adulthood, animals were perfused, and brains were collected and prepared for IHC. Tissue (including OFC and mPFC) were stained for WFA and PV using immunohistochemical procedures. Results indicate that while the expression of PV+ immunoreactivity (IR) is unchanged in the mPFC and OFC, density of WFA+IR is elevated within the OFC after AIE relative to water-treated controls. This suggests that AIE

induces an impairment in the molecular mechanisms underlying synaptic plasticity as evidenced by increased in PNNs. Future studies will investigate whether this increase in WFA is specific for PV or other types of inhibitory interneurons. These findings suggest that intermittent binge alcohol exposure during adolescence changes extracellular matrix components that regulate synaptic plasticity of inhibitory neurons such as PV. This reduced synaptic plasticity in the OFC may underlie reduced functional connectivity with other frontal and striatal regions and impaired cognitive flexibility after alcohol exposure.

Disclosures: **A. Gomez-A:** None. **V.A. Macht:** None. **H. Meeks:** None. **E. Sutherland:** None. **C.A. Boettiger:** None. **D.L. Robinson:** None. **C.A. Dannenhoffer:** None.

Digital Abstract Session

P059. Connectome and Structural Plasticity

Program #/Poster #: P059.05

Topic: B.07. Synaptic Plasticity

Support: NSF-HDR IDEAS Lab 1939987
NSF-HDR IDEAS Lab 1940202
NSF-HDR IDEAS Lab 1940162
NSF-HDR IDEAS Lab 1939999
NSF-HDR IDEAS Lab 1939992
NA
NA

Title: Understanding Neuronal Network Dynamics during Motor Skill Learning through a Model-Free Connectome Inference Method

Authors: ***S. MEAMARDOOST**[1], M. BHATTACHARYA[1], E. HWANG[2], T. KOMIYAMA[3], C. MEWES[4], L. WANG[5], Y. ZHANG[6], R. GUNAWAN[1];
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Abstract: The reconstruction of neuronal connectome from large-scale two-photon Calcium (Ca) neuronal images represents an active area of research in computational neuroscience. In this work, we developed a model-free method called FARCI (Fast and Robust Connectome Inference) [1] that provides a pipeline for inferring functional neuronal connectome from time-series Ca fluorescence data of neurons. FARCI combines non-negative deconvolution, thresholding, and spike smoothing procedure for Ca fluorescence data pre-processing and produces an undirected partial correlation network describing the neuronal connectome. We assessed the performance of FARCI using the gold standard Calcium fluorescence datasets from Neural Connectomics Challenge (NCC). Our results demonstrate that FARCI provides accurate

connectome with high computationally efficiency and scalability to large connectome. Notably, FARCI outperforms the winner of the NCC in terms of accuracy and robustness to noise levels, sampling rates, network densities, and hidden (missing) neurons. Furthermore, we applied FARCI to a real dataset [2] in order to infer neuronal connectome and characterize the connectome dynamics in mouse motor cortex region (L2/3) during a motor-skill learning involving a lever pressing task in response to an auditory cue. We showed that the neuronal connectome is dynamically rewired during motor skill learning and the rewiring takes place throughout 15-day period of learning and continues even after the cue-to-reward time does not improve further. Principal Component Analysis of the neuronal connectome from FARCI showed that learning explains the main variation in the connectome across different days of the learning. Our results further demonstrated that neuronal connectome rewires to have more complex and interconnected network configurations. But, in the later sessions, the connectome rewires and adopts a less connected network state while achieving the same cue-to-reward time. Thus, we posit that there exists a two-phase process for connectome rewiring in motor cortex during the above motor-skill learning: in the first phase, the connectome is rewired in order to maximize the reward - to shorten time to reward - by increasing interconnectedness of the neurons, and in the second phase, the connectome is rewired to achieve better network efficiency while maintaining motor performance. REFERENCES: [1] Meamardoost, Saber, et al. "FARCI: Fast and Robust Connectome Inference." bioRxiv (2020). [2] Peters, Andrew J., Simon X. Chen, and Takaki Komiyama. "Emergence of reproducible spatiotemporal activity during motor learning." Nature 510.7504 (2014): 263-267

Disclosures: S. Meamardoost: None. **M. Bhattacharya:** None. **E. Hwang:** None. **T. Komiyama:** None. **C. Mewes:** None. **L. Wang:** None. **Y. Zhang:** None. **R. Gunawan:** None.

Digital Abstract Session

P060. LTP Mechanism: Kinases and Intracellular Signaling

Program #/Poster #: P060.01

Topic: B.07. Synaptic Plasticity

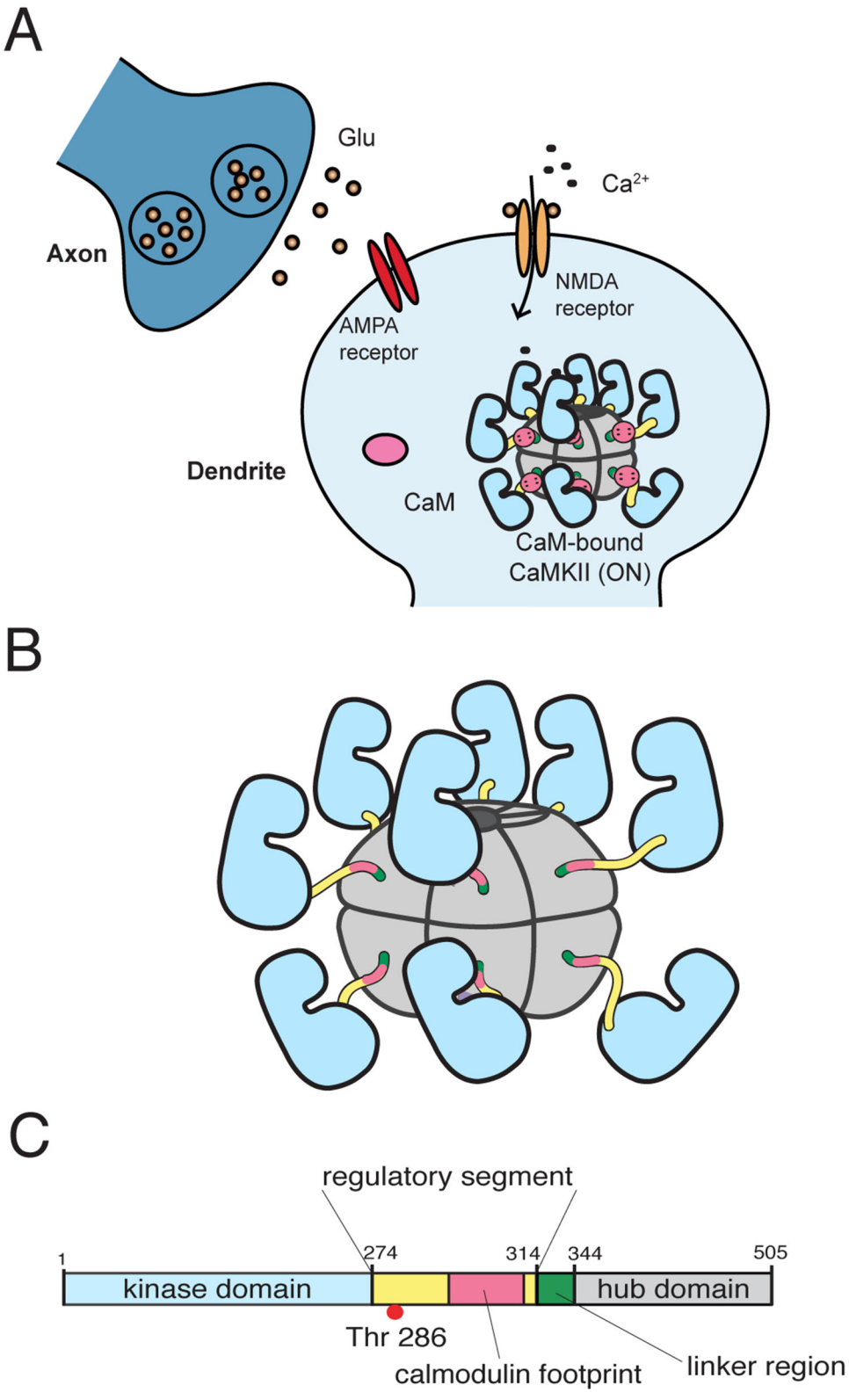
Support: T32 GM008515
R01123157

Title: Characterization of CaMKII holoenzyme stability

Authors: *A. P. TORRES-OCAMPO[1], C. ÖZDEN[1], A. HOMMER[1], A. GARDELLA[1], A. SAMKUTTY[1], E. LAPINSKAS[1], E. ESPOSITO[2], S. GARMAN[1], M. STRATTON[1]; [1]Univ. of Massachusetts-Amherst, Amherst, MA; [2]Malvern Analytics, Northampton, MA

Abstract: Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is a multimeric (12-14mer) Ser/Thr kinase necessary for long-term potentiation (LTP), the underlying cellular mechanism for memory and learning (Fig. 1A). Each CaMKII subunit is comprised of a kinase domain, regulatory segment, and hub domain that is responsible for oligomerizing the subunits into a

holoenzyme (Fig. 1B and 1C). Understanding the hub domain and its dynamics (i.e., how it assembles and falls apart) is crucial, and we have pursued experiments to interrogate holoenzyme stability. We employed differential scanning calorimetry (DSC) to measure the temperature at which the protein unfolds, which reveals the overall domain stability. The hub domain alone is extremely stable, while the kinase domain is quite unstable. Interestingly, the holoenzyme (with kinases attached to the hub) stability falls in the middle. The intermediate stability of the holoenzyme indicates that CaMKII domains are not acting independently (as beads on a string), because the holoenzyme would then unfold in two discrete transitions. These data also support a model where domains within the holoenzyme interact. We also employed newly developed mass photometry to determine the molecular weight (MW) of single CaMKII molecules in solution. We measured the MW of individual CaMKII molecules at decreasing concentrations to determine the threshold where the complex started to disassemble. We compared the hub alone to the holoenzyme and determined that the holoenzyme falls apart more easily. These data suggest that within the context of the holoenzyme, the kinase and hub domains influence one another, indicating that they likely interact. These new findings critically advance our understanding of the CaMKII assembly and have clear implications on understanding the mechanism of CaMKII subunit exchange, which will allow us to manipulate exchange and determine its functional role in signaling and LTP.


A
Glu
Ca2+
Axon
NMDA
receptor
AMPA
receptor
Dendrite
CaM
CaM-bound
CaMKII (ON)
B
C
regulatory segment
1
274
314
344
505
kinase domain
hub domain
Thr 286
calmodulin footprint
linker region

Disclosures: **A.P. Torres-Ocampo:** None. **C. Özden:** None. **A. Hommer:** None. **A. Gardella:** None. **A. Samkutty:** None. **E. Lapinskas:** None. **E. Esposito:** None. **S. Garman:** None. **M. Stratton:** None.

Digital Abstract Session

P060. LTP Mechanism: Kinases and Intracellular Signaling

Program #/Poster #: P060.02

Topic: B.07. Synaptic Plasticity

Support: NIH grant R37 MH057068 (T.C.S.)
NIH grant R01 MH115304 (T.C.S., A.A.F.)
NIH grant R01 NS108190 (P.B., T.C.S.)

Title: Persistently increased PKMζ traces hippocampal LTP maintenance and neuronal ensembles in spatial long-term memory storage

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Abstract: PKMζ, a neuron-specific atypical PKC isoform, is crucial for the maintenance of long-term potentiation (LTP) and long-term memory (LTM), during which the amount of the kinase is persistently increased (Sacktor & Fenton, 2018; Sacktor et al., 1993). Therefore, visualizing persistent increases in PKMζ might reveal traces of physiological LTP maintenance in the circuitry of the brain during long-term memory storage. Using quantitative immunohistochemistry validated by the lack of staining in PKMζ-null mice, we examined the amount and distribution of PKMζ in the hippocampal formation of wild-type mice during LTP maintenance and spatial long-term and remote memory storage.
In Schaffer collateral LTP maintenance for at least 2 h, compared to untetanized slices, PKMζ immunostaining increases in *stratum pyramidale* and in dendritic *strata oriens* and *radiatum* of CA1, that receive projections from the stimulated Schaffer collateral/commissural fibers, validating the rationale of the study design. During conditioned active place avoidance LTM storage, PKMζ increases in CA1 from 1 day to 1 month, paralleling the persistence of the memory in wild-type mice. In ArcCreERT2 x ChR2-eYFP mice designed to tag-memory activated neurons, the kinase preferentially increases in ensembles of CA1 pyramidal cells marked during memory training by the plasticity-dependent expression of ChR2-eYFP. Within these memory-tagged ensembles, PKMζ persistently accumulates in the principal cells in CA1, including the compartments of these cells in *strata pyramidale* and *radiatum* for at least 1 month. Because increased PKMζ is sufficient to potentiate synaptic transmission and intrahippocampal

applications of PKMζ inhibitors reverse the maintenance of late-LTP and erase the memory of active place avoidance conditioning at 1 month post-training (Ling et al., 2002; Pastalkova et al., 2006; Tsokas et al., 2016), we conclude that following memory training, the loci of persistently increased PKMζ represent sites of the endogenous molecular mechanism of LTP maintenance that sustains spatial long-term memory in hippocampus for at least a month.

Disclosures: **C. Hsieh:** None. **P. Tsokas:** None. **A. Grau-Perales:** None. **J. Bukai:** None. **K. Khanna:** None. **J. Chorny:** None. **C. Garcia-Jou:** None. **R.E. Flores-Obando:** None. **L.M.R. Valencia:** None. **P. Bergold:** None. **J.E. Cottrell:** None. **J.M. Alarcon:** None. **A.A. Fenton:** None. **T.C. Sacktor:** None.

Digital Abstract Session

P060. LTP Mechanism: Kinases and Intracellular Signaling

Program #/Poster #: P060.03

Topic: B.07. Synaptic Plasticity

Support: NIH R01NS036715

Title: Glua2 and NSF interaction is not required for protein synthesis-dependent LTP

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Abstract: Long-term potentiation (LTP), the most studied form of synaptic plasticity, is highly associated with learning and memory. It mainly involves two phases: early establishment and late maintenance. Many molecules have been implicated in the early phase of LTP, such as CaMKII, RAS, PKCs, GRIP1, SynGAP. However, the molecular mechanisms underlying LTP maintenance, which requires protein synthesis, remain elusive. N-ethylmaleimide sensitive fusion protein (NSF), which regulates synaptic targeting of AMPA receptors by interacting with the AMPAR subunit GluA2, has been reported to be necessary for LTP maintenance. Disrupting NSF-GluA2 interaction using a synthetic peptide, pep2m, impaired protein synthesis-dependent LTP. However, most of the evidence indicating a necessity of NSF-GluA2 binding in LTP heavily rely on the pharmacological intervention through the peptide pep2m. To further address the mechanism underlying LTP regulated by NSF-GluA2 interaction, we generated knockin mice in which GluA2 is mutated to prevent binding to NSF. We observe that the mutant mouse has normal synaptic transmission and intact LTP induction and maintenance at CA3-CA1 synapses compared to wild type (WT). Notably, pep2m still impairs LTP in the mutant mice. Furthermore, we utilize a more specific peptide to disrupt NSF-GluA2 binding, and again find LTP is not affected both in mutant and WT mice. These data indicate that the GluA2-NSF interaction is not required for protein synthesis-dependent LTP and that pep2m-mediated impairment on LTP is independent of NSF-GluA2 binding.

Disclosures: **Q. Zhu:** None. **H.L. Tan:** None. **A.J.H. Yong:** None. **R.C. Johnson:** None. **R.L. Huganir:** None.

Digital Abstract Session

P060. LTP Mechanism: Kinases and Intracellular Signaling

Program #/Poster #: P060.04

Topic: B.07. Synaptic Plasticity

Support: NIH 5R01NS036715
JHU Science of Learning Postdoc Fellowship

Title: Kibra is a crucial protein regulating human memory performance

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Abstract: The memory associated protein KIBRA is encoded by a gene that is significantly associated with human memory performance. Genetic deletion of *KIBRA* in mice impairs synaptic plasticity, as well as hippocampal-dependent contextual fear learning and memory. Trafficking of AMPA receptors is an important mechanism underlying various synaptic plasticity forms essential for learning and memory. Previous studies showed that KIBRA is a crucial scaffold protein associated with AMPA receptor complexes. However, the molecular basis through which KIBRA regulates AMPA receptor trafficking is unknown. We found that *KIBRA* knockout (KO) mice have altered brain anatomy and synapse function. *KIBRA* KO mice have reduced PKC signaling in the brain compared to wild-type animals. Following chemically induced long-term potentiation (LTP) or fear learning in adult mice, KIBRA translocates to the postsynaptic density (PSD) together with AMPA receptors and PKCs. Using biochemical and pharmacological approaches, we demonstrated that activation of PKCs enables KIBRA to recruit phosphorylated AMPA receptors to the PSD to initiate LTP and learning. We further performed transcriptomic and genetic analyses in human postmortem brain and behavioral and fMRI evaluations in living human subjects. We confirmed that *KIBRA* eQTLs in the brain are associated with human memory performance, and this association can be modified by eQTLs in genes encoding PKC. Recent studies showed Dendrin (encoded by *DDN* gene) recruits KIBRA to the membrane. We also demonstrated that genetic variation associated with *DDN* expression in the human brain modifies *KIBRA* genetic association with human memory performance. Our data reveal that KIBRA is a crucial protein modulating human memory performance.

Disclosures: **M. Tian:** None. **D. Chen:** None. **Q. Zhu:** None. **A.R. Graves:** None. **Z. Hou:** None. **H. Goldschmidt:** None. **L. Makuch:** None. **R. Johnshon:** None. **S. Mori:** None. **D.R. Weinberger:** None. **R.L. Huganir:** None.

Digital Abstract Session

P060. LTP Mechanism: Kinases and Intracellular Signaling

Program #/Poster #: P060.05

Topic: B.07. Synaptic Plasticity

Support: Campus Research Board Award, UIC

Title: "nmda receptor-mediated post-hypoxic potentiation in mice lacking glutamate antiporter, system x_c-"

Authors: *B. S. HEIT, A. CHU, J. E. RICHMOND, D. E. FEATHERSTONE, A. MCRAY, J. R. LARSON;
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Abstract: Ischemic stroke remains the leading cause of adult disability in the world. Efforts to reduce stroke severity, however, have been plagued by translational failure due to gaps in our understanding of cellular mechanisms leading to brain damage after metabolic insult. Loss of blood supply to brain tissue depletes neurons of energy, which induces a sequela of events within the neuronal network. Importantly, much of the "ischemic cascade" can be reproduced by transient deprivation of O_2 to *in vitro* hippocampal slices. The glial-bound cystine/glutamate antiporter, system x_c-, with specific subunit xCT, supplies 60-80% of ambient extracellular glutamate in the brain**.** Using slice electrophysiology, we have previously shown that xCT-mediated glutamate release influences both the rapidity and synchrony of depolarizing events after anoxia. Both genetic deletion and pharmacological antagonism of system x_c- provided ischemic neuroprotection by increasing latency to anoxic depolarization (AD), attenuating AD wave amplitudes, and extending AD wave durations. The sudden onset and rapid regenerative nature of AD, however, may obscure subtle, but important, antecedent differences. Partial ("graded") hypoxia, or reduced regional blood flow *in vivo*, is an ischemic condition that alters synaptic signaling, but is not noxious enough to induce glutamate excitotoxicity. Graded hypoxia elicits a suppression of synaptic transmission proportional to the degree of oxygen deprivation, ATP breakdown, and accumulation of extracellular adenosine. The present study employed graded hypoxia to better understand ischemia-induced alterations in neuronal responsiveness from WT and xCT KO ($xCT^{-/-}$)mice. Hippocampal slices from both genotypes were prepared, incubated, subjected to 30 minutes hypoxia, and fully re-oxygenated. Although WT and $xCT^{-/-}$ slices did not differ in hypoxia-induced synaptic suppression, mutant slices exhibited accelerated rate of recovery and post-hypoxic potentiation, which resembled LTP. Experiments using CPX, an A_1 adenosine antagonist, showed that this differential effect was not due to enhanced adenosine release in the $xCT^{-/-}$. Contrarily, further experimentation revealed that post-hypoxic potentiation in $xCT^{-/-}$ mice is driven by activation of NMDARs and enhanced calcium influx. Thus, post-hypoxic changes in mutant mice are propagated by mechanisms similar to those of LTP. This latter finding was remarkable as $xCT^{-/-}$ mice showed no differences in electrically-induced LTP compared to WT. Taken together, these data confirm system x_c- as a salient regulator of neuronal responses during ischemia and a therapeutic target ripe for exploration.

Disclosures: B.S. Heit: None. **A. Chu:** None. **J.E. Richmond:** None. **D.E. Featherstone:** None. **A. McRay:** None. **J.R. Larson:** None.

Digital Abstract Session

P060. LTP Mechanism: Kinases and Intracellular Signaling

Program #/Poster #: P060.06

Topic: B.07. Synaptic Plasticity

Support: R35 CA197622
R01 DK073368
FA9500-18-1-0051
R01 MH111516
DP2 MH107056
U01 NS094246
U24 NS109107

Title: An ultrasensitive biosensor for high-resolution kinase activity imaging in awake mice

Authors: *I. HONG[1], J.-F. ZHANG[2], B. LIU[1], A. MO[2], R. H. ROTH[3], B. TENNER[2], W. LIN[2], J. Z. ZHANG[2], R. S. MOLINA[4], M. DROBIZHEV[4], T. E. HUGHES[5], L. TIAN[6], R. L. HUGANIR[1], S. MEHTA[2], J. ZHANG[2];
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Abstract: Protein kinases control nearly every facet of cellular function. These key signaling nodes integrate diverse pathway inputs to regulate complex physiological processes, and aberrant kinase signaling is linked to numerous pathologies. While fluorescent protein-based biosensors have revolutionized the study of kinase signaling by allowing direct, spatiotemporally precise kinase activity measurements in living cells, powerful new molecular tools capable of robustly tracking kinase activity dynamics across diverse experimental contexts are needed to fully dissect the role of kinase signaling in physiology and disease. Here, we report the development of an ultrasensitive, second-generation excitation-ratiometric protein kinase A (PKA) activity reporter (ExRai-AKAR2), obtained via high-throughput linker library screening, that enables sensitive and rapid monitoring of live-cell PKA activity across multiple fluorescence detection modalities, including plate reading, cell sorting and one- or two-photon imaging. Notably, in vivo visual cortex imaging in awake mice reveals highly dynamic neuronal PKA activity rapidly recruited by forced locomotion.

Disclosures: I. Hong: None. **J. Zhang:** None. **B. Liu:** None. **A. Mo:** None. **R.H. Roth:** None. **B. Tenner:** None. **W. Lin:** None. **J.Z. Zhang:** None. **R.S. Molina:** None. **M. Drobizhev:**

None. **T.E. Hughes:** None. **L. Tian:** None. **R.L. Huganir:** None. **S. Mehta:** None. **J. Zhang:** None.

Digital Abstract Session

P060. LTP Mechanism: Kinases and Intracellular Signaling

Program #/Poster #: P060.07

Topic: B.07. Synaptic Plasticity

Support: NDSEG Fellowship
AFOSR MURI FA9550-18-1-0051

Title: Mechanochemical modeling of AMPAR trafficking

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Abstract: AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, is a ionotropic, glutamatergic receptor that is used as a readout for synaptic plasticity. AMPAR density at the postsynaptic density (PSD) is known to change during long term potentiation (LTP) and long term depression (LTD) triggered by spine activation. The signaling pathways underlying AMPAR density modification have been studied, however, the mechanisms of AMPAR trafficking and how signaling interacts with those mechanisms is less understood and currently controversial. Here we construct a deterministic, reaction-diffusion model of the simplified signaling network underlying AMPAR dynamics to elucidate the role of biophysical factors on signaling and trafficking modalities. We find that both AMPAR endocytosis and exocytosis, and AMPAR lateral membrane diffusion play important but temporally segregated roles in AMPAR trafficking at the PSD.

Disclosures: M. Bell: None. **P. Rangamani:** None.

Digital Abstract Session

P060. LTP Mechanism: Kinases and Intracellular Signaling

Program #/Poster #: P060.08

Topic: B.07. Synaptic Plasticity

Support: NIH Grant NS102490

Title: Comparing theories for the maintenance of LTP and long-term memory: computational analysis of the roles of kinase feedback pathways and synaptic reactivation

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Abstract: A fundamental problem in neuroscience is how memories are maintained from days to a lifetime, given turnover of proteins that underlie expression of long-term synaptic potentiation (LTP) or 'tag' synapses as eligible for LTP. One likely solution relies on synaptic positive feedback loops, prominently including persistent activation of Ca^{2+}/calmodulin kinase II (CaMKII) and self-activated synthesis of protein kinase M ζ (PKMζ). Recent studies also suggest positive feedback based on recurrent synaptic reactivation within neuron assemblies, or engrams, is necessary to maintain memories. The relative importance of these feedback mechanisms is controversial. To explore the likelihood that each mechanism is necessary or sufficient to maintain memory, we simulated maintenance of LTP with a simplified model incorporating persistent kinase activation, synaptic tagging, and preferential reactivation of strong synapses, and analyzed implications of recent data. We simulated three model variants, each maintaining LTP with one feedback loop: autonomous, self-activated PKMζ synthesis (model variant I); self-activated CamKII (model variant II); and recurrent reactivation of strengthened synapses (model variant III). Variant I requires and predicts that, for successful maintenance, PKMζ must contribute to synaptic tagging. Variant II maintains LTP and suggests persistent CaMKII activation could maintain PKMζ activity, a feedforward interaction not previously considered. However we note data challenging this feedback loop. In Variant III synaptic reactivation drives, and thus predicts, recurrent or persistent activity elevations of CamKII and other necessary kinases, plausibly contributing to empirically persistent elevation of PKMζ levels. Reactivation is thus predicted to sustain recurrent rounds of synaptic tagging and incorporation of plasticity-related proteins. We also suggest (model variant IV) that synaptic reactivation and autonomous kinase activation could synergistically maintain LTP. We propose experiments that could discriminate these maintenance mechanisms.

Disclosures: P.D. Smolen: None. **J.H. Byrne:** None. **D.A. Baxter:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.01

Topic: B.07. Synaptic Plasticity

Support: CARAS grant (Temple University)
Charles Kaufman Foundation Grant
NARSAD Young Investigator Award

Title: Molecular mechanisms of axon initial segment plasticity

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Abstract: The neuronal axon initial segment (AIS) is a region proximal to the soma and is responsible for action potential initiation. The AIS is a specialized compartment which contains a

high density of voltage-gated ion channels and has a unique cytoskeletal organization. The density of voltage-gated ion channels, including both sodium and potassium channels, as well as the length and diameter of the AIS determine the output of the neuron. Although once thought to be a rigid structure, it is now well accepted that AIS morphology continuously adapts to the surrounding environment to modulate neuronal excitability and maintain steady-state firing rates. More specifically, an increase in neuronal activity can lead to a decrease in AIS length, whereas a decrease in activity can lead to an increase in AIS length. These structural changes allow the neuron to maintain its activity within a given range, or setpoint, and can occur on a time scale of hours to days. However, despite the importance of AIS plasticity on neuronal excitability, the underlying molecular mechanisms regulating AIS length in response to neuronal activity remain largely unexplored. Here, we investigate the contribution of the protein Rem2, an activity-dependent negative regulator of intrinsic excitability, on AIS plasticity using a combination of immunolabeling and electrophysiology techniques in primary cortical neurons. Our studies, which explore the relationship between voltage-gated ion channel regulation and Rem2 function to influence AIS plasticity promise to yield significant insight into the regulation of intrinsic excitability.

Disclosures: G.D. Vitelli: None. **A. Ishaq:** None. **E. Spector:** None. **A.R. Moore:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.02

Topic: B.07. Synaptic Plasticity

Support: Ellison Medical Foundation
the Overland Foundation

Title: Role of transmitter plasticity in the generation of sustained fear

Authors: *H. LI[1], W. JIANG[1], C. CHEN[2], D. W. CLEVELAND[2], ***N. C. SPITZER**[1];
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Abstract: Fear is an essential emotion for survival (Rosen & Schulkin, 1998). However, when fear is sustained and disproportionate to the extent of the threat, the maladaptive response can lead to fear disorders, e.g. post-traumatic stress disorder (Fanselow & Lester, 1988). Patients with fear disorders show a shift in excitatory to inhibitory (E/I) balance in the brain (Fang et al., 2018). Neurotransmitter switching, known as the gain of one neurotransmitter and loss of another in the same neuron (Li & Spitzer, 2020), is a form of plasticity that regulates E/I balance (Spitzer, 2017) and may control sustained fear.Here we have identified a subset of serotonergic neurons in the lateral wings of the dorsal *raphe* (lwDR) of adult mice that have switched their co-transmitter from glutamate/vGluT3 (vesicular glutamate transporter type 3) to GABA/GAD67

(glutamic acid decarboxylase 67) two and four weeks after intense footshock stress. At both time points, shocked mice showed fear-related passive behaviors as they reduced exploration and increased freezing in a novel environment. We then investigated whether fluoxetine, a selective serotonin reuptake inhibitor (SSRI) that is a first-line pharmaceutical treatment for fear disorders affects transmitter switching and suppresses sustained and inappropriate fear. Our results show that immediate delivery of fluoxetine after the shock prevents both the glutamate-to-GABA switch in the lwDR and sustained fear responses. When the delivery of fluoxetine was delayed, e.g. starting two weeks after the shock, no rescue effect was seen for either the transmitter switch or the behavioral alterations. To investigate whether the loss of vGluT3/glutamate and gain of GAD1/GABA occur in the same neurons, we are now crossing a knock-in vGluT3-Cre transgenic mouse line with a Rosa-TdTomato reporter mouse line to permanently label vGluT3+ neurons and examine whether there is a higher number of TdTomato+GAD1+vGluT3- neurons in footshocked mice compared to controls. If so, this will suggest that the switch occurs in the same neurons and not in two different serotonergic neuronal populations. To study the causal relationship between the transmitter switch and sustained fear, we are using Cre-dependent adeno-associated viruses (AAVs) to express vGluT3 and/or suppress expression of GAD1 in serotonergic lwDR neurons and testing its impact on the stress-induced sustained fear responses. This study is expected to identify brain plasticity that links a prior strong fearful experience to subsequent long-lasting fear and passive behavior, and provide mechanistic understanding of the action of a pharmacological treatment for fear disorders.

Disclosures: H. Li: None. **W. Jiang:** None. **N.C. Spitzer:** None. **C. Chen:** None. **D.W. Cleveland:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.03

Topic: B.07. Synaptic Plasticity

Support: NIH-NINDS R21
W.M. Keck Foundation

Title: Transmitter switching contributes to two environmental models of neurodevelopmental disorders

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Abstract: Neurodevelopmental disorders including autism spectrum disorders are characterized by varying degrees of enhanced stereotypic behaviors, deficits in social interaction, and defects in communication. Early environmental factors play a key role and cortical neurotransmitter imbalance is a convergent phenotype seen across different types of disorders. Neurotransmitter

switching involves loss of one transmitter and gain of another. It often changes the sign of the synapse from excitatory to inhibitory or vice versa and causes changes in behavior (Spitzer, 2017). We are testing the hypothesis that transmitter switching contributes to these disorders in mouse models. We treat pregnant females with either Poly Inosine:Cytosine (Poly I:C) or valproic acid (VPA) at embryonic day E12.5. We find a transient decrease of 6000 GAD67+ neurons and an equal increase of vGluT1+ neurons in the medial prefrontal cortex (mPFC) at postnatal day P10 in experimental mice compared to saline-treated controls that has disappeared by P90. These changes do not result from altered number of precursors (Dlx2+ and Tbr1+) or apoptosis and occur specifically in PV+ and CCK+ neurons. P90 animals demonstrate enhanced stereotypy and deficits in social interaction compared to controls. We then generated PV-Cre/CCK-Cre mice to label neurons with Cre-dependent Herpes Simplex Virus (HSV)-GFP and search for vGluT1 expression in GFP+ neurons. Stereotaxic injection of P9 PV-Cre/CCK-Cre Poly I:C-treated embryos with Cre-dependent HSV-GFP revealed vGluT1+GFP+ neurons in the neonatal mPFC at P13 demonstrating expression of vGluT1 in a subset of PV-Cre/CCK-Cre neurons in response to maternal Poly I:C treatment in both male and female mice, providing strong evidence for transmitter switching. To test the role of the transmitter switch in emergence of behavioral disorders, we restored GAD67 specifically in PV and CCK neurons in the mPFC. Previous work shows that overriding one of the two transmitter changes in a switch is sufficient to prevent the change in behavior (Li & Spitzer, 2020). We stereotactically injected Cre-dependent HSV-GAD1-GFP in Poly I:C- treated male and female PV-Cre/CCK-Cre mice at P10 and scored them at P13. Overexpressing GAD67 in PV and CCK cells restored GAD67+GFP+ neurons in experimental mice and prevented gain of vGluT1 in GFP+ neurons unlike Poly I:C-treated controls injected with HSV-GFP. Restoring GAD67 in the mPFC of PV-Cre/CCK-Cre mice rescued altered behavior in experimental male mice. Our results add to growing evidence that alteration in signaling in the nervous system during the early stages of its construction can be detrimental to the function of the mature brain.

Disclosures: S.K. Godavarthi: None. **N.C. Spitzer:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.04

Topic: B.07. Synaptic Plasticity

Support: NIH Grant NS111562

Title: Developmental regulation of synaptic scaling and intrinsic homeostatic plasticity in mouse primary visual cortex

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Abstract: Homeostatic plasticity mechanisms are crucial for maintaining both neuronal and network excitability during development. Evidence shows that the two major forms of homeostatic plasticity, synaptic scaling (SS) and intrinsic homeostatic plasticity (IHP), cooperate to restore the excitability of pyramidal neurons in primary visual cortex (V1) following monocular deprivation (MD) in juvenile mice. However, less is known about how SS and IHP are developmentally regulated, especially after the critical period (CP) window has closed. One reason for this knowledge gap is the limitation of current tools for activity manipulations such as MD, which is age-sensitive and induces complex changes along the visual pathway. In this study, we have developed a chemogenetic approach to directly suppress neuronal activity. Specifically, we unilaterally expressed inhibitory DREADD hM4Di in L2/3 pyramidal neurons in mouse V1, and delivered clozapine dihydrochloride (CNO) to the animal during CP (P24-29), either via intraperitoneal injection or drinking water, to suppress the activity for 24 hours. We recorded miniature excitatory postsynaptic currents (mEPSCs) from both the hM4Di-positive neurons and the uninfected ones in the control hemisphere. Under both delivery methods, we observed a global increase in the average mEPSC amplitude of hM4Di-positive neurons, indicating enhanced excitatory synaptic strengths. Moreover, this increase required protein interactions involving GluA2 C-tail, a hallmark of SS. We further assessed the intrinsic excitability by measuring the frequency of evoked firing in response to depolarizing current steps (f-I curves) following the same manipulation, and found that hM4Di-positive neurons exhibited an upward-shifted curve, indicating increased intrinsic excitability. Importantly, this same paradigm failed to induce changes in either mEPSC amplitudes or f-I curves in shank3 knockout mice, a model for autism spectrum disorder that shows defects in engaging homeostatic plasticity. Finally, we conducted the same experiments on older mice (P45-50) that are outside the visual system CP. While SS was still present in adult animals, hM4Di-mediated inhibition induced no change in f-I curves. Our data show that in L2/3 pyramidal neurons SS persists into adulthood while IHP does not. Thus, these two forms of homeostatic plasticity can dissociate from each other as animals age. We propose that SS and IHP subserve distinct functions within neural circuits and can be turned on and off to suit different developmental needs.

Disclosures: W. Wen: None. **G. Turrigiano:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.05

Topic: B.08. Intrinsic Membrane Properties

Support: NIH P50 MH094268

Title: The effect of probiotics and gut microbiome on homeostatic balance of neuronal excitability and emotional behaviors

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Abstract: The effect of probiotics and gut microbiome on homeostatic balance of neuronal excitability and emotional behaviors Authors*Adrian Lee[1], Juhyun Kim[2], Mikhail V. Pletnikov[3]; [1]Department of Neuroscience, Johns Hopkins University School of Medicine; [2]Department of Psychiatry Behavioral Science, Johns Hopkins University School of Medicine; [3]Department of Physiology and Biophysics, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo.**DisclosuresAdrian Lee:** None. **Juhyun Kim:** None. **Mikhail V. Pletnikov**: None.**Abstract:**The microbiota-gut brain axis is a bidirectional communication system between the central and the enteric nervous system which links emotional and cognitive centers of the brain with peripheral intestinal functions. Emerging evidence have shown a strong relationship between the alteration of gut microbiome and brain disorders including neurodegenerative and psychiatric disorders. In this regard, Germ-Free (GF) mice have been a prime model for investigating the effect of gut microbiota on host function and are known to display reduced anxiety-like behavior. Using probiotics, we investigated the neurophysiological mechanism underlying gut microbiome-mediated neural circuit alterations. 5-month-old male GF mice, 6 with a daily gavaging vehicle of an LB broth solution as a control group, and 6 with the probiotic mixture of Lactobacillus and Bifidobacterium are used for this experiment . After 2 weeks-period of oral gavage, behavior tests using an open field, zero maze, and y maze and electrophysiological recordings were conducted to determine behavioral and neuronal alterations. We found that probiotic treatments to GF mice increased anxiety-like behaviors. Whole-cell patch clamp recordings performed on acute brain slices showed that the intrinsic excitability of CA1 hippocampal pyramidal neurons and amygdala excitatory neurons were significantly increased in the probiotics-treated GF mice. Interestingly, when conventionalized GF (EX-GF) mice, which had restored gut microbiome by oral gavage of stools from SPF mice, were fed with probiotics, the probiotics-EX-GF mice showed decreased intrinsic excitability in hippocampal CA1 neurons. Our result suggests that probiotics treatment changes neuronal excitability in a gut-microbiome status-specific manner, maintaining homeostatic balance of intrinsic firing capability.

Disclosures: A.C. Lee: None. **J. Kim:** None. **M. Pletnikov:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.06

Topic: B.07. Synaptic Plasticity

Support: NIH Grant F31 MH115611
NIH Grant R01 MH071739
NIH Grant T32 MH19524

Title: Synaptic homeostasis is a damped oscillatory response emerging from dynamic appropriation of hebbian plasticity

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Abstract: Homeostasis is a property of biological systems that serves to maintain functional stability through forms of negative feedback. In the nervous system, this is exhibited as homeostatic plasticity, whereby neurons alter their properties in response to prolonged changes in activity levels. While homeostatic plasticity is conceptually thought to oppose destabilizing positive feedback like “Hebbian” synaptic plasticity, many molecular components involved in one are crucial for the other. Both regulate synaptic strength through the control of AMPA receptors at excitatory synapses through the action of intracellular calcium, calcium/calmodulin (Ca^{2+}/CaM) dependent kinases and phosphatases. These overlapping players raise questions about the extent to which neuronal homeostasis is mechanistically distinct from Hebbian plasticity. Furthermore, computational studies using homeostatic synaptic scaling rules find that the temporal relationship between Hebbian and homeostatic plasticity remains unresolved. These observations raise several questions: What is the time course of homeostatic adaption? What distinguishes the overlapping components of homeostatic and Hebbian plasticity? What mechanistic role do they play in homeostatic responses? We discovered that homeostatic synaptic plasticity is a slow dampened oscillatory response. Furthermore, we identified that the voltage activation of Cav1.2, a calcium channel linked to both Hebbian and homeostatic plasticities, tunes these dynamics. By manipulating the voltage activation of Cav1.2, we found that Ca^{2+}/CaM kinases and phosphatases both act on AMPARs to homeostatically regulate synaptic weight by transiently appropriating elements involved in long-term potentiation (LTP) and depression (LTD). These novel findings suggest that a Hebbian-like feedback loop is nested within homeostatic negative feedback. Building on our experimental observations, we propose a novel model of synaptic homeostasis incorporating the dynamic interactions between Hebbian and Homeostatic mechanisms to elicit synaptic homeostasis.

Disclosures: S.D. Sun: None. **D. Levenstein:** None. **B. Li:** None. **N. Mandelberg:** None. **B.S. Suutari:** None. **N. Chenouard:** None. **G. Tian:** None. **S. Sanchez:** None. **R.W. Tsien:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.07

Topic: B.07. Synaptic Plasticity

Support: Research Grants Council of Hong Kong (HKUST16102019, HKUST16149616 and HKUST16102717; Collaborative Research Fund C6027-19GF)
National Key R&D Program of China (2017YFE0190000 and 2018YFE0203600)
Area of Excellence Scheme of the University Grants Committee (AoE/M-604/16)
Theme-based Research Scheme (T13-605/18W)
Innovation and Technology Commission (ITCPD/17-9)
Guangdong Provincial Key S&T Program (2018B030336001)
Shenzhen Knowledge Innovation Program (JCYJ20180507183642005 and JCYJ20170413173717055)

Title: Astrocyte-secreted IL-33 mediates homeostatic synaptic plasticity in the adult hippocampus

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Abstract: Hippocampal synaptic plasticity is important for learning and memory formation. Homeostatic synaptic plasticity is a specific form of synaptic plasticity that is induced upon prolonged changes in neuronal activity to maintain network homeostasis. While astrocytes are important regulators of synaptic transmission and plasticity, it is largely unclear how they interact with neurons to regulate synaptic plasticity at the circuit level. Here, we show that astrocyte-secreted interleukin-33 (IL-33) actively regulates homeostatic synaptic plasticity in the adult mouse hippocampus. Local suppression of neuronal activity in the hippocampal cornu ammonis 1 (CA1) increases IL-33 expression and secretion by astrocytes. Moreover, IL-33 signals hippocampal neurons to promote excitatory synapse formation and enhance synaptic transmission through the synaptic recruitment of scaffold protein, PSD-95. Furthermore, this astrocytic IL-33 signaling is required for the chronic neuronal activity blockade-induced increase of CA3-CA1 excitatory synapses in the adult mouse hippocampus. These results collectively reveal an important role of astrocytic IL-33 in mediating the negative-feedback signaling mechanism in homeostatic synaptic plasticity, providing insights into how astrocytes maintain hippocampal network homeostasis.

Disclosures: Y. Wang: None. **W. Fu:** None. **K. Cheung:** None. **K. Hung:** None. **A.K.Y. Fu:** None. **N.Y. Ip:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.08

Topic: B.07. Synaptic Plasticity

Support: NIH Grant R01NS095123

Title: Modulation of synapticplasticity with transcranialdirect current stimulationin single neurons

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Abstract: Background: Lasting therapeutic effects of transcranial direct current stimulation are thought to be mediated by synaptic plasticity. Direct current stimulation (DCS) is known to affect synaptic long-term potentiation (LTP) in vitro. We hypothesized that this is the result of a modulation of somatic spiking with DCS in the postsynaptic neuron, as opposed to indirect network effects. Previous studies with population activity provided only indirect evidence for this hypothesis. Here we aim to directly record somatic spiking in a postsynaptic neuron during LTP induction with concurrent DCS. Methods: We recorded single-neuron activity by patching the soma of individual CA1 pyramidal neurons in a rodent in-vitro slice preparation. LTP was induced with theta-burst stimulation (TBS) applied concurrently with DCS. To specifically test the causal role of somatic polarization during DCS, we manipulated this polarization via patch pipette current injections. To explain the observed effects, we used a computational multi-compartment neuron model that captures the effect of electric fields on membrane polarization and activity-dependent synaptic plasticity. Results: We find that TBS-induced LTP was enhanced when paired with anodal DCS as well as depolarizing current injections. In both cases, somatic spiking during the TBS was increased, suggesting that evoked somatic activity is indeed the primary factor affecting LTP modulation. However, the boost of LTP with DCS was less than expected given the increase in spiking activity alone. In some cells, we also observed spontaneous somatic spiking during DCS, suggesting that DCS also modulates LTP via spontaneous network activity. The computational model reproduces the observed effects of DCS on LTP and suggests that these effects are driven by both direct changes in postsynaptic spiking and indirect changes due to network activity Conclusion: DCS enhances synaptic plasticity by increasing postsynaptic somatic spiking, but we also find that an increase in network activity may limit this enhancement.

Disclosures: F. Farahani: None. **G. Kronberg:** None. **H.V. Oviedo:** None. **L.C. Parra:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.09

Topic: B.07. Synaptic Plasticity

Support: INSPIRE FELLOWSHIP

Title: Implications of presynaptic design on short-term plasticity in hippocampal mossy fiber

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Abstract: Several important functions at the mossy fiber (MF) synapses are associated with profound short-term plasticity (STP) at this synapse. For example, the MF terminal triggers an action potential after a threshold number of stimuli arrives, independent of the incoming stimulus rate. This invariance to rate may be essential for filtering non-coding signals, and is governed by STP. We developed a physiologically realistic spatial model of the MF bouton to explore the role of the structural and functional relationship that regulate synaptic function. MFs, unlike the CA3 presynaptic terminal, have several release sites, each seemingly associated with a distinct source of calcium (voltage-dependent calcium channels, VDCC). Accordingly, most studies consider MF as an extension of the CA3 terminal with multiple autonomous lines of signal transmission. In a direct contrast, our in-silico approach shows not only crosstalk across multiple release sites exists, but also this communication is crucial for the observed plasticity. As presynaptic activity progresses, nonlinear coupling between calcium released through VDCC clusters and the buffering by intrinsic calcium buffers is orchestrated. Apart from the coupling between the release sites, the biophysical properties specific to a family of calcium sensors for vesicle release, synaptotagmin-7 (Syt7) contribute to the observed facilitation. Each of these (crosstalk between AZs, buffers saturation, Syt7) makes a distinct contribution essential for the synapse to carry-out important calculations. MFs indirectly inhibit CA3 synapse via barely plastic inhibitory interneurons (apart from direct excitation to the CA3 pyramidal). We show that this network motif along with STP can trigger phase precession in CA3 place cell firing activity essential for spatial navigation. Our quantitative synaptic level computational model showcases how details of synaptic organization and biophysical properties of molecular machinery at the synapse transcend levels of organization and has a profound influence on behavior.

Disclosures: **N. Singh:** None. **S. Nadkarni:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.10

Topic: B.07. Synaptic Plasticity

Support: HKRGC grant ECS 21103818
HKRGC grant ECS 27103715
HKRGC grant GRF 17128816
HKRGC grant GRF 11104320
HK HMRF grant 03143096
City University of Hong Kong 7200494
City University of Hong Kong 7004586

Title: Target-specific control of piriform cortical output via recruitment of distinct inhibitory circuits

Authors: H. JIANG[1], A. GUO[1], A. CHIU[1], H. LI[1], C. S. W. LAI[3], ***C. G. LAU**[2];
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Abstract: Neural circuits in anterior piriform cortex (APC) are regulated by ongoing sensory activity, but the underlying circuit mechanisms remain elusive. Here we examined the hypothesis that recurrent inhibition differentially regulates layer 2 (L2) principal neuron types, semilunar (SL) and superficial pyramidal (SP) cells. Patterned optogenetic stimulation revealed that recurrent inhibition was stronger in L1 for SL cells but stronger in L3 for SP cells. This target-specific, differential inhibition across layers was largely attributed to the parvalbumin (PV), but not somatostatin (SST), interneuron. Intriguingly, activity deprivation via naris occlusion (NO) revealed that experience specifically regulated the PV, but not SST, circuit, but the overall target specificity was preserved. Together, these results indicate target-specific inhibitory wiring and heightened inhibitory plasticity of PV cells, implicating these mechanisms in odor processing.

Disclosures: H. Jiang: A. Employment/Salary (full or part-time):; City University of Hong Kong. **A. Guo:** A. Employment/Salary (full or part-time):; City University of Hong Kong. **A. Chiu:** A. Employment/Salary (full or part-time):; City University of Hong Kong. **H. Li:** A. Employment/Salary (full or part-time):; City University of Hong Kong. **C.S.W. Lai:** A. Employment/Salary (full or part-time):; The University of Hong Kong. **C.G. Lau:** A. Employment/Salary (full or part-time):; City University of Hong Kong. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; City University of Hong Kong.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.11

Topic: B.07. Synaptic Plasticity

Support: NIDCD DC012557

Title: Lateralized Short-Term and Long-Term Plasticity of Somatostatin-Positive Interneurons in Female Mouse Auditory Cortex

Authors: *S. C. SONG[1], R. C. FROEMKE[2];
[1]Skirball Inst. for Biomolecular Med., New York Univ., New York, NY; [2]Otolaryngology, NYU Med., New York, NY

Abstract: Lateralized Short-Term and Long-Term Plasticity of Somatostatin-Positive Interneurons inFemale Mouse Auditory CortexSong SC[1-4], Froemke RC[1-61] Skirball Institute for Biomolecular Medicine, New York University School of Medicine, NewYork, NY, USA[2] Neuroscience Institute, New York University School of Medicine, New York, NY, USA[3] Department of Otolaryngology, New York University School of Medicine, New York, NY, USA[4] Department of Neuroscience and Physiology, New York University School of Medicine, NewYork, NY, USA[5] Center for Neural Science, New York University, New York, NY, USA[6] Howard Hughes Medical Institute Faculty ScholarThere are multiple forms of synaptic plasticity, and distinct inputs onto the same postsynapticneuron can have qualitatively different learning rules (Maheux et al. 2015). There is growingevidence that cortical inhibitory synapses are highly plastic, including differences in cell-type-specific GABAergic inputs onto layer 2/3 pyramidal neurons (Chiu et al. Neuron 2018). In theauditory cortex, we have found that an unusual left-lateralized form of short-term inhibitorydepression might help enable longer-term changes to both excitation and inhibition for enhancedresponses to pup calls in maternal mice (Marlin et al. Nature 2015, Schiavo et al. Nature 2020).Here we examined short-term and spike-timing-dependent plasticity (STDP) of differentinhibitory inputs onto layer 2/3 pyramidal neurons of adult mouse auditory cortex in slices.STDP is a method for inducing input-specific and long-term modifications of synaptic strength,where short-term plasticity reflects the dynamic synaptic strength based on recent history. Thesemechanisms might underlie the synchronization of stimulus-evoked inhibitory input duringdevelopment (Dorrn et al. Nature 2010, Field et al. Neuron 2020) and after conditioning ormaternal experience.We utilized isolated expression of channelrhodopsin-2 in various GABAergic interneurons toselectively activate these inputs. Somatostatin interneurons are distinct physiologically,expressing STDP (169% potentiated current, $n = 11$, $p < 0.05$) as compared to PV, VIP andLayer-1 interneurons. Somatostatin also demonstrate differential depression to repetitivestimulation ($df = 3$, $F = 3.26$, $p < 0.05$), depending on hemisphere, as compared to PV. Our datasuggest somatostatin interneurons are critical for laterality in the auditory cortex.

Disclosures: S.C. Song: None. **R.C. Froemke:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.12

Topic: B.07. Synaptic Plasticity

Support: NIH Grant NS094184
NIH Grant EY022388

Title: A driver input from cortical layer 2/3 to layer 5

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Abstract: We used a slice preparation to study the synaptic properties of the layers 2/3 to 5 projection in mouse somatosensory cortex. We specifically identified these inputs to layer 5 cells as driver or modulator. Driver inputs evoke large, depressing EPSPs with no mGluR component; modulators evoke smaller, facilitating EPSPs and can activate mGluRs. Recording single cells in layer 5, we used glutamate uncaging to minimal electrical stimulation to localize and activate presynaptic neurons in layers 2/3. As a control, we used the same approach to examine the synapse from layer 6 to 4, which has previously been shown to be modulator. 70% of layer 5 cells received a driver input from layers 2/3, and the rest, modulator input. A driver component was most pronounced for cells of layer 5a (75% of cells), which exclusively innervate other cortical areas, compared to cells of layer 5b (67% of cells), which additionally project subcortically. Because a driver phenotype is associated with the transmission of information and receptive field properties, these findings indicate an important contribution of layer 2/3 to cortical efferents to other cortical areas and subcortical targets.

Disclosures: B.J. Carroll: None. **S.M. Sherman:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.13

Topic: B.07. Synaptic Plasticity

Support: NIH Grant NS111986

Title: Endocannabinoid and BDNF-mediated enhancement of theta burst stimulation-induced long-term potentiation

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Abstract: It is widely accepted that exogenous cannabinoids impair short-term memory and cognition in humans and other animals. This is likely related to the inhibition of long-term potentiation (LTP) by global, sustained activation of CB1 cannabinoid receptors in the presence of exogenous agonists. The release of endogenous cannabinoid ligands, on the other hand, may enhance synaptic plasticity in a spatially and temporally specific manner. The functional roles of endocannabinoids are complex because they can modulate synaptic transmission via suppression of both GABA and glutamate release, with opposing effects on postsynaptic excitability.
In the present studies, we examined the role of endocannabinoid signaling in LTP by recording field excitatory postsynaptic potential (fEPSPs) in the CA1 stratum radiatum in hippocampal slices from juvenile mice. LTP was induced by theta-burst stimulation (TBS) of the Schaffer collaterals. Significant LTP (~50% increase from baseline) was induced by either 1 or 3 trains of TBS. This potentiation was significantly inhibited by preventing cannabinoid receptor activation with the CB1R antagonist NESS 0327 (~25% increase from baseline). In addition, LTP was

inhibited to a similar extent by preventing the synthesis of the endocannabinoid 2-AG using the DAG lipase α inhibitor DO34.
These results suggest that activation of CB1 receptors by 2-AG enhances TBS-induced LTP, leading to the hypothesis that the predominant endocannabinoid effect under these conditions is to suppress inhibition. We therefore examined LTP while blocking inhibitory synapses using the GABA-A receptor blocker picrotoxin (PTX). The addition of PTX alone caused a slight, but not significant, increase in LTP. Interestingly, PTX completely prevented the effect of blocking CB1 receptors, indicating that intact GABAergic transmission is required for the endocannabinoid effect. The presence of PTX did not unmask any enhancing effect of CB1 receptor blockade, suggesting minimal endocannabinoid modulation of glutamate release under these conditions.
We have previously shown that TBS stimulation can cause a BDNF-dependent increase in endocannabinoid release at inhibitory synapses. Consistent with this, we found that blocking TrkB receptor activation with ANA-12 inhibited LTP to the same extent as blocking CB1 receptors. Interestingly, however, the effect of TrkB antagonism on LTP was still observed in the presence of PTX. This suggests that endogenous BDNF may enhance LTP by acting directly on glutamatergic synapses under these conditions. Ongoing experiments are further exploring the role of BDNF-eCB interactions in the modulation of LTP.

Disclosures: **F. Lemtiri-Chlieh:** None. **E.S. Levine:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.14

Topic: B.07. Synaptic Plasticity

Support: P50 DA044118
NIMH training grant T32 MH119049-01
National Science Foundation Graduate Research Fellowship under Grant No.DGE-1839285

Title: Sexually Dimorphic Effects of Adolescent THC on LTP in Hippocampus

Authors: A. A. LE[1], ***J. QUINTANILLA**[1], M. AMANI[1], C. M. GALL[2], G. LYNCH[3];
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Abstract: Detrimental effects of adolescent cannabinoid exposure on memory have been described but the neurobiological bases for these changes and long term effects of cannabis exposure on specific forms of synaptic plasticity are unknown. The present studies evaluated enduring effects of adolescent tetrahydrocannabinol (THC) exposure on synaptic plasticity in hippocampus. Male and female rats were given daily injections of 5mg/kg THC or vehicle (Veh) over postnatal days 30 to 45. At least 1 month later, hippocampal slices and field recordings were

used to evaluate synaptic transmission and long-term potentiation (LTP) in the Commissural/Associational (C/A) innervation of CA1 and the lateral perforant path (LPP) innervation of the dentate gyrus. Input-output curves were used to assess the relationship between the number of axons stimulated and the size of the post-synaptic response in both fields: there were no group differences in these measures. We then tested for treatment effects on frequency facilitation to a 10-pulse train applied at 10, 20 and 40 Hz. THC treatment had no effect on the typical response facilitation in field CA1 in males or females. Similarly, for the LPP the groups exhibited nearly identical responses, with an initial increase in response size followed by a decrease toward the latter half of the train. These results indicate that adolescent THC treatment had no clear influence on basic synaptic transmission in either subfield. However, THC effects were evident in studies of LTP. Theta burst stimulation of C/A afferents to CA1 elicited robust and comparable LTP in slices from Veh- and THC-treated males (n=6,7; p=0.655, veh vs THC). However, in females CA1 LTP was robust in the Veh group but failed to stabilize in the THC group (n=13/grp; p<0.00003). Similar effects on LTP were evident for the LPP: Potentiation induced by high frequency (100Hz) stimulation was robust in males receiving Veh or THC (n=7grp; p=0.77, Veh vs THC) whereas in females LPP LTP was stable in the Veh-group but significantly attenuated in those receiving THC (n=12,14; p=0.006 Veh vs THC). These findings demonstrate that a 2-week period of THC treatment, initiated in early adolescence, has long term effects on LTP in hippocampus of female but not male rats. CA1 LTP is expressed postsynaptically, is sexually dimorphic with female dependence on estrogen, and is not dependent on endocannabinoid function. In contrast, LPP LTP is presynaptic and endocannabinoid dependent (Wang et al., 2016, 2018 A,B). The distinctions between these pathways suggest different mechanisms that may be vulnerable to cannabinoid exposure during the adolescent period.

Disclosures: **A.A. Le:** None. **J. Quintanilla:** None. **M. Amani:** None. **C.M. Gall:** None. **G. Lynch:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.15

Topic: B.07. Synaptic Plasticity

Support: NIH Grant R01AA016022

Title: Effect of estrus and estrogen receptor activity on LTP in the dorsal striatum

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Abstract: The striatum is involved in a variety of learning behaviors such as motor control and habit formation. Two subregions, the dorsomedial striatum (DMS) and the dorsolateral striatum

(DLS) are responsible for goal-directed and habitual learning, respectively. The dorsal striatum also is implicated in the development of drug addiction: drug use becomes more habitual as behavioral control over drug use switches from the DMS to the DLS. Sex differences are found in drug addiction, where women tend to progress more rapidly into habitual drug use than men, but men demonstrate more goal-seeking behaviors than women. This may be due to the cellular actions of estrogen, a hormone that fluctuates across the menstrual cycle in humans and across the estrous cycle in most non-primate mammals. Estrogen influences striatal-dependent learning and neuronal physiology and has direct effects on structural plasticity in the hippocampus. To further investigate the effect of sex differences and estrogen on striatal learning, we used field recording to measure long-term potentiation (LTP), a form of learning at the cellular level, in brain slices of male and female C57Bl6 mice. We first demonstrated that the appropriate theta burst stimulation (TBS) frequencies to induce LTP in the DMS and DLS in male mice were 10.5 Hz and 5.0 Hz, respectively. We then tested these frequencies in female mice, which were grouped based on estrus status. We found that 10.5 Hz TBS induced LTP in the DMS of non-estrus female mice but not in the DMS of female mice that were in estrus. We also investigated which of the three major types of membrane estrogen receptors in the striatum - ER-α, ER-β, and GPER - are mediating the effects of estrus. Finally, using each region's TBS frequency, we tested whether LTP in the two regions differed in the requirement of two key receptors: dopamine D1 receptors and TrkB, and found that both were necessary for LTP in both regions. These results further the understanding of sex differences in the mechanisms underlying striatal-based learning and may have implications for more individualized treatment of drug addiction.

Disclosures: V. Lewitus: None. **K.L. Blackwell:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.16

Topic: B.07. Synaptic Plasticity

Support: NCATS 1UH2 TR0022082
NIH P50 AA22534
Sanofi Pharmaceutical

Title: Long term potentiation enhancement in prenatal alcohol- exposed animals following administration of the histamine H3 receptor inverse agonist SAR152954

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Abstract: Cognitive impairments are common consequences of Fetal Alcohol Spectrum Disorder (FASD). The hippocampus is particularly susceptible to the effects of prenatal alcohol exposure (PAE) which persist throughout an individual's life. Previous studies have identified deficits in long-term potentiation (LTP) in perforant pathway to dentate gyrus synapses as a robust consequence of PAE. Although there have been numerous investigations, PAE-induced deficits in synaptic plasticity remain poorly understood. Currently, there are no known, clinically effective pharmacotherapeutic interventions for these deficits. This study sought to investigate the effects of PAE on LTP in the dentate gyrus and the H3 receptor inverse agonist, SAR152954, as a possible agent to reverse deficits in LTP associated with PAE. We utilized a rat model of moderate PAE (60 mg/dl peak blood alcohol content). Urethane-anesthetized adult male Long-Evans rats (N=27; PAE = 18) were implanted with monopolar electrodes in the dorsal dentate gyrus and in the entorhinal cortical perforant pathway. Evoked responses were recorded at 20KHz (Stimuli: 100ms, 1/30s, 400mA). An Input-Output (I/O) curve (50-500 uA) was obtained to determine the EC40 (40% of maximal response). Rats were given a single injection of SAR152954 (0.1 or 1.0 mg/kg) or saline (vehicle) 30 min prior to the recording session. Baseline recordings were obtained for 20 min. Synaptic potentiation was induced by 5 trains of high frequency stimulation (HFS; 400Hz, 25ms duration) with 30s inter-train intervals. Post-HFS recordings were obtained for 60 min. Fractional change of the post-HFS fEPSP relative to baseline was calculated for 1 min intervals. PAE rats displayed reduced LTP relative to controls ($p = 0.05$). PAE rats receiving 0.1 mg/kg SAR152954 did not show reversal of PAE-induced deficits ($p > 0.8$). However, PAE rats receiving the 1.0 mg/kg dose) reversed PAE-induced deficits to levels comparable to the control animals ($p > 0.9$) and greater than PAE animals that received either the vehicle or low dose SAR152954 conditions ($p = 0.024$). These results indicate that H3 receptor inverse agonist SAR152954, reverses PAE-induced deficits in LTP and offer a possibility for investigating agents with this mechanism of action as novel pharmacological interventions for FASD-associated cognitive impairments. Support: Sanofi Pharmaceutical for the generous donation of SAR152954, NCATS 1UH2 TR0022082, 1P50 AA22534.

Disclosures: M. Goncalves-Garcia: None. **S. Davies:** None. **D.D. Savage II:** None. **D.A. Hamilton:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.17

Topic: B.07. Synaptic Plasticity

Support: NIH/NINDS 3R01NS089578-03S1

Title: Dendritic spine abnormalities and behavioral deficits in forebrain-specific Par1c/MARK1 knockout mice

Authors: *E. C. KELLY-CASTRO[1], M. SUN[1], H. ZHANG[2];
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Abstract: Dendritic spines are dynamic postsynaptic structures that play an important role in cognitive functions, such as learning and memory. The dysregulation of spine size, shape, and density may lead to learning and memory deficiencies, as well as neurological disorders such as autism spectrum disorders (ASD). Previous studies from our laboratory have demonstrated that partitioning defective 1 c (Par1c), also known as microtubule affinity regulating kinase 1 (MARK1), regulates dendritic spine morphogenesis and plasticity in cultured hippocampal neurons. Interestingly, studies have found multiple single nucleotide polymorphisms of MARK1 associated with ASD and bipolar disorder. However, the role of Par1c/MARK1 in synaptic plasticity and cognitive functions *in vivo* is still unknown. Therefore, we developed a conditional knockout (cKO)MARK1 mouse model to examine the effects of MARK1 depletion on spine morphogenesis and cognitive functions such as learning and memory. In this mouse model, MARK1 is depleted postnatally from pyramidal neurons of the forebrain including the hippocampus and the cerebral cortex. We found that dendritic spine density, width, and length are significantly decreased in the hippocampal CA1 region of the MARK1 conditional KO mice compared to wild type controls. The MARK1 cKO mice also show an increase of stubby spines and a reduction in mushroom-shaped spines. Furthermore, MARK1 cKO mice show a defect in spatial learning in the Morris water maze test and significantly reduced anxiety in the elevated plus-maze. Together, our studies point to an important role for Par1c/MARK1 in regulating dendritic spine morphogenesis, spatial learning and anxiety-related behavior *in vivo*.

Disclosures: E.C. Kelly-Castro: None. **H. Zhang:** None. **M. Sun:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.18

Topic: B.07. Synaptic Plasticity

Support: This work was supported by Air Force Office of Scientific Research (AFOSR) grant 20RHCOR04.

Title: Vagus nerve stimulation induced cognitive enhancement in healthy male rats: Hippocampal DCX and BDNF correlates

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Abstract: Although vagus nerve stimulation (VNS) (an FDA approved treatment for epilepsy and treatment-resistant depression) has been reported to improve learning and memory, little is

understood about the mechanisms responsible for this cognitive enhancement. This study investigates the cognitive effects of a single 30 min session of VNS. Neuroplasticity marker brain derived nerve growth factor (BDNF) and neurogenesis marker doublecortin (DCX) were examined to determine VNS associated changes in the hippocampus. Male Sprague-Dawley rats (N = 30) 10-12 weeks of age were implanted with an electrode cuff around the VN. Fifteen 100 μs biphasic pulses at 30 Hz, 0.8 mA constant current were administered to the VNS rats every 18 seconds for 30 minutes after behavioral training for the Novel Object Recognition (NOR) and Passive Avoidance Task (PAT) tests. SHAM rats received the same treatment but no VNS. Behavior experimenters were blinded to treatment groups. Twenty-four hours later, stimulated rats demonstrated enhanced performance in NOR ($p < 0.05$, n = 11-13) and PAT ($p < 0.05$, n = 14) compared to SHAM rats. Rat brains were perfused and fixed 48 hours post-VNS for immunohistochemical (IHC) BDNF and DCX analyses. Experimenters were blinded while counting BDNF/DCX immunopositive cells. BDNF expression after VNS (compared to SHAM in t-tests) was significantly greater in the CA2 ($p < 0.05$, n = 7) and approached significance in the CA1 ($p = 0.08$, n = 7). Although DCX expression did not show any significant group differences in hippocampal regions (t-tests, $p > 0.05$, n = 7-9), DCX expression in the CA3 was significantly positively correlated to novel object preference score in NOR among VNS rats only (Pearson's correlation, $r^2 = 0.633$, $p < 0.05$, n = 7). Behavioral results demonstrate that cognitive performance can be enhanced when VNS is administered after training. IHC results suggest hippocampal BDNF and DCX expression may mediate VNS induced cognitive enhancement. A better understanding of the interplay between VNS and hippocampal neuroplasticity may improve the utilization of VNS for cognitive enhancement.

Disclosures: L.K. Olsen: None. **R.J. Moore:** None. **N.A. Bechmann:** None. **S.D. Cunningham:** None. **C.N. Hatcher-Solis:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.19

Topic: B.07. Synaptic Plasticity

Support: R01MH092926
R01NS095123

Title: Synaptic evidence for the cumulative effects of transcranial direct current stimulation in spaced learning

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Abstract: Background: Transcranial direct current stimulation (tDCS) is thought to improve learning by modulating Hebbian synaptic plasticity. The majority of in vitro studies supporting this theory use electric fields that are much higher than those generated with transcranial stimulation in humans (20V/m vs 1 V/m). The effectiveness of tDCS has also been called into question because of the relatively small effect sizes. There is therefore a need to improve the effectiveness of tDCS at realistic field intensities. Here we leverage the observation that effects of learning in humans are known to accumulate over multiple bouts of learning, known as spaced learning. This cumulative effect has been replicated in synaptic long term potentiation (LTP) experiments in vitro.
Hypothesis: We propose that effects of DCS on synaptic LTP can accumulate over time in a spaced learning paradigm.
Methods: We studied the cumulative effects of DCS at various intensities applied during the induction of LTP in the CA1 region of rat hippocampal slices using theta burst stimulation (TBS).
Results: DCS applied during repeated bouts of TBS resulted in an increase of LTP. Spaced learning saturated quickly with strong TBS protocols, thus obscuring cumulative effects of DCS. However, with weaker TBS, we saw a cumulative effect of spaced learning, including a cumulative boost of LTP with electric fields of lower intensity.
Conclusions: These results support the notion that the effects of tDCS accumulate through an increasing synaptic strength after repeated bouts of learning.

Disclosures: **M. Sharma:** None. **F. Farahani:** None. **L. Parra:** None. **G. Kronberg:** None.

Digital Abstract Session

P061. Plasticity: From Homeostatic To Short- and Long-Term Plasticity Ex Vivo and in Vivo

Program #/Poster #: P061.20

Topic: B.07. Synaptic Plasticity

Support: NIH ReBUILDetroit Grant
NIH Grant GM118981
NIH Grant GM118982
NIH Grant GM118983

Title: Traumatic stress exposure alters protein composition of perineuronal nets in the mouse hippocampus

Authors: *S. I. BUHAY[1,2], V. M. CHIU[2], M. ABBAS[2], J. RUAN[2], A. C. CONTI[2];
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Abstract: Post-traumatic stress disorder (PTSD), which can manifest itself as behavioral avoidance, emotional numbing, and/or reliving of trauma, can be described as a crippling pathological condition that arises from experiencing a traumatic event. In the United States, the

lifetime prevalence of PTSD has been found to be 7.3%. PTSD is known to induce behavioral and psychological changes including impaired fear learning, memory, and extinction of conditioned fear, all of which involve deviations in neuroplasticity. Perineuronal nets (PNNs) have been implicated in stabilizing and protecting synaptic connections predominantly formed during critical periods of neural development, however, they are regulated throughout a lifetime and may change in response to various assaults on the brain. The effect of high-level stress events, such as those that induce PTSD, on the morphology, regulation, and associated effects of PNNs on neuroplasticity is presently unknown. We aimed to identify and quantify PNNs across the dorsal hippocampal regions of adult male C57BL/6 mice exposed to mouse single-prolonged stress (mSPS), an animal model that shows validated symptoms akin to humans with PTSD, or control conditions. To examine the effect of PTSD on PNN expression patterns and associated proteins (aggrecan, brevican, and phosphacan) levels, immunofluorescence (IF), immunoblotting, and real-time polymerase chain reaction (RT-PCR) experiments were performed. Using *Wisteria floribunda agglutinin* (WFA) to identify aggrecan-containing PNNs and antibodies directed against parvalbumin (PV), we detected PNN expression surrounding PV+ neurons in the dentate gyrus and CA3 of the dorsal hippocampus (dHC) of both mSPS and control mice. In addition, RT-PCR analysis indicated that aggrecan mRNA levels did not change in mSPS-exposed mice compared to controls. However, immunoblotting results revealed a decrease in phosphacan protein levels in the dHC of mSPS-exposed mice compared to controls. These results support previous studies that indicate PNN disruption can impair long-term fear memory and suggest that alterations in the protein composition of PNNs may contribute to PTSD symptom development or presentation related to dHC function.

Disclosures: S.I. Buhay: None. **V.M. Chiu:** None. **M. Abbas:** None. **J. Ruan:** None. **A.C. Conti:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.01

Topic: B.07. Synaptic Plasticity

Support: NIH Grant NS089578

Title: Mechanism of Par3-mediated regulation of dendritic spine plasticity

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Abstract: Dendritic spines are small, highly polarized protrusions on excitatory neurons serving as sites ofpostsynaptic input. Plasticity of dendritic spines is necessary for learning, while stable dendritic spinesare thought to encode long-term memories. The polarized nature of dendritic spines suggests theirplasticity and stability may be mediated by polarity proteins. The polarity protein Partitioning defective(Par) 3 (Par3) regulates mature dendritic spine formation in vitro,

and several single nucleotidepolymorphisms (SNPs) and copy number variation (CNV) of Pard3, which encodes Par3, are associatedwith intelligence, schizophrenia, and autism spectrum disorder (ASD). Together, these data implicatePar3 in mature dendritic spine stabilization, which may play a role in cognition and social interaction.However, the mechanisms of Par3 in dendritic spine plasticity and cognition *in vivo* remains completelyunknown. We established a novel Par3 mouse model to conditionally knockout Par3 in postnatalforebrain pyramidal neurons. We found that loss of Par3 *in vivo* increases immature dendritic spines andincreases overall dendritic spine density in the CA1 region of the hippocampus. In addition, loss of Par3*in vivo* alters spatial learning in the Morris water maze and phosphorylation of cytoskeletal regulatingproteins. Together, our data suggest that Par3 plays a role in regulation of mature dendritic spinestability and cognitive functions.

Disclosures: **M. Voglewede:** None. **H. Zhang:** None. **M. Sun:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.03

Topic: B.07. Synaptic Plasticity

Support: NIH Grant 1ZIANS002994
NIH Grant 1ZIANS003140

Title: Biochemical analysis of synaptic plasticity changes induced by dark rearing in mice.

Authors: *J. D. BADGER, M. VIEIRA, T. NGUYEN, J. PASKUS, R. LOMASH, J. JEONG, K. W. ROCHE;
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Abstract: A robust method for inducing synaptic plasticity in vivo in mice is important to expand upon findings from in vitro approaches. In particular, for biochemical analyses of changes in synaptic proteins, it is critical to activate a large number of synapses with a single perturbation. Two common methods include environmental enrichment and chemically induced stimulation. However, these assays can be either difficult to control (eg. ensuring animal participation) or too over-powering (eg. differentiating between synaptic activation and seizures). Thus, we have opted for a more natural in vivo paradigm. Dark rearing studies in rats have shown that this protocol leads to a delay in the well-characterized developmental NMDAR subunit switch (Carmignoto & Vicini 1992 Science). Interestingly, brief light exposure after a period of dark rearing induces rapid and transient NMDA receptor-dependent cortical responses (Quinlan & Bear 1999 PNAS; Philpot & Bear 2001, Neuron). This method has the advantage of being relatively simple to implement and also includes appropriate control groups, thus it is powerful for measuring the induction of protein changes. Using mice, we divide littermates into 3 groups at P21: One group is reared in a normal light/dark cycle (LR); one group is reared in complete dark for 5 days (DR; importantly, the brain is dissected in dark until optic nerve

severed); and a third group reared in the same 5 day dark environment, but then exposed to light for 2 hours before euthanasia (DR+LE). The visual cortex is then collected for all 3 groups, lysed, and western blots performed. Here we present data evaluating post synaptic proteins, including NMDA receptor subunits and associated proteins, in mice subjected to this DR+LE method. We measured the changes in protein levels in SPM (synaptic plasma membranes) or PSD (post-synaptic density) fractions comparing the 3 groups of animals: LR; DR; and DR+LE. As expected, we saw an increase in GluN2A in the SPM fraction in mice that were dark reared then exposed to light, compared to the dark reared group, which is in accordance with previously published data demonstrating activity-dependent plasticity induced by this protocol (Quinlan & Bear 1999 PNAS). We then evaluated posttranslational modifications of several synaptic proteins. We found that this in vivo activity protocol induced dynamic regulation of a variety of phosphorylation events in receptor subunits (eg. GluN2A-phosphoS1459) and adhesion molecules (eg. NLGN1 and NLGN3), leading to both increases and decreases. Thus, we are able to measure rapid changes in synaptic enrichment and biochemical correlates using the dark rearing paradigm in mice.

Disclosures: **J.D. Badger:** None. **M. Vieira:** None. **T. Nguyen:** None. **J. Paskus:** None. **R. Lomash:** None. **J. Jeong:** None. **K.W. Roche:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.04

Topic: B.07. Synaptic Plasticity

Support: This research is funded by the Research Council of Lithuania (Flagship ERA-NET Joint Transnational Call JTC 2019 in synergy with the Human Brain Project, No. S-FLAG-ERA-20-1/2020-PRO-28).

Title: Synaptic cluster-shaped plasticity in hippocampal Schaffer collateral to CA1 pyramidal cell synapses

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Abstract: Memory formation in the hippocampal CA1 area is influenced by multiple biochemical and electrophysiological mechanisms at individual neurons and synapses triggered by on-going neuronal activity. However, not only the temporal, but also the spatial organization of synaptic inputs plays an important role in shaping synaptic plasticity at hippocampal Schaffer collateral to CA1 pyramidal cell synapses. In our study we modeled synaptic plasticity at hippocampal CA3-CA1 synapses and explored the influence of the spatial organization of the inputs on the long-term-potentiation (LTP)/long-term-depression (LTD) induction. We employed

a hippocampal CA1 pyramidal neuron model (Migliore et al., PLOS Comp Biology 2018) and used the modified NMDAr-dependent voltage-based synaptic plasticity model (Clopath et al., Front. Synaptic Neurosci., 2010). Synaptic clusters of the varying radius were formed on the proximal apical dendrites, located in stratum radiatum, and consisted up to fifty AMPA/NMDA synapses. All synapses were stimulated simultaneously applying the protocols used in the experimental studies of synaptic plasticity: low-frequency (LF) stimulation at 1 Hz for 900 s for LTD induction and high-frequency (HF) stimulation consisting of continuous 100 Hz tetanization for 1 s repeated twice at 2 s interval for LTP induction. Spatially clustered synapses underwent strong LTP following the HF stimulation and enhanced LTD after the LF stimulation. Increased radius of the synaptic clusters led to the reduced synaptic modifications or no changes for both HF and LF stimulation protocols. The cooperativity rules governing the induction of synaptic LTP and LTD strongly depend on the synaptic cluster radius and dendritic location and imply that synaptic clustering of the multiple excitatory synaptic inputs shapes synaptic modifications. Structural organization of synaptic inputs contributes to memory formation mechanisms in the hippocampal CA1 area and needs to be explored further both experimentally and theoretically.

Disclosures: J.J. Dainauskas: None. **A. Saudargienė:** None. **H. Marie:** None. **M. Migliore:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.05

Topic: B.07. Synaptic Plasticity

Support: R15 NIMH MH101700
GM103475 (PRINBRE)
RCMI NIMHD MD007579
RISE GM082406

Title: Plasticity of GluN1 at ventral hippocampus synapses in the infra limbic cortex

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Abstract: The fear circuit involves connections between the ventral hippocampus (vHPC) and the infralimbic cortex (IL). The vHPC routes information about the environment to IL which modulates the expression of fear via the amygdala. Although IL is not thought to play a role in fear conditioning, recent experiments suggests that synaptic plasticity is occurring in IL during auditory fear acquisition as measured by changes in the NMDA receptor-mediated currents in male rats. Based on our laboratory's previous electrophysiological studies, wehypothesized that

fear conditioning decreases NMDA receptors on vHPC-to-IL synapses in rats. To evaluate synaptic changes in NMDA receptors at this specific synapse, we injected AAV particles expressing EYFP into the vHPC of male and female rats to label vHPC projections with EYFP. Since GluN1 is an obligatory subunit in all NMDA receptors, we measured GluN1 as a measure of total NMDA receptors. To test for NMDA receptor changes in the vHPC-to-IL synapses after fear learning, we used fluorescence active cell sorting (FACS) to quantify synaptosomes isolated from IL tissue punches that were positive for EYFP and GluN1. We compared rats exposed to auditory fear conditioning (AFC), contextual fear conditioning (CFC), or context and tone exposure. Females and males showed similar freezing behavior after AFC and CFC. More EYFP+/GluN1+ IL synaptosomes were isolated from male rats exposed to AFC than those exposed to context and tones only or CFC suggesting that AFC altered NMDA receptor expression in males. In contrast, females showed similar levels of EYFP+/GluN1+ IL synaptosomes in all three behavioral groups. These findings suggest that AFC induces synaptic plasticity of NMDA receptors in the vHPC-to-IL pathway in males while female rats rely on different synaptic mechanisms to encode AFC.

Disclosures: Y. Castillo-Ocampo: None. **J.T. Porter:** None. **A. Hernandez:** None. **P. Lopez:** None. **M. Colon:** None. **Y. Gerena:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.07

Topic: B.07. Synaptic Plasticity

Support: University of Wisconsin-Madison ICTR

Title: Neuronal RhoA signaling is controlled by DISC1 with consequences on prefrontal cortical-dependent synapse stability and cognition

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Abstract: Cognitive impairments are a core feature of many neuropsychiatric disorders, including, but not limited to, bipolar disorder, schizophrenia, and major depressive disorder. These impairments have proven very difficult to alleviate in most patients, resulting in impaired daily functioning. Consequently, much attention has been devoted to understanding the molecular and biochemical mechanisms that control cognitive processes and phenotypes. Genome wide association studies have identified the RhoA small GTPase as a potential hub gene relevant for human general cognitive function. Further, murine translational studies have found that excessive RhoA signaling is associated with impaired cognition, which is thought to be caused by RhoA's effects in destabilizing dendritic spines, the sites of most excitatory connections in the brain. Despite the involvement of RhoA in cognition, the precise mechanisms that control RhoA activation in neurons have received little attention. PDZ-RhoGEF is a RhoA-

specific guanine nucleotide exchange factor (GEF) whose expression is restricted to the central nervous system, with levels highest in the neocortex. Further, among RhoA-specific GEFs, PDZ-RhoGEF shows an unusually potent ability to activate RhoA. Thus, understanding the mechanisms controlling PDZ-RhoGEF function will have implications for understanding the broader regulation of RhoA in neurons. Here we show that PDZ-RhoGEF reduces dendritic spine density in cortical neurons via its ability to activate RhoA. Consistent with these spine phenotypes, we found that PDZ-RhoGEF overexpression in the prefrontal cortex of mice using viral-mediated gene transfer impairs executive function and episodic memory. Further, we show that PDZ-RhoGEF activity is largely controlled by its interactions with the DISC1 scaffold-like protein, such that DISC1 occludes PDZ-RhoGEF's ability to activate RhoA. Consistent with this, disruption of PDZ-RhoGEF's ability to bind DISC1 exasperates the adverse effects of PDZ-RhoGEF on dendritic spine stability. Current studies are aimed at determining if DISC1 hypofunction leads to PDZ-RhoGEF disinhibition and concomitant RhoA hyperactivity-mediated dendritic spine destabilization. These findings not only characterize the involvement of PDZ-RhoGEF in the control of neuronal morphological and behavioral phenotypes, but also identify a previously unrecognized involvement of DISC1 in the control of RhoA signaling in neurons via PDZ-RhoGEF.

Disclosures: **K.J. Bjornson:** None. **B. Kermath:** None. **A. Sawa:** None. **M.E. Cahill:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.08

Topic: B.07. Synaptic Plasticity

Support: NIH Grant R01NS062736
NIH Grant T32 GM099608

Title: Saturation of Structural Plasticity at Individual CA1 Dendritic Spines

Authors: ***J. C. FLORES**, K. ZITO;
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Abstract: Learning is crucial for survival. One intriguing aspect of learning in humans is that efficiency of learning can be improved when there are breaks between episodes of learning. Long-term potentiation (LTP) of synaptic strength has been proposed as a cellular basis of learning and memory. Notably, LTP can saturate in the hippocampus and that this saturation can lead to deficits in behavioral learning tasks. Importantly, saturation of LTP is released over time. Thus, it is possible that release from saturation of LTP in the hippocampus contributes to the increased efficacy of spaced learning over massed learning. We propose that LTP saturates at individual synapses. In the hippocampus, most excitatory synapses occur at small protrusions on dendrites called dendritic spines. The size of a spine is strongly correlated with the strength and size of its associated synapse. Spines are dynamic and have been shown to exhibit long-term

growth, or structural LTP (sLTP), in response to high-frequency stimulation in vitro and during learning in vivo. Here, we use 2-photon glutamate uncaging and time-lapse imaging to show that structural LTP can saturate at individual dendritic spines for at least 30 minutes after high frequency stimulation. We also show that saturation of sLTP is specific to the stimulated spine. Notably, individual spines are released from saturation and can undergo further structural LTP if the second stimulus is administered at least 60 minutes after the initial stimulus. Furthermore, we show that, if the intensity of the second stimulus is increased, spines can exhibit more structural LTP, suggesting that a metaplastic shift in threshold for sLTP is responsible for the observed saturation. We hypothesize that saturation of sLTP is due to altered function of specific signaling components involved in LTP. Using 2-photon FRET fluorescence lifetime imaging and a genetically-encoded CaMKII FRET probe, we show that 30 minutes following sLTP induction at single spines, CaMKII activity is significantly reduced. We are currently investigating potential postsynaptic mechanism for postsynaptic saturation of sLTP. This work will further our understanding of the signaling mechanisms that limit LTP and thus will lead to a better understanding of learning.

Disclosures: J.C. Flores: None. **K. Zito:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.09

Topic: B.07. Synaptic Plasticity

Support: NIH Grant 1 R15 NS101608-01A1

Title: Investigating the effects of rab11 mutations on the localization of cell adhesion molecules and endocytic proteins at the glutamatergic drosophila neuromuscular junction

Authors: *I. SMITH, F. L. LIEBL;
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Abstract: Chromodomain helicase DNA-binding (CHD) domain proteins are chromatin remodelers whose loss of function are associated with pathologies such as CHARGE syndrome, epilepsy, and autism spectrum disorders. Kismet is the *Drosophila* ortholog of CHD7 and CHD8 and restricts synaptic levels of cell adhesion molecules (CAMs) and promotes endocytosis at glutamatergic neuromuscular junctions (NMJs). Kis also promotes the expression and synaptic localization of Rab11, which is a GTPase important for endosomal trafficking. Thus, Rab11 is critical for the synaptic localization of proteins including CAMs and endocytic proteins. In an effort to tease out the mechanism that links Kismet and these phenotypes, we are examining the effects of dominant negative and constitutively active *rab11* mutations on Fasciclin II, the *Drosophila* ortholog of NCAM, and the endocytic markers Endophilin A and Dynamin. If the results of these experiments mimic the phenotype observed in *kismet* loss of function mutants, then Kismet may promote Rab11 function to ensure appropriate levels of endocytic proteins and

CAMs at the synapse. These results will further illuminate the relationship between chromatin remodelers and the localization of synaptic proteins to aid in the understanding of CHARGE syndrome and autism spectrum disorders.

Disclosures: I. Smith: None. **F.L. Liebl:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.10

Topic: B.07. Synaptic Plasticity

Support: SNSF-Professorship grant (PP00P3_144816)
ERC StG ('SynDegrade')

Title: Homeostatic control of transsynaptically aligned glutamate receptor rings

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Abstract: The molecular organization of synapses is a key determinant of neural information processing. Neurotransmitter receptors form clusters within individual synapses. How these receptor clusters arrange within the postsynaptic density, and how this arrangement relates to presynaptic nano-architecture remains enigmatic. Moreover, it is currently unknown how synaptic nano-architecture is modulated during homeostatic plasticity. Using stimulated emission depletion microscopy (STED), we discovered that postsynaptic glutamate receptors (GluRs) are organized in ring-like arrays composed of ~6 sub-diffraction GluR 'clusters' at the *Drosophila* neuromuscular junction. Strikingly, postsynaptic GluR rings precisely aligned with presynaptic rings formed by the C-termini of the active zone scaffold Bruchpilot (Brp), suggesting transsynaptic alignment. Moreover, while GluRs containing the GluRIIA subunit predominantly localized to rings, GluRIIB-containing receptor clusters were found both, inside and outside of the rings, implying a GluR subunit-specific nano-organization. Furthermore, we observed rings of the auxiliary GluR subunit neto-β (neuropilin and tolloid-like-β), which co-localized with GluR rings, and transsynaptically aligned with Brp. Neto-β clusters were also found outside of transsynaptically-aligned rings, and GluR rings were more pronounced in *neto*^{109} mutants, because of a predominant decrease in GluR fluorescence intensity outside the rings. Interestingly, we uncovered rapid and sequential modulation of aligned Brp-GluR rings during homeostatic plasticity induced by GluR perturbation. Application of the GluR antagonist philanthotoxin-433 (PhTX) resulted in a pronounced, scaled increase of GluRIIC fluorescence intensity within minutes upon receptor perturbation. Additionally, PhTX treatment led to a significant increase in receptor cluster number within the ring. PhTX also increased Brp-fluorescence intensity and Brp-cluster number without affecting Brp-ring diameter, consistent with previous work. We also provide evidence that GluR regulation precedes Brp modulation

during homeostatic plasticity. Together, our findings provide evidence for transsynaptically-aligned rings that undergo rapid changes during synaptic plasticity.

Disclosures: P. Muttathukunnel: None. **P. Frei:** None. **M. Müller:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.11

Topic: B.07. Synaptic Plasticity

Support: NIH Grant 1 R15 NS101608-01A1

Title: Investigating the relationship between Kismet, cell adhesion molecules, and endocytosis at the drosophila neuromuscular junction

Authors: *G. JENKINS[1], F. L. LIEBL[2];
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Abstract: Kismet (Kis) is the ortholog of chromodomain helicase DNA (CHD) binding protein 7, which, if mutated, is causative for CHARGE syndrome. CHD proteins alter gene expression of proteins important for synaptic plasticity. Proteins required for synaptic plasticity include cell adhesion molecules (CAMs) Neurolgins (Nlgs) and Neurexins, which stabilize and align the pre- and postsynaptic cell. CAMs are overexpressed, and endocytosis is decreased in *kis* loss of function mutants. To understand endocytic protein localization in *kis* mutants, we examined the protein levels of α-adaptin and β-adaptin, both of which link clathrin to membranes. There was no significant difference between *kis* mutants and control α-adaptin levels but, *kis* mutants possessed lower β-adaptin levels, a finding that may partially explain the decrease in endocytosis. To determine whether Kis promotes endocytosis by restricting the expression of *nlg2*, we are currently knocking down Nlg2 and assessing the levels of the endocytic proteins, Endophilin A (EndoA) and Dynamin (Dyn). EndoA and Dyn curve the membrane for vesicle formation and pinch the vesicle off from the membrane, respectively. We will next examine overexpression of Nlg2 to determine if overexpression of Nlg2 will increase EndoA and Dyn levels, similar to *kis* mutants. These experiments will aid in understanding how Kis regulates proteins important for synaptic plasticity and how disfunction of Kis and its orthologs can lead to neurodevelopmental diseases.

Disclosures: G. Jenkins: None. **F.L. Liebl:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.12

Topic: B.07. Synaptic Plasticity

Support: R.K MH113858
S.S. 2T32MH016259-39A1
S.J.H. MGH Research Scholars Program

Title: Novel modulators of Arc function for the development of pro-cognitive therapeutics in neuropsychiatric disorders

Authors: *S. SANTARRIAGA[1,2], K. GERLOVIN[1,2], S. A. REIS[1,2], J. LALONDE[1,2], S. J. HAGGARTY[1,2], R. KARMACHARYA[1,2];
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Abstract: There is convergent genetic evidence for the involvement of multiple components of the Arc (Activity-regulated cytoskeleton-associated protein; Arg3.1) protein complex in glutamatergic dysregulation in neuropsychiatric disorders. Functional studies of the consequence of loss of Arc on synaptic plasticity and memory consolidation in rodent systems further suggest that deficits in Arc may contribute to the pathophysiology of cognitive impairments in neuropsychiatric disorders. Conversely, overexpression of Arc has been shown to increase dendritic spine density and to restore plasticity in the adult cortex in animal studies. We conducted a high-content, image-based screen of a library of approved drugs and known bioactive small molecules in mouse cortical neurons to identify potentiators of neuronal activity-dependent induction of Arc protein. Based on our results, we performed a preliminary structure-activity relationship assay and identified specific chemical moieties that modulate Arc levels in human cortical neurons differentiated from induced pluripotent stem cells (iPSCs) of schizophrenia subjects and healthy subjects. Our studies will lead to the development of novel chemical probes to enable dissection of Arc biology in synaptic function and to undertake pre-clinical testing of our hypotheses related to the role of Arc modulation to ameliorate cognitive deficits in neuropsychiatric disorders.

Disclosures: S. Santarriaga: None. **K. Gerlovin:** None. **S.A. Reis:** None. **J. Lalonde:** None. **S.J. Haggarty:** F. Consulting Fees (e.g., advisory boards); Psy Therapeutics, Frequency Therapeutics, Souvien Therapeutics, Vesigen Therapeutics, Amgen, AstraZeneca, Biogen, Merck, Regenacy Pharmaceuticals, Sunovion, Syros Pharmaceuticals, JW Pharmaceuticals. **R. Karmacharya:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.13

Topic: B.07. Synaptic Plasticity

Support: T32 HD041921-18

Title: Maternal sleep disordered breathing during pregnancy is a previously unrecognized facilitator of autism-relevant neuronal and behavioral aberrations in the offspring

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Abstract: Sleep disordered breathing (SDB) is characterized by recurring breathing cessations during sleep, causing intermittent hypoxia (oxygen deprivation) often several hundred times per night. Obstructive sleep apnea is the most common form of SDB in pregnancy, with a prevalence of 10-32%. Maternal SDB confers risk for complications during pregnancy, which have detrimental maternal-fetal outcomes. Complications known to result from gestational SDB are all known risk factors for the development of neurodevelopmental disorders, such as autism spectrum disorder (ASD). We posit that SDB during pregnancy may act as a priming experience for the development of long-term adverse behavioral and cognitive outcomes in the offspring. To investigate the consequences of maternal SDB on offspring, we subjected pregnant rat dams to chronic intermittent hypoxia or normoxia from gestation day 10-21 (GIH and GNX, respectively). Our findings indicate that GIH male offspring exhibit deficits in several behaviors in conjunction with an increased dendritic spine density within pyramidal neurons of the medial prefrontal cortex. Spine time-course results indicate that these spine aberrations likely arise from a lack of the normal pruning of synapses starting in late childhood and extending into early adolescence. The mTOR kinase signal transduction pathway has emerged as a critical regulator of dendritic spine formation, and dysregulation of key components of this pathway are implicated in the etiology of the increased dendritic spine density found in ASD. Our preliminary data indicate that mTOR signaling shows a biphasic pattern in male GIH offspring such that mTOR signaling is increased in juvenile offspring yet decreased in adult offspring, relative to male GNX offspring These data indicate that excessive mTOR signaling may be responsible for the lack of spine pruning in male GIH offspring, while the subsequent decrease in mTOR signaling at later stages of development could be compensatory to prevent further surges in spine numbers. Taken together, these findings indicate that maternal sleep disordered breathing not only induces autism-relevant behavioral impairments, but also mimics the heightened cortical synaptic connectivity phenotypes of ASD, largely in a sexually dimorphic manner.

Disclosures: A.M. Vanderplow: None. **B.A. Kermath:** None. **C.R. Bernhardt:** None. **K.T. Gums:** None. **E.N. Seablom:** None. **A.C. Ewald:** None. **M.V. Jones:** None. **T.L. Baker:** None. **J.J. Watters:** None. **M.E. Cahill:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.14

Topic: B.07. Synaptic Plasticity

Support: CAPES

CNPq
PROPPi

Title: Plastic effects of caffeine in the superior colliculus of adolescent rats under normal and temporal retinal damage

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Abstract: Introduction: Caffeine is the most consumed psychoactive drug in the world. Our previous data have shown that caffeine induces plasticity in the retinotectal pathway of rats under normal conditions and after temporal retinal lesion (TRL), both within and outside the critical period. The aim of my work is to investigate the cellular and molecular mechanisms underlying these plastic effects. **Methods:** Lister Hooded rats (approved project 802 by CEUA) were divided into four different groups: normal animals treated with saline (1) or caffeine (2), animals submitted to TRL on postnatal day 21 (PND21) treated with saline (3) or caffeine (4). Treatment with caffeine (30 mg / kg, ip.) or saline was done daily between PND20 and PND27. In PND28, the animals were euthanized with anesthetic overdose, perfused and processed for immunofluorescence and subsequent analysis using a deconvolution microscope. **Results:** The analysis with Iba-1, a marker of microglial cells in the brain indicates that in the control group the microglial cells are branched and have a reduced cell body. On the other hand, treatment with caffeine promotes activation of these cells that partially retract their extensions. In the group with retinal injury, caffeine appears to decrease the activation of these cells compared to the normal group with caffeine. We also performed the immunostaining of the GluN1 and GluN2B subunits of the NMDA glutamate receptor. Since we only have only performed these experiments one time, our experimental number is one and we don't have statistical analysis. Our results showed that caffeine increases the expression of the GluN1 subunit in the visual layer of the superior colliculus in the control condition, which is different from the group that received saline, where the subunits are much more present in the deep non-visual areas. In the TRL group, caffeine decreases the expression of this subunit on the side contralateral to the TRL. Regarding the GluN2B subunit, the group with TRL and caffeine treatment showed an increase in the expression of this subunit in the visual layers on the side contralateral to the lesion and a decrease on the ipsolateral side in relation to the group with lesion and saline. **Conclusion:** Our preliminary results show caffeine modulation of the analyzed markers, both in the plasticity induced by temporal retinal injury and in the natural plasticity of the visual system. Taken together, these results indicate that caffeine has different effects under normal conditions and with injury. In addition, they suggest that caffeine-induced plasticity involves glial cells and the glutamatergic system.

Disclosures: A.D. Campos: None. **P. Campello-Costa:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.15

Topic: B.07. Synaptic Plasticity

Support: NIH Grant R56NS112207
NIH Grant R21NS102991

Title: Extracellular Vesicles Derived from Mesenchymal Stem Cells Modulate Microglia-Synapse Structural Interactions after Cortical Injury in Rhesus Monkeys

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Abstract: Microglia as the immune cells of the brain promote neuronal plasticity after cortical injury by contacting synaptic elements to eliminate damaged synapses, or release neurotrophic factors to facilitate synapse-turn over. Our previous work showed that treatment with mesenchymal derived extracellular vesicles (MSC-EVs) enhanced post-injury recovery in aged rhesus monkeys by promoting a shift in microglial from pro- to anti-inflammatory morphology, reducing injury-related neuronal hyperexcitability, and enhancing dendritic and synaptic plasticity in perilesional cortex. MSC-EVs are nanovesicles providing cell-to-cell signaling which can modulate neuro-immune interactions to facilitate restorative processes. Here, we assessed whether EV treatment alters synaptic markers and synapse-microglia contacts after cortical injury, by comparing tissue from the same cohort of non-lesion monkeys and lesion monkeys with vehicle (Veh) or EV treatment (EV) used in our previous studies. For Veh and EV, cortical injury was surgically induced in the hand representation of the primary motor cortex (M1). Monkeys then received either 4×10^{11} EVs/kg or vehicle (PBS) intravenously at 24 hrs and at 14 days post-injury. We used immunohistochemical labeling to quantify markers for excitatory (VGLUTs, GluRs) and inhibitory synapse (VGAT, GABARs), and microglia (Iba-1, P2RY12) in perilesional M1 gray matter directly underlying the damaged pial surface, and in gray matter of premotor cortex (PMC) with intact pial surface ~2mm distal to the lesion. The perilesional M1 showed lesion-related decreases in VGLUT2, VGLUT-microglia contacts, and $GABA_a$ α1 receptor expression. These results are consistent with previous work in rodents that phasic glutamatergic and GABAergic transmission decreased after injury. Interestingly, qPCR analyses showed elevated gene expression of tonic $GABA_a$ ∂ and $GABA_b$ ß receptor subunits that mediate tonic inhibitory currents in Veh and EV relative to non-lesion controls. No significant treatment effect was found for synaptic markers expression in perilesional M1. However, in PMC area more distal to the lesion, we found loss of VGLUT2 and GLUR2/3 in Veh but increased GLUR2/3 receptors in EV, consistent with our previous electrophysiological findings. By combining microglial labeling and intracellular filling of pyramidal neurons in PMC, we further showed greater microglial-spines contacts in Veh but not in EV compared to the non-lesion control. Our results suggest that EV treatment might dampen microglial-mediated synapse loss in PMC to regulate synaptic activity in these compensatory networks and hence facilitate recovery of function.

Disclosures: **Y. Zhou:** None. **V. Go:** None. **D.L. Rosene:** None. **B. Buller:** None. **T.L. Moore:** None. **M. Medalla:** None.

Digital Abstract Session

P062. Structural Plasticity of Synapses

Program #/Poster #: P062.16

Topic: B.06. Synaptic Transmission

Support: NIH grant 1R01NS092705
NIH grant 1R01NS107453
NIH grant T32NS007453-16

Title: Microrna-324-5p regulates dendritic spine density, morphology, and expression of cytoskeletal proteins in the hippocampus

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Abstract: microRNAs are an emerging class of synaptic regulators. These small noncoding RNAs post-transcriptionally regulate gene expression, thereby altering neuronal pathways and shaping cell-to-cell communication. Though several miRNAs have been identified that regulate the synapse, the full range of synaptic miRNA regulators has not been identified. Here, we demonstrate that miRNA-324-5p regulates dendritic spines, small protrusions along the dendrite that comprise the majority of excitatory synapses in the brain. We demonstrate that both acute and long-term loss of miR-324-5p alter the density, morphology, and channel composition of dendritic spines in the hippocampus. To assess the effect of acute loss of miR-324-5p function, we intracerebroventricularly (ICV) administered a miR-324-5p-specific antagomir (inhibitor) to Thy1-eGFP^{hemi} mice, which exhibit sparse labeling of neurons throughout the brain. Confocal imaging of hydrogel-cleared brain sections revealed reduced dendritic spine density and altered spine morphology with antagomir treatment. To assess the effect of long-term, complete miR-324-5p loss, we generated CRISPR-Cas9-mediated *Mir324* knockout (miR324 KO) mouse model. Characterization of this model reveals no gross morphological or behavioral changes. Interestingly, analysis of Golgi-stained hippocampal dendrites revealed a similar reduction in spine density in miR324 KO mice to that seen in antagomir-treated mice. RNA-sequencing identified altered biological pathways in the miR-324 KO hippocampus that may contribute to the mechanism of miR-324-5p-mediated spine regulation, including cytoskeletal organization. Western-blot and quantitative Real-Time PCR also revealed changes in protein and mRNA levels for potassium channels, cytoskeletal components, and synaptic markers. We used both male and female mice in these studies and did not detect sex-specific differences. Experimenters and analyzers were blind to conditions, and studies were fully powered (power≥0.8) or indicated

as preliminary. Our findings suggest that miR-324-5p is an important and essential regulator of dendritic spines, as loss of miR-324-5p function or expression leads to significant changes in dendritic spine density, morphology, and channel composition. Future work will assess brain functional consequences of the effects of miR-324-5p loss on neuronal morphology.

Disclosures: E. Parkins: None. **J. Rymer:** None. **Y. Hu:** None. **D. Tiwari:** None. **C. Gross:** None.

Digital Abstract Session

P063. Transcription and Translation in Plasticity

Program #/Poster #: P063.01

Topic: B.07. Synaptic Plasticity

Title: Dopamine D1R and cAMP/PKA signaling stimulates Brd4 recruitment to chromatin to regulate gene expression in striatal neurons

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Abstract: The dopamine D1 receptor (D1R) is a $G\alpha_{s/olf}$-coupled receptor expressed throughput the forebrain, where it regulates motor behavior, reward, motivational states and cognitive performance. D1R is most abundant in the striatum where it couples primarily to $G\alpha_{olf}$ and activates a cAMP/PKA/DARPP-32 signalling cascade that increases neuronal excitability and facilitates structural and synaptic plasticity. Many dopamine dependent striatal functions, such as goal-directed learning and motor learning, depend on transcription and *de novo* protein synthesis. Transcription can be regulated by D1R effectors including PKA and ERK1/2 which can translocate to the nucleus to phosphorylate transcription factors including CREB. Dysregulated transcription linked to D1R hyperactivation contributes to therapy-associated motor disorders such as L-DOPA-induced dyskinesia and to behaviors associated with addiction to drugs of abuse. However, our understanding of the mechanisms by which D1R regulates gene expression downstream of PKA and ERK1/2 activation remain incomplete. In this regard, the chromatin reader Brd4 has previously been found to regulate striatal gene expression in response to drugs of abuse. Brd4 is activated by phosphorylation upon which it interacts with acetylated histones and recruits transcriptional activators including P-TEFb. Here we demonstrate that D1R activation of cAMP/PKA signalling increases Brd4 recruitment to dopamine-induced immediate early genes, and that knockdown or inhibition of Brd4 bi-directionally modulates D1R-induced gene expression. Hence, our findings identify Brd4 as a novel regulator of D1R-dependent transcription and highlight the potential for disrupted striatal function as an adverse effect in the clinical use of Brd4 inhibitors.

Disclosures: J. Jones-Tabah: None. **R.D. Martin:** None. **J.J. Chen:** None. **J.C. Tanny:** None. **P.B.S. Clarke:** None. **T.E. Hébert:** None.

Digital Abstract Session

P063. Transcription and Translation in Plasticity

Program #/Poster #: P063.02

Topic: B.07. Synaptic Plasticity

Support: NIMH R00MH109626

Title: Cis and trans features of dendritically localized transcripts in the Hippocampus

Authors: *J. PARK, S. FARRIS;
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Abstract: Local translation is required for the synaptic changes underlying learning and memory in the hippocampus. This process relies on the efficient localization of RNAs to their target synapses, which is mediated by RNA binding proteins (RBP). However, the precise mechanisms regulating the trafficking, processing, and translation of appropriate transcripts at hippocampal synapses remains unclear. In order to elucidate how hippocampal neurons regulate the subcellular localization of RNAs, we performed both *in silico* and *in vivo* analyses to uncover cis and trans factors involved in RNA localization at synapses. Among the hippocampal subregions (CA1, CA2, CA3, Dentate Gyrus), we focused on CA2 for its unique synapse-specific plasticity-resistant phenotype. An understudied subregion, CA2 has been reported to have both long-term potentiation (LTP)-competent and LTP-resistant synapses, making it an ideal neuronal population to study local translation factors involved in learning at individual synapses. A previous study utilized laser capture microdissection and RNAseq to identify dendritically localized transcripts in each subregion of the mouse hippocampus. Since sequence elements within the untranslated regions (UTRs), have been shown to mediate RNA trafficking and translational regulation via trans-acting RBPs, we used this dataset to identify enriched sequence motifs in the 5' and 3' UTRs of dendritically localized transcripts in the CA2. We also identified RNA binding proteins that are predicted to bind these transcripts. Referencing the expression levels of these RBPs in the CA2 subregion led to the identification of high-probability candidates for regulating CA2 transcript localization. Furthermore, we focused on alternatively spliced transcripts that show compartment- and/or subregion-specific splice variant expression. SHANK2, a synaptic scaffolding protein, has two splice variants with alternate 5' UTRs. Using splice variant-specific smFISH, we show subregion and compartment specific enrichment of the longer *Shank2* variant in CA2 compared with neighboring hippocampal subregions. Currently, we are using single molecule fluorescence in situ hybridization combined with immunohistochemistry (smFISH-IHC) to confirm candidate RNA-RBP interactions and identify behavior-induced changes in these interactions at hippocampal synapses in vivo. Results from these studies will provide mechanistic insight to how localized RNAs are regulated to support the synaptic changes required for encoding hippocampal-dependent memories.

Disclosures: J. Park: None. **S. Farris:** None.

Digital Abstract Session

P063. Transcription and Translation in Plasticity

Program #/Poster #: P063.03

Topic: B.07. Synaptic Plasticity

Support: NIH Grant R15 NS on "Investigating How Chromatin Remodeling Affects Endocytosis and Synaptic Organization"

Title: Investigating potential interactions between Kismet and Amyloid precursor protein-like at the Drosophila neuromuscular junction

Authors: *B. PRATES DE OLIVEIRA ALBANEZ;
Southern Illinois Univ. Edwardsville.

Abstract: Investigating potential interactions between Kismet and Amyloid precursor protein-like at the *Drosophila* neuromuscular junctionKismet (Kis) is the *Drosophila* ortholog of the mammalian chromatin remodeling proteins CHD7 and CHD8. Mutations in *CHD7* and *CHD8* are causative for CHARGE syndrome and implicated in autism spectrum disorders, respectively. Interestingly, *kis* mutants exhibit similar phenotypes as are found in animal models of Alzheimer's Disease (AD) suggesting Kis may influence synaptic mechanisms that underlie AD pathology. AD is characterized by accumulation of neurotoxic Aβ fragments from amyloid precursor protein (APP or APPL in *Drosophila*) cleavage, resulting in loss of neuronal synapses and cognitive decline. Similar to *kis* loss-of-function mutants, overexpression of human APP and BACE (β-site APP cleaving enzyme) at the *Drosophila* neuromuscular junction impaired endocytosis, larval locomotion, and increases the transcript levels of *Neuroligin 2*, which encodes a cell adhesion molecule that stabilizes synaptic connections. The pathway by which Kis influences APPL remains unclear. We discovered that Kis represses *appl* expression in motor neurons. Therefore, Kis could affect APPL three ways, including upstream by direct control, in cooperation with AICD (a fragment generated during APP cleavage), or by acting as a cofactor to promote AICD transcriptional activity. To better characterize APPL synaptic function, first we will examine the expression and localization of the cell adhesion molecule, FasII, and the glutamate receptor subunit, GluRIIC, in animals overexpressing APP and BACE, expressing full-length APPL, constitutively secreted APPL, and a secretion-deficient APPL. Then we will construct double mutants and overexpress Kis in *Drosophila* lacking *appl* to examine endocytosis. In conjunction, these experimental results will contribute to better understand how chromatin remodeling protein Kis influences APPL at the synapse.Authors: Bruna Prates and Faith Liebl, PhD.

Disclosures:

Digital Abstract Session

P063. Transcription and Translation in Plasticity

Program #/Poster #: P063.04

Topic: B.07. Synaptic Plasticity

Support: CIHR

Title: Isoflurane Anesthesia Induced Sex-Related Differences in Cortical EEG Dynamics and Gene Expression

Authors: R. TADAVARTY[1], T. MARIAM[1], X. DONG[1], S. SINHA[1], S. KHAN[1], S. LIU[2], T. CHEUNG[2], G. GIAEVER[1], C. NISLOW[1], *P. J. SOJA[1];
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Simon Fraser Univ., Burnaby, BC, Canada

Abstract: Exposure to inhalational anesthesia (IA) adversely affects cognition and increases the risk of dementia, leading to post-operative cognitive dysfunction (POCD). Interestingly, POCD may preferentially last in males from several months to years. We recently found that following prolonged exposure to surgical levels of isoflurane anesthesia (ISO), long-term depression (LTD) of hippocampal pEPSPs, once established, can be reversed in female rats but not males. A failure of LTD reversal in males might contribute to cognitive "inflexibility" following ISO exposure. The mechanisms behind this observation are not known. The expression of synaptic plasticity (long-term potentiation; LTP and LTD) and its maintenance critically depends on gene expression. Given that exposure to IA can alter gene expression in the brain, we hypothesized that a prolonged exposure to ISO induces selective changes in gene expression in male vs female rats which underlie sex-differences in de-depression following LTD. Accordingly, adult male or female SD rats were initially anesthetized in an induction chamber, their trachea intubated and head mounted in a stereotaxic frame. Cortical EEG was recorded using stainless steel screw electrodes positioned in S1 bilaterally. After 5h exposure to ISO, S1 cortical tissue was dissected and RNA-seq was used to quantify changes in the transcriptome. Differential expression analyses was performed on these datasets to identify cohort-specific changes. We analyzed the expression of over 13,000 genes, of which ~225 were found to be down-regulated selectively in males. Gene ontology of these differentially expressed genes allowed us to functionally group them into clusters potentially affecting aspects of synaptic transmission, protein phosphorylation, calcium signaling, neuronal activity and neurotransmitter release. EEG slow wave activity (SWA) in male rats revealed oscillatory behavior characterized by robust "up"-states and "down"-states that was marginally present in females. Relative power in "up"-states of male rats was confined to the δ band and was ~3-fold greater than "down"-states. Our results disclose for the first time that males and females differ in EEG SWA oscillation dynamics, spectral content and gene expression following exposure to (IA).

Disclosures: **R. Tadavarty:** None. **T. Mariam:** None. **X. Dong:** None. **S. Sinha:** None. **S. Khan:** None. **P.J. Soja:** None. **S. Liu:** None. **T. Cheung:** None. **G. Giaever:** None. **C. Nislow:** None.

Digital Abstract Session

P063. Transcription and Translation in Plasticity

Program #/Poster #: P063.05

Topic: B.07. Synaptic Plasticity

Support: MOE2017-T3-1-002
MOE2018-T1-002-033
NAP

Title: Local regulation and function of Importin-β1 during transcription-dependent plasticity

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Abstract: Activity-dependent transcription is critical for the encoding of long-term memories. Regulated nuclear entry of soluble proteins is one method to relay synaptic signals to the nucleus to couple neuronal excitation with transcription. To date, the role of importin-β1 in the nuclear shuttling of proteins during activity-dependent transcription has always been inferred but not directly investigated. In this study, we demonstrate the activity-dependent nuclear accumulation of importin-β1 from the soma and the synapto-dendritic compartments. Importantly, inhibition of importin-β1 mediated nuclear import during synaptic stimulation impairs long-term plasticity. We show evidence that importin-β1 mRNA-ribosome complex is distributed throughout the synapto-dendritic compartment and the synaptic stimulation induces importin-β1 local protein synthesis. Finally, we identified candidate proteins that associate with importin-β1 at the synapse and characterize NDRG1 as an importin-β1 interactor that undergoes activity-dependent translocation into the nucleus. Collectively, our results highlight the crucial role of importin-β1 in nuclear import of proteins during long-term plasticity.

Disclosures: **Y. Lee:** None. **T. Chng:** None. **S. Navakkode:** None. **C. Tan:** None. **S. Sze:** None. **S. Sajikumar:** None.

Digital Abstract Session

P063. Transcription and Translation in Plasticity

Program #/Poster #: P063.06

Topic: B.07. Synaptic Plasticity

Support: NINDS Grant R01NS036715

Title: Experience-induced remodeling of the post-synaptic proteome and phosphoproteome: New aspects of learning-induced dynamics of synaptic proteome

Authors: *S. HEO[1], T. KANG[2], A. BYGRAVE[1], M. R. LARSEN[2], R. L. HUGANIR[1,3];
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Abstract: The post synaptic densities (PSD) of excitatory synapses are key nodes in the regulation of synaptic plasticity and contain thousands of proteins. To gain new mechanistic insight of experience-induced proteome dynamics, we examined the learning-induced changes in PSD proteome and phosphoproteome. Mice were trained using inhibitory avoidance (IA) paradigm and hippocampal PSD fractions were prepared for quantitative proteomic analysis. We used iTRAQ (Isobaric Tags for Relative and Absolute Quantitation) mass spectrometry combined with phosphopeptide enrichment to quantify the protein expression and phosphorylation events in hippocampal PSD following IA training (IA) or unpaired foot shock (Shock) in mice. We detected and analyzed 6,200 proteins and 3,700 phosphoproteins. Strikingly, of the significantly IA or unpaired shock regulated proteins and phosphoproteins, a large fraction showed decreased abundance and phosphorylation. Gene ontology analysis of proteins and phosphoproteins that were regulated by IA were annotated for an involvement in regulation of glutamate receptor functionality, calcium signaling, and synaptic plasticity. We also identified synaptic kinases, phosphatases and their respective autophosphorylation sites regulated by IA training or unpaired foot shock. Together, these results unravel the dynamic remodeling of the PSD upon IA learning or unpaired foot shock and serve as a resource for elucidating the synaptic proteome dynamics induced by experience-dependent plasticity.

Disclosures: S. Heo: None. **T. Kang:** None. **A. Bygrave:** None. **M.R. Larsen:** None. **R.L. Huganir:** None.

Digital Abstract Session

P064. Long-Term Depression (LTD)

Program #/Poster #: P064.01

Topic: B.07. Synaptic Plasticity

Support: NIH Grant R21MH116315

Title: Co-agonist Occupancy as a Determinant of Bidirectional Plasticity

Authors: *E. V. BARRAGAN[1,2], J. GRAY[2,3];
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Abstract: Synaptic plasticity, or the ability of synapses to undergo bidirectional changes in strength, underlies a vast number of complex cognitive processes. NMDA receptors (NMDARs) are master regulators of synaptic plasticity but it remains unclear how this one receptor is able to

mediate both depression and potentiation of synaptic strength. NMDARs are unique among receptors in that they require co-agonist binding - either glycine or D-serine - to the glycine-site on the GluN1 subunit, in addition to glutamate binding. It has been recently shown that LTD can occur during competitive antagonism of the NMDAR co-agonist site. This can also reverse the direction of plasticity following an LTP-inducing stimulus. Based on these results, we hypothesize that glycine-site occupancy dictates the directionality of synaptic plasticity. To investigate this hypothesis, we pharmacologically manipulated glycine-site occupancy while administering plasticity-inducing stimuli. We show that blocking the glycine-site results in decreased synaptic strength during LTD and LTP-inducing paradigms, shifting the plasticity curve toward synaptic depression. Similarly, increasing extracellular co-agonist concentration results in increased synaptic strength following LTD induction, which would indicate a shift toward favoring synaptic potentiation. These results support our hypothesis that the glycine-site serves to influence the direction of synaptic plasticity, highlighting it as an attractive target for treatment of the vast range of psychiatric and neurologic disorders that arise due to dysfunction in NMDAR function and synaptic plasticity.

Disclosures: E.V. Barragan: None. **J. Gray:** None.

Digital Abstract Session

P064. Long-Term Depression (LTD)

Program #/Poster #: P064.02

Topic: B.07. Synaptic Plasticity

Support: European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 720270 (Human Brain Project SGA1)
European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 785907 (Human Brain Project SGA2)
European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 945539 (Human Brain Project SGA3)
Academy of Finland (decision No. 297893)
Academy of Finland (decision No. 318879)
Academy of Finland (decision No. 326494)
Academy of Finland (decision No. 326495)

Title: In silico analysis of biochemical mechanisms responsible for astrocyte modulation of synaptic plasticity in developing somatosensory cortex

Authors: *T. MANNINEN[1], A. SAUDARGIENE[2], M.-L. LINNE[1];
[1]Tampere Univ., Tampere, Finland; [2]Lithuanian Univ. of Hlth. Sci., Kaunas, Lithuania

Abstract: Astrocytes have critical roles in controlling synapse development, functionality, plasticity, and elimination (Allen and Eroglu, 2017), but the biochemical and biophysical mechanisms responsible for these phenomena are not fully understood. The mechanisms involved seem to depend not only on the developmental stage of an animal but also on the brain area, neural circuitry, as well as on the experimental technique used to characterize the phenomena. In our previous study, we developed a computational model of astrocyte-neuron interactions at a layer 4 to layer 2/3 synapse in somatosensory cortex during postnatal development (Manninen et al., 2020). The model links presynaptic, postsynaptic, and astrocytic mechanisms to the time window of spike-timing-dependent long-term depression (t-LTD) induction (Min and Nevian, 2012; Banerjee et al., 2014). In brief, we showed that postsynaptically released endocannabinoids increase astrocytic calcium signaling which in turn induces exocytosis of glutamate. Glutamate exocytosis from an astrocyte can further activate presynaptic N-methyl-D-aspartate receptors coupled with calcineurin signaling and influence synaptic properties. These complex signaling cascades between the neurons and the adjacent astrocyte can induce t-LTD which is sensitive to temporal difference between post- and presynaptic activity. The work involved using a multitude of data from molecular, biochemical, and electrophysiological experiments in rodent somatosensory cortex during postnatal development. Recent studies have indicated increased motility of fine astrocyte processes during synaptic activation, particularly with long-term plasticity changes (Bernardinelli et al., 2014; Sakert et al., 2017). Structural reorganization of a fine astrocyte process can thus be hypothesized to change the microenvironment around the synapse. It is of interest to analyse the influence of extracellular glutamate in such conditions, using the previously developed *in silico* model. In the present study we explored the amount of glutamate spillover required to induce t-LTD with and without astrocyte activation. The latter condition may occur when fine astrocyte processes retract from the close vicinity of the synapse during the learning process but also during injury. Our results show that during postnatal development astrocytes take part in synaptic computations and facilitate the biochemical processes for plasticity, a precondition for learning and memory. Computational studies of synaptic functions promote development of new hypotheses that can be tested experimentally.

Disclosures: **T. Manninen:** None. **M. Linne:** None. **A. Saudargiene:** None.

Digital Abstract Session

P064. Long-Term Depression (LTD)

Program #/Poster #: P064.03

Topic: B.07. Synaptic Plasticity

Support: NIH Grant AA07462
NIH Grant AA026488
NIH Grant AA023507

Title: Input-selective adenosine a_1 receptor-mediated synaptic depression of excitatory transmission in dorsal striatum

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Abstract: The medial (DMS) and lateral (DLS) dorsal striatum differentially drive goal-directed and habitual/compulsive behaviors, respectively, and are implicated in a variety of neuropsychiatric disorders. These subregions receive distinct inputs from cortical and thalamic regions which uniquely determine dorsal striatal activity and function. Adenosine A_1 receptors (A1Rs) are prolific within striatum and regulate excitatory glutamate transmission. Thus, A1Rs may have regionally-specific effects on neuroadaptive processes which may ultimately influence striatally-mediated behaviors. The occurrence of A1R-driven plasticity at specific excitatory inputs to dorsal striatum is currently unknown. To better understand how A1Rs may influence these behaviors, we first sought to understand how A1Rs modulate these distinct inputs. We evaluated A1R-mediated inhibition of cortico- and thalamostriatal transmission using *in vitro* whole-cell, patch clamp slice electrophysiology recordings in medium spiny neurons from both the DLS and DMS of C57BL/6J mice in conjunction with optogenetic approaches. In addition, conditional A1R KO mice lacking A1Rs at specific striatal inputs to DMS and DLS were generated to directly determine the role of these presynaptic A1Rs on the measured electrophysiological responses. Activation of presynaptic A1Rs produced significant long-term synaptic depression (A1R-LTD) of excitatory transmission in the both the DLS and DMS of male and female animals. Our findings indicate that A1R-LTD at corticostriatal and thalamostriatal inputs to DLS can be additive and that A1R-LTD in DMS occurs primarily at thalamostriatal inputs. These findings advance the field's understanding of the functional roles of A1Rs in striatum and implicate their potential contribution to neuropsychiatric diseases.

Disclosures: B.M. Fritz: None. **F. Yin:** None. **B.K. Atwood:** None.

Digital Abstract Session

P065. Spike-Timing Dependent Plasticity

Program #/Poster #: P065.01

Topic: B.07. Synaptic Plasticity

Support: NIH 2R01MH101297
NSF CRCNS 1516235
Northwestern University WCAS summer grant

Title: Clustered synaptic plasticity in a dendrite CA1 compartmental model

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Abstract: A hippocampal CA1 place cell is a pyramidal neuron that fires at specific locations in an environment. Recent experimental studies have shown that in novel environments place

activity appears to begin first in dendrites, followed by full somatic place cell firing. Such experiments raise questions concerning how dendritic inputs lead to the formation of CA1 place cells.We have created a compartmental model of a CA1 place cell dendrite and soma with a simplified version of a biophysically-motivated, calcium-based synaptic plasticity model (Rubin et al., J. Neurophysiol., 2005, 93, 2600-2613). First, we verified that the simplified model is capable of reproducing the spike-timing-dependent plasticity rules exhibited by the full model when a single synapse is stimulated. Then, we introduced two spatially-separated synaptic inputs, and paired their stimulations with a somatic action potential. When the distance between the synapses is varied, the synaptic potentiation increases when the synapses are in close proximity with one another, i.e., clustered synaptic potentiation is produced.We also performed simulations to confirm that calcium diffusion is responsible for these spatially-dependent plasticity changes. If we increased the rate of the calcium pump in a compartment between the two stimulated synapses, the distance-dependent effect upon synaptic plasticity was significantly reduced. Moreover, the effect of artificially changing the calcium diffusion coefficient showed a positive correlation with the length scale over which the synaptic potentiation is increased. These two simulations demonstrate that the diffusion of calcium signals from nearby synapses was the primary reason for the distance dependence of the synaptic plasticity.We constructed this model with the formation of place cells in mind, but it is fairly general and so could be relevant for other neurons with extensive dendritic arbors with many synaptic inputs, such as those investigated by Makino and Malinow (Neuron, 2011, 72:1001-1011).

Disclosures: Z. Wu: None. **D.B. Poll:** None. **D.A. Dombeck:** None. **W.L. Kath:** None.

Digital Abstract Session

P066. Dendritic Integration, Neural Oscillations, and Plasticity

Program #/Poster #: P066.01

Topic: B.08. Intrinsic Membrane Properties

Support: NIH RO1 MH115832

Title: The role of Kv2 and Nav1.6 in Adaptive Firing in Place Fields within the CA1 region of the Hippocampus: A Computational and Experimental Approach

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Abstract: CA1 place cells in the hippocampus receive synaptic input from the CA3 region and the entorhinal cortex. *In vivo* this synaptic input has been shown to depolarize the cell as the animal enters a place field and return to baseline as the animal leaves. Our experimental collaborators (see Combe et al. poster) replicate this *in vitro* (slice electrophysiology) through

triangular current ramps of varying durations (1-10 s), delivered either to the soma or dendrites. From their experimental data, we have found that under control conditions, CA1 neurons fire more on the up ramp than the down ramp, and this is accentuated in the dendrites compared to the soma. We have also found spike height decreases in the dendrites during repetitive firing. We believe this to be the result of slow cumulative inactivation of the Nav1.6 voltage gated Na+ channel, which has been shown to increase in dendrites with distance from the soma. In the presence of cholinergic agonists, these CA1 neurons fire more on the down ramp than the up ramp, a possible consequence of the activation of the Ca2+- activated nonspecific current ICAN mediated by TRP channels. We calibrated a five state Markov model of Nav1.6 to fit time, voltage and spatial dependences of slow inactivation according to the literature, and inserted it into our previously published, morphologically realistic, multicompartment model of a CA1 pyramidal cell implemented in NEURON. We simulated triangular current ramps delivered either to the soma or proximal dendrites (~200 um from the soma) to reproduce the in vitro experiments.
With the calibrated Markov model of Nav1.6, our previously published model produced a burst in response to the application of triangular current ramps. In vitro experiments showed the same burst response when triangular ramps were delivered in the presence of Guangxitoxin-1E, a Kv2.1 specific blocker. After the insertion of a model of Kv2, we restored repetitive single spiking during the ramp and replicated the adaptation in the soma, increased adaptation in the dendrites and decrease in spike height. From our modeling results, we find the Kv2 channel is active during afterhyperpolarization and suggest it may be necessary to prevent burst firing in these neurons. The addition of a phenomenological model of TRPM4 shifted the rate response to cause more firing on the down ramp than the up ramp.
Our results suggest 1) the slow inactivation of Nav1.6 contributes to the adaptation seen in CA1 pyramidal cells under control conditions, 2) the afterhyperpolarization mediated by Kv2 is necessary to prevent burst firing in these neurons, 3) TRPM channels contribute to the acceleration seen in these cells in the presence of acetylcholine.

Disclosures: C.M. Upchurch: None. **C.L. Combe:** None. **C.J. Knowlton:** None. **S. Gasparini:** None. **C.C. Canavier:** None.

Digital Abstract Session

P066. Dendritic Integration, Neural Oscillations, and Plasticity

Program #/Poster #: P066.02

Topic: B.08. Intrinsic Membrane Properties

Support: USC Provost Undergraduate Research Fellowship

Title: When should, and how can, a dendrite provide sharp thresholding, despite natural input variability?

Authors: *T. RAMDAS[1], B. W. MEL[2];
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Abstract: Experimental and modeling studies over the past 25 years inform us that dendrites can function as separately thresholded learning and signaling units, potentially affording neurons with greater computational power and increased storage capacity. In previous studies that explored the role that dendritic compartmentalization may play in memory formation, we found that storage capacity is maximized when dendrites are sharply thresholded, with cleanly separated sub- and suprathreshold response ranges. From a biophysical perspective, configuring a dendrite to produce effectively binary-valued outputs given natural input variability is non-trivial. Even in the simple case of focal excitation at different dendritic shaft locations, the amplitudes of dendritically-evoked NMDA spikes measured at the soma vary essentially continuously. We carried out compartmental modeling studies of a canonical spiny basal dendrite in search of biophysical conditions that maximize the dendrite's propensity to generate sharply thresholded, rather than continuously varying, voltage responses at the soma despite fluctuations in the patterning of activated synapses. Spines were outfitted with synapses consisting of NMDA and AMPA conductances whose activations (with a burst of 3 inputs at 200 Hz) were modeled as independent Bernoulli random variables. We studied the effects of peak synaptic conductance, spine neck resistance, and spine density, all potentially varying with dendritic shaft location, on the distribution of somatic responses. Our main new finding is that a decaying spine density moving outward along a dendrite leads to a strikingly bimodal NMDA-dependent somatic voltage distribution as compared to either uniform or increasing spine density, which led to more graded responses. To quantify bistability, we measured the widest "gap" along the voltage axis separating sub- and suprathreshold ranges containing no more than 2% of total cases. The peri-threshold voltage gap for the decreasing density case (15 mV) is significantly wider than that for the constant density case (3 mV) and the increasing density case (1 mV). Interestingly, data from a recent EM study indicates that spine density does indeed fall off moving distally along dendrites in hippocampal pyramidal neurons, which the authors showed was consistent with a 2-layer integration scheme in these cells. Our finding adds a normative account for the observed spine density gradient and provides further insight into the brain's subtle biophysical strategies for optimizing the computing capabilities of neural tissue.

Disclosures: **T. Ramdas:** None. **B.W. Mel:** None.

Digital Abstract Session

P066. Dendritic Integration, Neural Oscillations, and Plasticity

Program #/Poster #: P066.03

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant NS094643
Startup Funds, UT Austin
UT System STARS Award
Child Neurology Foundation PERF Elterman Grant
Child Neurology Society Philip R. Dodge Young Investigator Award

Title: Physiologically and anatomically distinct projection neurons connect mediodorsal thalamus to medial prefrontal cortex

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Abstract: At the heart of the prefrontal executive and limbic networks is the mediodorsal thalamus (MD). Despite the importance of MD in a broad range of behaviors and neuropsychiatric disorders, virtually nothing is known about the physiology of the different populations of neurons in MD. Here, we injected the retrograde tracer cholera toxin subunit B (CTB) into the medial prefrontal cortex (mPFC) of adult (8-12 week old) male and female wildtype mice. We prepared acute brain slices and used whole cell electrophysiology to measure and compare the intrinsic properties of the neurons in ipsilateral MD that project to mPFC (MD→mPFC neurons). MD→mPFC neurons are located predominantly in the medial (MD-M) and lateral (MD-L) subnuclei of MD. We found that that MD-M→mPFC neurons have longer membrane time constants, higher membrane resistance, less Hyperpolarization and Cyclic Nucleotide gated (HCN) channel activity, and more readily generate action potentials compared to MD-L→mPFC neurons. Additionally, MD-M→mPFC neurons have larger and more complex dendritic arbors compared to MD-L→mPFC neurons. These data demonstrating that the two populations of MD→mPFC neurons have distinct physiologies and morphologies suggests a differential role in thalamocortical information processing and potentially behavior.

Disclosures: P. Lyuboslavsky: None. **A. Kizimenko:** None. **A.C. Brumback:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.01

Topic: B.08. Intrinsic Membrane Properties

Support: NS R35111562
NS F32101832

Title: Activity labeling in vivo using CaMPARI2 reveals cell type-specific intrinsic and synaptic differences between neurons with high and low firing rate set points

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Abstract: To enable robust network stability in the face of dynamic perturbations, rodent neocortical pyramidal neurons actively maintain their mean firing rates within a target range, termed their firing rate set point (FRSP). Remarkably, while these set points can span several orders of magnitude within a transcriptional cell type, individual pyramidal neurons reliably return to their own specific FRSP during prolonged activity perturbations, demonstrating that these individual set points are actively maintained. High and low FR neurons in rodent frontal

cortex and hippocampus are differentially modulated by sleep states, suggesting that they are functionally distinct and may play unique roles in information storage and transmission. However, little is known about the factors that generate this broad distribution of FRSPs in different cell types *in vivo*.
To investigate the differences between high and low activity neurons within single cell types, we developed an approach for permanently labeling pyramidal neurons according to their *in vivo* mean firing rates before *ex vivo* analysis of their synaptic and intrinsic properties. To permanently label neurons in monocular visual cortex of freely behaving mice *in vivo* based on their mean firing rate, we used the permanent green-to red Ca^{2+}- and UV-dependent photoconversion of CaMPARI2 as a proxy for mean neuronal activity. Red/green ratios following 30-minute *in vivo* photoconversion were lognormally distributed, mirroring *in vivo* firing rates, and neurons with greater *in vivo* photoconversion had higher firing rates *ex vivo*. During *ex vivo* photoconversion, neurons with higher firing rates underwent greater changes in CaMPARI2 red/green ratio. These data show that CaMPARI2 labeling is sensitive enough to detect differences in mean firing rates, and to differentiate between neurons with low and high FRSPs.
We went on to characterize the properties that differentiate high from low FR neurons in different cell types. We found that high activity layer 4 (L4) pyramidal neurons had greater intrinsic excitability and received stronger inputs from other L4 pyramidal neurons, although there were no consistent differences in total E/I ratio between these neurons. In contrast, we then found that in L5 TLX-3-positive neurons (a subset of thin-tufted, regular spiking pyramidal neurons), high activity neurons received greater excitatory input but had no increase in intrinsic excitability. Thus, both intrinsic excitability and intralaminar excitatory synaptic strength can be important contributors to the broad range of FRSPs observed within single cell types *in vivo*, though their relative importance varies across cell types.

Disclosures: **N. Trojanowski:** None. **G. Turrigiano:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.02

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant 1ZIANS003135-08

Title: Probing Fidelity of High-Frequency Firing in Midbrain Dopaminergic Neuron Axons

Authors: *A. SUKHAREV, P. F. KRAMER, R. ZHANG, Z. KHALIQ;
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Abstract: Dopamine release in the striatum is key for motor execution and reinforcement learning. Striatal dopamine that is released following stimulation of dopaminergic neuron axons undergoes dramatic short-term depression at physiological rates (5-40 Hz) through mechanisms

that are incompletely understood. Past studies examining evoked dopamine have focused on calcium coupling and vesicular release mechanisms, while others have posited that the highly branching axonal architecture could increase the likelihood of failure during action potential propagation. However, whether intrinsic axonal firing mechanisms contribute to the observed depression has not been directly tested. Here, we obtain axonal recordings using a combination of cell-attached, whole-cell, and perforated-patch configurations to test the frequency dependence of action potential firing in both the unbranched dopaminergic axons of the medial fiber bundle and the profusely branching terminal processes within the dorsal striatum of adult mice. Trains of action potentials were tested over a range of stimulation frequencies. Axonal action potentials were evoked by either using brief current pulses applied directly through the recording electrode or by placement of a bipolar stimulation electrode within the striatal tissue slices to stimulate propagated spikes. In the main dopaminergic axons, we found action potential firing was completely reliable with no failures observed during 5-pulse trains at 100 Hz (current injected, 8 out of 9 axons; stimulated, 11 of 13 axons). The high maximal firing rates that we observed in dopaminergic axons contrasts with the relatively low maximal firing rates of 30 Hz and below that have been consistently reported in studies of somatic dopaminergic neuron firing. In separate experiments, we tested evoked firing in the thin, highly branching striatal dopaminergic axons. We found that striatal dopaminergic axons could reliably fire action potentials above 100 Hz. Thus, this data demonstrates that action potential firing in axons can follow at rates that are significantly higher than those observed in the soma. We are currently testing whether propagation throughout the branching axonal arbor in striatum occurs reliably during firing rates that are seen during physiologically relevant tonic and phasic firing patterns.

Disclosures: **A. Sukharev:** None. **P.F. Kramer:** None. **R. Zhang:** None. **Z. Khaliq:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.03

Topic: B.08. Intrinsic Membrane Properties

Support: NSERC Discover Grant RGPIN-2019-04989

Title: Sex-specific electrophysiological and morphological characterization of regular-firing and burst-firing pyramidal neurons within layer 5 of the mouse prefrontal cortex

Authors: ***A. PATEL**, A. NGUYEN, C. D. BAILEY;
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Abstract: The medial prefrontal cortex (mPFC) plays an important role to support cognitive functioning. Pyramidal neurons within cortical layer 5 form the primary signalling output from this region. These neurons may be categorized as either regular-firing or initial burst-firing, based on threshold responses to positive current injection. These distinct firing patterns are believed to provide distinct contributions toward information transmission between brain

regions. The electrophysiological and morphological properties of these mPFC neurons have not been compared within developing male and female mice. We completed this comparison using whole-cell electrophysiology and biocytin neuron labelling in young postnatal mice (age 15-20 days) of both sexes. We first observed a striking sex difference, in that the proportion of regular-firing neurons was greater in males than in females. Within each sex, regular-firing neurons were generally more excitable than burst-firing neurons. They had a greater membrane resistance, spike adaptation ratio, and input-output firing response, in addition to a lower-magnitude afterhyperpolarization potential. Regular-firing neurons also exhibited a lower frequency of excitatory postsynaptic potentials (EPSCs). Detailed morphological analysis revealed a difference between neuron types in male mice only, such that regular-firing neurons had a greater amount of apical dendrite matter distal to the soma and a greater amount of basal dendrite matter proximal to the soma. Between the sexes, we found that burst-firing neurons had a greater amount of apical and basal dendrite matter in females than in males. Ongoing experiments aim to determine the projection targets for each type of pyramidal neuron within each sex. These novel findings demonstrate sex-differences in the proportion and morphology of regular-firing and burst-firing pyramidal neurons within mPFC layer 5, which may impact the development of cognitive circuits involving this brain region.

Disclosures: **A. Patel:** None. **A. Nguyen:** None. **C.D. Bailey:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.04

Topic: B.08. Intrinsic Membrane Properties

Support: NS077675

Title: Mild head trauma-induced impairment of functional efficacy of both L5 basket cells and chandelier cells

Authors: ***A. C. HARRIS, Jr.**, K. M. JACOBS, M. AFRIFA;
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Abstract: Alterations in the balance of excitation and inhibition within neocortical networks are consistent features of mild traumatic brain injury (TBI). Diffuse axonal injury is borne by neocortical parvalbumin+ interneurons (PV) at the same rate (~10%) as pyramidal neurons. PV interneurons contain both basket (BCs) and chandelier cells (ChCs), and the disruption of ChCs might catalyze the emergence of excitatory-inhibitory imbalance secondary to the release from inhibition of large numbers of pyramidal neurons at the axon initial segment. We labeled ChCs, by crossing Nkx2.1Cre^{ER} with Ai32 mice and administered 0.25 mg tamoxifen at P0. In a separate cohort Ai32 were crossed with PV-Cre mice to target BCs. Mice of each cohort were randomized to receive a sham or mild central fluid percussion injury (1.6-1.7 atm, righting time: 5-7 min). Results reported as mean ±SEM; t-tests were used to compare TBI with sham, with

$p<0.05$ considered significant. Neurons of each cohort were targeted for patch clamp recordings. Despite sharing classification as fast-spiking interneurons, the intrinsic membrane properties of BCs and ChCs differed on many metrics. The input resistance, the ratio of the height of the first to the second action potential (AP), the AP half-width, the total adaptation, and the after-hyperpolarization amplitude were significantly higher in ChCs compared to BCs, while the maximum AP frequency and rheobase were significantly lower in ChCs compared to BCs. In BCs, the AP half-width was significantly longer in TBI compared to sham (0.57 ±0.03 msec TBI; 0.44 ±0.02 msec sham, n = 9 TBI and 18 sham neurons), while the maximum firing frequency was significantly lower (188 ± 9.0 Hz TBI; 234 ±9.4 Hz sham). ChCs of L5 had a significantly lower maximum frequency in the TBI group compared to sham (119.5 ±12.0 Hz TBI; 164.2 ±10.6 Hz, sham, n= 5 TBI and 9 sham neurons). Furthermore, the relative decrement in AP amplitude throughout a train of APs was significantly larger in TBI compared to sham. There were no differences in the intrinsic properties of L2/3 ChCs in TBI compared to sham. These results suggest trauma-induced reduction of the functional efficacy of both L5 BCs and ChCs, which might favor a shift toward excessive excitatory tone within neocortical networks.

Disclosures: **A.C. Harris:** None. **K.M. Jacobs:** None. **M. Afrifa:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.05

Topic: B.08. Intrinsic Membrane Properties

Support: NIH/NIMH Grant U01MH114812

Title: Single-neuron models explain role of A-type potassium channels in response diversity of human supragranular pyramidal cells

Authors: ***T. CHARTRAND**, B. E. KALMBACH, A. NANDI, A. BUCHIN, J. BERG, S. A. SORENSEN, J. A. MILLER, J. T. TING, C. KOCH, E. S. LEIN, C. A. ANASTASSIOU; Allen Inst. for Brain Sci., Seattle, WA

Abstract: The study of human single neurons from surgically resected tissue has advanced dramatically in recent years, showing that human cortical neurons exhibit many unique physiological properties compared to their rodent counterparts. Because these findings are of potentially high relevance in understanding human brain circuits as well as the treatment of disease, linking observed differences to the underlying ion channel properties and gene expression is critical for understanding mechanisms and predicting effects of pharmacological interventions. Data-driven biophysical models are a valuable tool in the study of ion channel function, as detailed simultaneous recordings of distinct ion channels are possible only in silico. To fully realize this potential, neuronal models based on multi-modal single-cell data with accompanying gene expression are necessary. Based on recently published patch-seq data from human supragranular pyramidal cells, we developed detailed biophysical models of human single

neurons using a computational optimization pipeline with single-neuron electrophysiology and morphology as input.
We present evidence that the unique bursting or fast spike frequency adaptation dynamics observed at response onset in a subtype of deep layer 3 pyramidal cells in primate may be determined by A-type potassium channels. We provide support from both simulations of detailed biophysical models and data mining for correlations between ion channel genes and electrophysiology features. The correlation analysis points to a role for the K-channel subunit gene KCNAB1, which confers A-type fast inactivation to the composite channel . Fine-tuning the A-type conductance parameter in our models produces a close match to the experimental bursting dynamics. Recording and decomposition of the ion channel currents involved in these spike train simulations show that the faster A-type currents reduce the total K-current during spike repolarization, minimizing the size of the post-spike AHP and thus permitting faster reactivation of sodium channels early in the spike train. This adaptation permits rapid amplification at the onset of synaptic input, possibly reflecting a specialization of deep layer 3 cells to their predominantly feedforward connections in the cortical circuit.

Disclosures: **T. Chartrand:** None. **B.E. Kalmbach:** None. **A. Nandi:** None. **A. Buchin:** None. **J. Berg:** None. **S.A. Sorensen:** None. **J.A. Miller:** None. **J.T. Ting:** None. **E.S. Lein:** None. **C. Koch:** None. **C.A. Anastassiou:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.06

Topic: B.08. Intrinsic Membrane Properties

Support: JSPS KAKENHI 18K06514

Title: An experimental test for the ectopic origin of the 4-AP-induced burst of hippocampal mossy fibers

Authors: *H. KAMIYA;
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Abstract: The application of a potassium channel blocker 4-aminopyridine (4-AP) to the hippocampal slice preparation causes hyperexcitability and elicits robust burst discharges in the CA3 region. However, it remains to be determined how a blockade of voltage-dependent potassium channels by 4-AP leads to the generation of burst discharges. A previous study demonstrated that the blockade of potassium channels at the distal axons is sufficient for the generation of the burst discharges, and it suggested the ectopic origin of the burst discharges from the distal portions of the mossy fibers. To test for the burst generation of the mossy fibers from the distal axons, an attempt was made to disconnect the distal axons from the soma and the proximal axons. For this purpose, a surgical cut was made between the dentate gyrus and the CA3 region in the mouse hippocampal slices. The mossy fiber boutons discharged spontaneous

action potentials at low frequency in the control condition, possibly reflecting spontaneous discharges of the granule cells, while they were almost abolished after the surgical cut. The focal application of 100 μM 4-AP to the CA3 region elicited robust burst discharges of the mossy fiber boutons. After the surgical cut, on the other hand, the focal application of 4-AP did not evoke the burst firings of the mossy fiber boutons. These findings suggest that the 4-AP-induced bursts are generated from the distal axons of the mossy fibers, while the invasion of the propagating spontaneous action potentials from the soma is required for triggering the burst discharges from distal axons.

Disclosures:

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.07

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant R01 MH115832

Title: Cholinergic modulation of responses to depolarizing current ramps in CA1 hippocampal pyramidal neurons

Authors: *C. L. COMBE, C. C. CANAVIER, S. GASPARINI;
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Abstract: *In vivo* measurements show that CA1 place cell firing rates within the place field are influenced by novelty and previous exposure to the environment. Although synaptic plasticity likely contributes to modulation, the intrinsic properties of CA1 pyramidal neurons, such as adaptation, also influence the firing rate. To isolate these intrinsic contributions, we injected triangular depolarizing current ramps of various durations (1-10 s) to approximate the spatially-tuned, temporally-diffuse depolarizing synaptic input received by these neurons while traversing a place field. These ramps were applied to CA1 pyramidal neurons *in vitro* (slice electrophysiology, male rats, 7-10 weeks old) and *in silico* (multicompartmental model, see companion poster Upchurch et al). We found that under control conditions, CA1 neurons responded with more action potentials and at higher frequencies on the ascending phase than the descending phase of the depolarizing ramps. The cholinergic agonist carbachol (2 μM) caused the rate responses to shift, with more action potentials fired on the descending phase of the ramps. In order to tease out which aspects of cholinergic activation are responsible for this shift, we investigated the involvement of several currents associated with rate adaptation and known to be modulated by cholinergic activity. Pharmacological blockers of SK, M-type, A-type, and ERG currents did not shift the response, nor did blocking SK and M-type together. Flufenamic acid (FFA) is a wide-spectrum antagonist for the Ca2+-activated nonselective cationic current (ICAN) carried by TRP channels. When applied after carbachol, FFA was effective in reversing the effect of cholinergic activation. Further experiments with blockers specific for TRPC and

TRPM channels suggest that currents mediated by these channels are responsible for the increase in the firing rate on the down ramp in the presence of carbachol. In order to investigate cholinergic modulation in a more nuanced fashion, we looked at dose response effects of acetylcholine (ACh), the endogenous neuromodulator, on the spike ratio (spikes on the up-ramp/spikes on the down-ramp). We found a significant effect of ACh dose on the spike ratio (control 2.5 ± 0.4; 2 μM ACh 1.4 ± 0.1; 10 μM ACh 1.1 ± 0.1; 15 μM ACh 1.1 ± 0.1; $P<0.001$, n = 11). These results suggest that the extracellular concentration of ACh may be important in the modulation of firing rate responses within a cell's place field. Our results suggest that modulation of intrinsic ion channels by experience and novelty-related fluctuations in cholinergic activity may contribute to the shifts observed *in vivo* in peak firing rate as the place field is traversed.

Disclosures: C.L. Combe: None. **C.C. Canavier:** None. **S. Gasparini:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.08

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant R37AA009986
NIH Grant P50AA010761

Title: Ethanol decreases excitability of orbitofrontal cortex neurons via dopamine D1-like receptor activation of astrocytic glycine release

Authors: S. NIMITVILAI-ROBERTS, D. GIOIA, *J. J. WOODWARD;
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Abstract: Alcohol addiction is associated with dysfunction of brain circuitry including areas of the orbitofrontal cortex (OFC). Recent findings from this laboratory demonstrate that ethanol reduces the intrinsic excitability of OFC neurons in naïve mice via activation of strychnine-sensitive glycine receptors. Withdrawal from chronic intermittent ethanol exposure enhances current-evoked OFC firing, and diminishes acute ethanol inhibition of spiking by blunting its activation of a glycine receptor-dependent tonic current. Although the mechanism linking ethanol to the release of glycine is currently unknown, several studies have identified astrocytes as a source of neurotransmitters including glycine. Furthermore, dopamine has been reported to cause a functional reversal of the astrocytic glycine transporter 1 (GlyT1) via Gq-coupled receptor activation of PLC/PKC pathway resulting in elevated extracellular glycine levels. We reported previously that like ethanol, dopamine increased a glycine-mediated tonic current in lOFC neurons. In this study, we used whole-cell patch-clamp electrophysiology to examine whether ethanol inhibition of OFC spiking involves the release of glycine from astrocytes and whether this release is dopamine receptor dependent. Ethanol, applied acutely, decreased spiking of lOFC neurons and this inhibition was blocked by a non-transportable GlyT1 blocker,

monoamine transporter inhibitors and a D1 but not D2 receptor blocker. Ethanol enhanced a tonic current in lOFC neurons with no further increase following subsequent exposure to dopamine suggesting that ethanol and dopamine may share a common pathway. Altering astrocyte function by inhibiting glial metabolism or by suppressing intracellular astrocytic calcium signaling decreased, but did not fully eliminate ethanol inhibition of lOFC neuron firing. Likewise, blocking the astrocyte-specific Kir4.1 channel, known to induce depolarization of astrocytes and reversal of GlyT1, reduced but did not abolish ethanol inhibition of OFC firing. However, when both astrocytic calcium signaling and Kir4.1 channels were suppressed, ethanol no longer inhibited lOFC neuron firing. Ethanol inhibition of lOFC neuron firing was also prevented in slices treated with inhibitors that target phospholipase C or conventional isoforms of protein kinase C. Finally, using sharp electrodes to record from astrocytes, we found that bath application of a Kir4.1 blocker, D1 agonist or ethanol depolarized OFC astrocytes. We propose that acute ethanol induces a GlyT1-dependent release of astrocytic glycine via activation of D1-like receptors and subsequent inhibition of Kir4.1 channels.

Disclosures: S. Nimitvilai-Roberts: None. **D. Gioia:** None. **J.J. Woodward:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.09

Topic: B.08. Intrinsic Membrane Properties

Support: DARPA Contract Nº N6523619C8017

Title: Qualitative Examination Of Temporal Interference Mechanisms For Cortical Neurons

Authors: *S. C. MARTINEZ[1], P. GROVER[1], A. L. BARTH[2];
[1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Carnegie Mellon U., Pittsburgh, PA

Abstract: Motivation: Temporal Interference (TI) stimulation has been proposed as a neuromodulation technique for the non-invasive stimulation of deep brain regions, without causing shallow stimulation. This technique consists of an interplay between two high-frequency sinusoidal waves with a small difference in frequencies. Localized wave summation of these sinusoids can drive neural activity, where a single sinusoid application is insufficient. The mechanisms underlying TI stimulation phenomenon are still not well understood and more experimental studies, hand-in-hand with modelling, are needed. Methods/approach: In this experimental study, we qualitatively evaluate the efficacy of TI stimulation for cortical neurons. We performed whole-cell current-clamp recordings in acute brain slices from the mouse cortex (postnatal day (P18)). Waveforms were applied in kHz frequency range using a bipolar probe, maintaining a distance of approximately 100 um from the patched neuron. A cell was said to exhibit TI stimulation if it was stimulated by the summation of two sinusoids of slightly different kHz frequency and equal amplitude, but not by a pure sinusoid of equal kHz carrier frequency and twice the amplitude. Results: We observe that cortical neurons can exhibit TI stimulation

Fig.1. Furthermore, consistent with recent predictions in [Cao et al.,'20], our exploratory results suggest that a) higher current amplitudes are required to observe TI stimulation when the sinusoidal carrier frequencies are increased. b) an increase of the carrier frequency provides more reliable TI stimulation. Early results on effects of modulation frequency are also reported. Conclusions: We provide TI experimental validation in cortical neurons and provide some insights in the amplitudes and frequencies required to produce such results. Further exploration of cell-types would be insightful to gain a better understanding of how TI stimulation could affect different brain regions.

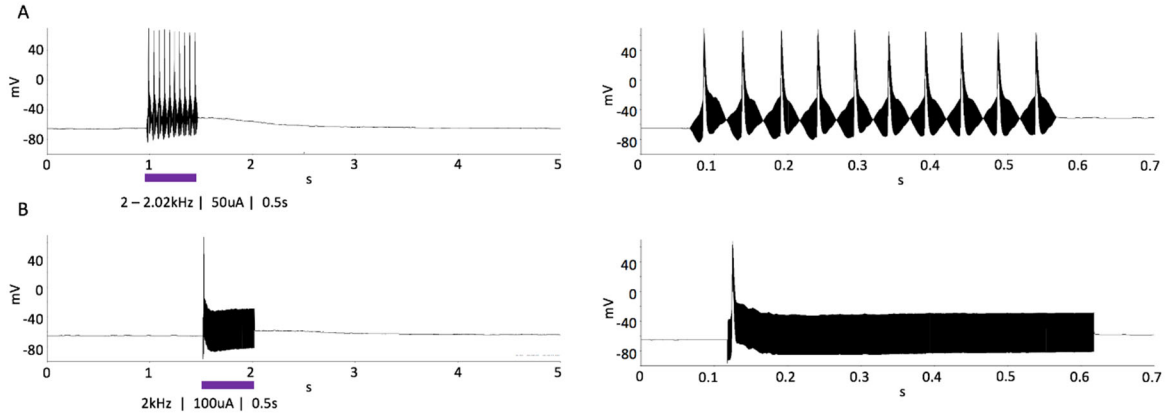

Figure 1. Temporal Interference Stimulation in a Cortical Pyramidal Neuron. (A and B) Example trace of a whole-cell current-clamp recoding in a L2-3 cortical pyramidal neuron of a mouse brain slice (A); left, applied the summation of two sinusoids of 50 uA each and 2-2.02kHz respectively with a total duration of 0.5s (purple waveform representation); right, magnified view of the left example trace. trace (B); left, applied one sinusoid of 100uA and 2kHz with a total duration of 0.5s (purple waveform representation); right, magnified view of the left example trace.

Disclosures: S.C. Martinez: None. **P. Grover:** None. **A.L. Barth:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.10

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant RF1MH124909

Title: Tacs induces neural entrainment in neocortical neurons models

Authors: *H. TRAN, S. SHIRINPOUR, A. OPITZ;
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Abstract: Transcranial alternating current stimulation (tACS) is a non-invasive neuromodulation method which can directly modulate the ongoing activity through neural entrainment, i.e. the firing activity of neurons is synchronized with the external oscillations. tACS thus holds promise as a new treatment option for neuropsychiatric disorders characterized by pathological brain oscillations. However, neural responses to oscillating electric fields depend on the intrinsic

properties of cells and have to be carefully characterized. Here, we perform a computational study to investigate the relationship between electric field strength and neural entrainment in morphologically realistic neocortical neurons. For this, we use cable theory together with proper biophysics to model morphologically realistic models of neurons with synaptic input to create ongoing spiking activity. We explored the effect of different tACS electric field strengths (10Hz) by investigating the subthreshold neuron depolarization - we computed the polarization length - for each cell type and then quantifying the neural entrainment by computing the phase-locking value (PLV) of spikes times across neurons and field strengths. Due to their morphology, pyramidal cells tend to have a larger polarization length resulting in higher phase-locked spiking to the applied oscillation. Both pyramidal cells and interneurons did not show a significant increase in their firing rate for tACS amplitudes achievable in humans. Our results are consistent with experimental literature and can further help to distinguish the effects of oscillating electric fields on single neurons and their spiking activity. Future steps will include to model the effect of tACS on neuronal populations. Modifying the dynamics of the network will allow us to reproduce specific spiking pattern and to better understand how tACS can affect them.

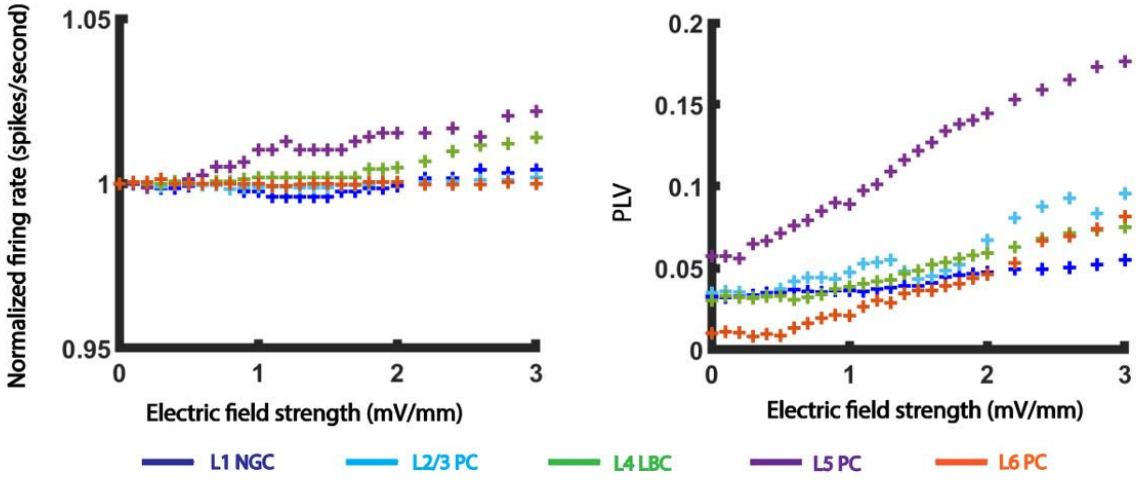


Figure. Left. *Normalized firing rate. All the cells exhibited a light increase in the firing right compared to the baseline* ***Right.*** *Pyramidal cells exhibited a stronger PLV compared to the interneurons with the electric field strength.*

Disclosures: H. Tran: None. **S. Shirinpour:** None. **A. Opitz:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.11

Topic: B.08. Intrinsic Membrane Properties

Support: CIHR

Title: Regional phase amplitude coupling modulation in response to localized subcutaneous alternating current stimulation

Authors: *M. BELL VILA[1], P. BAZIGALUPPI[2], M. KOLETAR[2], B. STEFANOVIC[2,1];
[1]Univ. of Toronto, Toronto, ON, Canada; [2]Sunnybrook Res. Inst., Toronto, ON, Canada

Abstract: Subcutaneous alternating current stimulation (sACS) is a method of electrical stimulation in which the current is applied as a wave, oscillating in amplitude at a given frequency. It has been shown to have the potential to entrain cortical networks, and be localized via targeted placement of stimulating electrodes. Further, previous studies indicate low frequency to high frequency phase amplitude coupling (LF-HF PAC) is correlated with neurological disability in numerous diseases and has been linked to the degree of motor dysfunction in Parkinson's disease (Devergnas et al., 2019). We here investigated the potential of LF-HF PAC as a reporter of disability in stroke and its amenability to modulation via sACS. To determine the feasibility of localized sACS modulating LF-HF PAC, we delivered stimulation through 3-electrode arrays positioned on the skulls over the sensorimotor cortex of 5 naive six-month old Sprague-Dawley rats, while simultaneously recording electrophysiological responses via an intracerebral electrode array. sACS was delivered at high (120Hz) and low (1Hz) frequencies at a 20µA amplitude. Stimulation was administered for a duration of 1 minute. LF-HF PAC was evaluated using an algorithm by Tort et al. (2010). We observed an LF-HF PAC response between pairs of stimulating electrodes while leaving distal regions unaffected. High frequency stimulation resulted in a suppression of neuronal power and LF-HF PAC, with opposite observed effects for low frequency stimulation. These findings demonstrate the possibility of delivering localized sACS while measuring regional LF-HF PAC responses, as well as a frequency dependent attenuation or potentiation effect on neuronal activity.

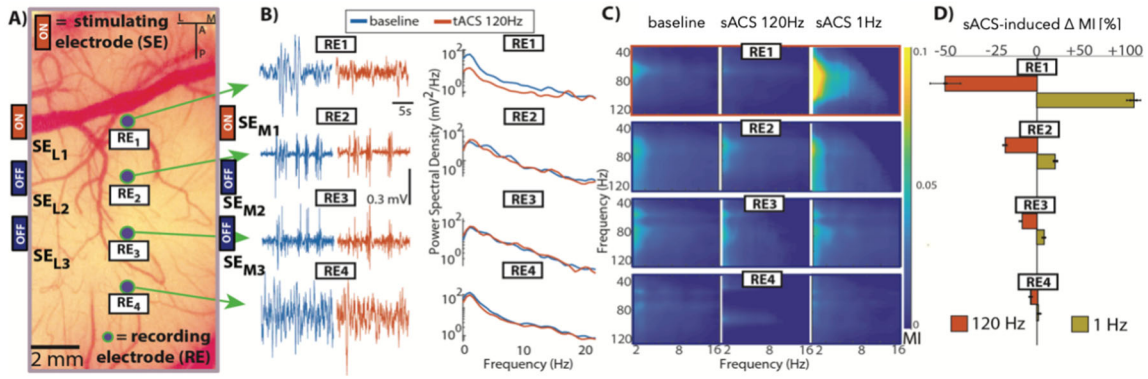


Fig 1. sACS induces frequency-dependent modulation of LF-HF PAC and neuronal power (120-Hz eliciting attenuation and 1-Hz, potentiation), measured from recording electrodes (RE). The use of a stimulating electrode (SE) array permits localization of sACS effects in naive rats.

Disclosures: **M. Bell Vila:** None. **P. Bazigaluppi:** None. **M. Koletar:** None. **B. Stefanovic:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.12

Topic: B.08. Intrinsic Membrane Properties

Support: National Health and Medical Research Council Grant APP1144145
Australian National University

Title: Er81 transcription factor fine-tunes striatal cholinergic interneuron activity and drives habit formation

Authors: Y. RANJBAR-SLAMLOO, ***N. Y. AHMED**, A. S. AL ABED, L. GAO, Y. SONTANI, A. RCOM-H'CHEO-GAUTHIER, E. ARABZADEH, N. DEHORTER;
Eccles Inst. of Neurosci., John Curtin Sch. of Med. Research, Australian Natl. Univ., Canberra, Australia

Abstract: The molecular mechanisms tuning cholinergic interneuron (CIN) activity, although crucial for striatal function and behaviour, remain largely unexplored. Previous studies report that the Etv1/Er81 transcription factor is vital for regulating neuronal maturation and activity. Whilst Er81 is known to be expressed in the striatum during development, its specific role in defining CIN properties and the resulting consequences on striatal function is unknown. We report here that Er81 is expressed in CINs and its specific ablation leads to prominent changes in their molecular, morphological and electrophysiological features. In particular, the lack of Er81 amplifies intrinsic delayed-rectifier and hyperpolarization-activated currents, which subsequently alters the tonic and phasic activity of CINs. We further reveal that Er81 expression is required for normal CIN pause and time-locked responses to sensorimotor inputs in awake mice. Overall, this study uncovers a new cell-type specific control of CIN function in the striatum which drives habit formation in adult male mice.

Disclosures: Y. Ranjbar-Slamloo: None. **N.Y. Ahmed:** None. **A.S. Al Abed:** None. **A. RCom-H'cheo-Gauthier:** None. **Y. Sontani:** None. **L. Gao:** None. **E. Arabzadeh:** None. **N. Dehorter:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.13

Topic: B.08. Intrinsic Membrane Properties

Support: NIH U10 AA008401

Title: Kcnj6 gene variants associated with alcohol use disorder increase excitability in a human ipsc-derived neuron model.

Authors: *D. POPOVA[1], M. YOUSSEF[2], P. ZALAMEA[2], J. A. TISCHFIELD[1], P. A. SLESINGER[3], Z. PANG[4], R. P. HART[2];
[1]Human Genet. Inst. Rutgers Univ., Piscataway, NJ; [2]Rutgers Univ., Piscataway, NJ; [3]Professor, Dept. of Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY; [4]Child Hlth. Inst., New Brunswick, NJ

Abstract: Alcohol use disorders (AUDs) are leading contributors to preventable mortality and morbidity worldwide. A family study identified genome-wide association of theta event-related oscillations, observed in alcoholics and in their offspring, with single nucleotide polymorphisms (SNPs) in *KCNJ6*, which encodes the inwardly-rectifying potassium channel GIRK2. We hypothesize that alcoholism-associated *KCNJ6* SNPs affect excitability of neurons, altering their response to alcohol. To test this hypothesis we prepared 8 human iPSC lines (4 affected/alcoholics/major allele and 4 unaffected/non-alcoholics/minor allele, sex balanced) from the Collaborative Study on the Genetics of Alcoholism repository collection and generated glutamatergic neurons using Ngn2 induction. Morphological and electrophysiological properties of neurons were examined in parallel with cytochemical detection of GIRK2 expression. We found that basal morphology (soma size, solidity and circularity) and electrophysiological properties (capacitance, resistance, release of glutamate, resting membrane potential and basal firing) of neurons generated from major and minor allele carriers were unchanged. However, affected minor allele carriers show a reduction in punctate GIRK2 expression, detected in presynaptic compartments of neurons, which was paralleled by increased excitability of the neurons measured in action potential induction protocol. Furthermore, even though chronic alcohol treatment affects excitability of the neurons, the treatment eliminates most differences between affected and unaffected groups/individuals. Single-cell RNAseq also identifies that *KCNJ6* minor allele neurons exhibit decreased expression of pathways associated with neuronal and synaptic function. The original KCNJ6 minor allele-associated phenotype, higher theta power in a monetary gambling task, predicts that affected subjects may exhibit increased neural excitability and reward processing, with modulation by alcohol. We conclude that there are physiological differences between neurons from affected and unaffected subjects associated with excitability, possibly due to altered GIRK2 expression, and that alcohol “rescues” excess excitability.

Disclosures: D. Popova: None. **M. Youssef:** None. **P. Zalamea:** None. **J.A. Tischfield:** None. **P.A. Slesinger:** None. **Z. Pang:** None. **R.P. Hart:** None.

Digital Abstract Session

P067. Neuronal Firing Properties: Control, Modulation and Molecular Mechanisms

Program #/Poster #: P067.14

Topic: B.08. Intrinsic Membrane Properties

Title: Mathematical simulation predicts regulation of neuronal excitability by changes in the membrane capacitance

Authors: *E. PETROFF, V. SNITSAREV;
Montclair State Univ., Montclair, NJ

Abstract: Mathematical modeling of experimentally obtained action potentials (APs) provides a powerful tool for understanding the intrinsic mechanisms of AP generation and regulation of excitability, as well as helps to formulate further hypotheses to be tested experimentally. In our model of an A-type neuron [https://www.mathworks.com/matlabcentral/fileexchange/18812-nodose-neuron-action-potential-firing], the resting membrane potential (MP) settles to -56 mV. In response to progressively increasing current injections from 0.025 to 0.045 nA with 0.005 nA increments, the model responds with generating 0, 1, 2, 4, and 6 APs, and adapts within 1 second to MPs of -48, -46.4, -44.6, -42.6, and -40.5 mV, respectively. The model behaves similar to an A-type neuron. For example, eliminating the fast tetrodotoxin-sensitive voltage-gated Na^+ channel leads to elimination of APs. *In situ*, membrane capacitance (Cm) increases upon caveolae or synaptic vesicle opening. Changes in Cm can potentially affect excitability. We tested how changes in Cm affect excitability in this model. An increase in Cm by 10% from its default value of 32.5 pF results in 0, 1, 2, 2, and 3 APs and following adaptation to -48, -46.4, -44.6, -42.6, and -40.3 mV in response to 0.025, 0.03, 0.035, 0.04 and 0.045 nA current injections, respectively. A decrease in Cm by 10% from its default value of 32.5 pF results in 0, 1, 4, 10 APs and adaptation to -48, -46.4, -44.6, and -42.8 mV in response to 0.025, 0.03, 0.035, and 0.04 nA current injection, respectively; while in response to 0.045 nA a non-adapting train of APs at 25 Hz was elicited. At 0.4 nA current injection, a minimum decrease in Cm by 2.5% was required to add one AP to the adapting train of four APs, and a minimum increase by 2% was required to eliminate one AP from the adapting train of four APs. Notably, these changes in excitability were not directly related to the changes in the level of depolarization. Based on this simulation, we hypothesize the following. First, fusion of synaptic vesicles increases Cm in the presynaptic terminal and could decrease AP firing to provide the local negative-feedback regulation of the synaptic release. Then, upon synaptic vesicle internalization that decreases Cm, the local excitability could increase to potentiate the following synaptic release. Second, a decrease in Cm due to a decrease in the neuronal surface area increases neuronal excitability to the point of making a neuron spontaneously active at the resting membrane potential (model of epilepsy). Conversely, an increase in Cm due to an increase in the neuronal surface area, makes a neuron less excitable (depression). We are currently testing these hypotheses experimentally.

Disclosures: E. Petroff: None. **V. Snitsarev:** None.

Digital Abstract Session

P068. Oscillations and Synchrony: EEG Studies

Program #/Poster #: P068.01

Topic: B.09. Network interactions

Support: Fondecyt 1170027

Title: Envelope analysis of the human alpha rhythm

Authors: *V. M. HIDALGO[1], J. DIAZ[2], J. MPODOZIS[1], J. C. LETELIER[1];
[1]Biol. of Cognition Lab., Univ. of Chile, Santiago, Chile; [2]Intl. Inst. for Integrative Sleep Med., Univ. of Tsukuba, Tsukuba, Japan

Abstract: The origin of human alpha rhythm has been a matter of debate since Lord Adrian attributed it to synchronous neural populations. Although some authors reported Gaussian characteristics for the alpha band, their EEG Gaussianity results were disregarded in favor of Adrian's explanation. We revisit this problem using the EEG envelope analysis (envEEG) − a method relying on the fact that the coefficient of variation of the envelope (CVE) for continuous-time zero-mean Gaussian white noise (as well as for any filtered sub-band) is equal to $\sqrt{((4-\pi)/\pi)} \approx 0.523$, thus making the CVE a fingerprint for Gaussianity (Diaz et al. 2018, DOI:10.1016/j.neuroimage.2018.01.063). The CVE of sampled and finite zero-mean Gaussian white noise, however, is a random variable whose probability distribution must be estimated by numerical simulations and three CVE classes are established: low (CVE<1%), mid (1%≤CVE≤99%), and high (99%<CVE). Low CVE values denote rhythmic signals, consistent with synchronization of coupled oscillators, while medium CVE values represent Gaussian noise. High CVE values represent pulsating activity, consistent with concurrent neuronal activity. We analyzed the "Leipzig Study for Mind-Body-Emotion Interactions" dataset, containing EEG data from 203 subjects with envEEG. Each subject's continuous recording consists of 16 blocks of 60[s], 8 with eyes-closed (EC) and 8 with eyes-open (EO) interleaved. We filtered the data in the alpha band (8-13[Hz]) and segmented it in 24[s] epochs with 50% overlap. For each epoch, we calculated a) the envelope using the Hilbert Transform b) the CVE and c) the mean of the envelope (ME) as a measure of total energy. We analyzed 22893 EC and 22634 EO epochs and found 44% of EC epochs were in the Gaussian class, while 9% and 46% presented low and high CVE values, respectively. For EO epochs, 55% were Gaussian, 43% showed high CVE values and only 0.6% presented low CVE values. When ME values from each individual recording were log-transformed and normalized, the overall positive change in energy from EO to EC was about 2 standard deviations regardless of initial CVE values thus recuperating, within the perspective of envEEG, the classic result about alpha power and visual attention. Fourier analysis showed that epochs from all three CVE classes have the canonical spectral peak at ≈10[Hz] demonstrating that this same peak could be produced by rhythms, pulsating ripples or Gaussian noise. This technique opens a new facet for neural signal analysis relating temporal modulations (CVE) to signal energy.

Disclosures: V.M. Hidalgo: None. **J. Diaz:** None. **J. Mpodozis:** None. **J.C. Letelier:** None.

Digital Abstract Session

P068. Oscillations and Synchrony: EEG Studies

Program #/Poster #: P068.02

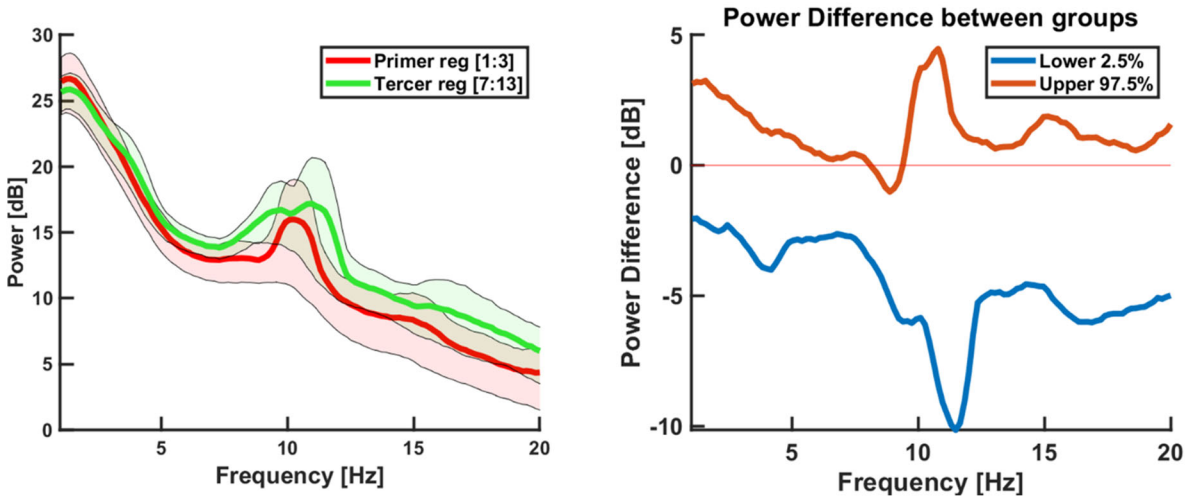
Topic: B.09. Network interactions

Support: FONDEF ID19I10345
ICN09_015

Title: Effect of repeated exposure to Sevoflurane on physiologic and electroencephalographic features in pediatric patients undergoing radiation therapy

Authors: S. MADARIAGA[1,2], *C. DEVIA[6,1], V. LUCERO[7], S. RAMIREZ[7], M. GANGA[7], N. VALLS[7], R. TORRES[7], N. VILLABLANCA[7], J. EGAÑA[3,4], F. MALDONADO[3], A. PENNA[3,5], R. GUTIERREZ[3,5];
[1]Biomed. Neurosci. Inst., Santiago, Chile; [2]Dept. of Neurosci., [3]Dept. of Anesthesia and Perioperative Medicine, Clin. Hosp., [4]Biomed. Neurosci. Inst., [5]Cica, Univ. de Chile, Santiago, Chile; [6]Dept. of Neurosci., Univ. De Chile, Santiago, Chile; [7]Inst. Nacional del Cancer, Santiago, Chile

Abstract: Repeated exposure to drugs, such as opioids or benzodiazepines, leads to pharmacological tolerance. However, it has not been established whether the repeated exposure to sevoflurane, an inhalational anesthetic used especially in children, induces pharmacological tolerance. Here, we conducted an observational study in infants (n=11) requiring general anesthesia for radiotherapy treatment under a standardized protocol. Only sevoflurane was administered for Induction (8%) and Maintenance (2.5%) throughout all radiotherapy sessions. We record the "Induction time", defined as the time between the beginning of the anesthetic administration and the insertion of the laryngeal mask once the unconsciousness is established. Every third session we recorded electroencephalographic signals with 4 frontal electrodes. We performed power spectral analysis over cleaned electroencephalographic windows recorded during Maintenance. To compare spectral power through radiotherapy sessions we used a multitaper frequency-domain bootstrap method. Based on this statistical we performed a pot-hoc paired t-test. Throughout sessions, we found no differences in the Induction time. Our preliminary results show an attenuation in alpha-band activity through sessions. Further paired comparison showed a significant decrement in alpha-band power between the first and the last recorded session. Overall, our results suggest that repetitive exposure to sevoflurane does not induce tolerance. (Funds: FONDEF ID19I10345, ICN09_015)



Disclosures: **S. Madariaga:** None. **C. Devia:** None. **V. Lucero:** None. **S. Ramirez:** None. **M. Ganga:** None. **N. Valls:** None. **R. Torres:** None. **N. Villablanca:** None. **J. Egaña:** None. **F. Maldonado:** None. **A. Penna:** None. **R. Gutierrez:** None.

Digital Abstract Session

P068. Oscillations and Synchrony: EEG Studies

Program #/Poster #: P068.03

Topic: B.09. Network interactions

Support: FONDEF ID19I345
Milenio Scientific Initiative ICN09_015 (Biomedical Neuroscience Institute))

Title: Remifentanil enhances propofol hypnotic clinical and electroencephalographic effect in humans during loss of consciousness

Authors: B. ABURTO[1], C. F. NAVARRO[1], *J. I. EGANA[2], F. MALDONADO[2], C. DEVIA[1], R. GUTIERREZ[2], A. PENNA[2];
[1]Dept. de Neurociencias, [2]Dept. de Anestesiología y Medicina Perioperatoria, Univ. de Chile, Santiago, Chile

Abstract: Introduction:
General anesthesia is a reversible pharmacological state in which patients are unconscious making surgery possible. As part of balanced general anesthesia, it is common to use the synergy between the hypnotic (propofol) and the opioid (remifentanil) to achieve the desired clinical effect. However, the electroencephalographic correlate of this synergy has not been fully studied.
Methods:
Here, we registered patients during anesthesia induction with a 4-frontal electrodes EEG monitor using two protocols. 1) PROPO (n=10): propofol infusion until Loss of Consciousness (LOC). 2) REMI-PROPO (n=10): remifentanil and propofol infusion until LOC. The EEG spectrum was obtained for both protocols during all induction until after LOC. We compared the spectra from both groups using bootstrapping analysis.
Results:
•PROPO patients required higher propofol dose to achieve LOC compared to REMI-PROPO.
•REMI-PROPO patients have lower spectral alpha power before LOC, and lower delta power after LOC than patients in PROPO protocol.
•When PROPO had the same propofol concentration of REMI-PROPO after LOC onset, PROPO had lower alpha power compared to REMI-PROPO.
Discussion:
•Remifentanil enhances propofol hypnotic clinical effect, which is correlated with electroencephalographic activity.
•Electroencephalographic synergy persists once the LOC is reached, evidenced by a higher delta power in those patients who only reach LOC with propofol, which is consistent with the higher concentration of propofol required to reach LOC.

•Lower alpha power in PROPO at the same dose of propofol in REMI-PROPO is consistent with the fact that PROPO patients have not reached the LOC at this dose.

Disclosures: B. Aburto: None. **C.F. Navarro:** None. **J.I. Egana:** None. **F. Maldonado:** None. **C. Devia:** None. **R. Gutierrez:** None. **A. Penna:** None.

Digital Abstract Session

P068. Oscillations and Synchrony: EEG Studies

Program #/Poster #: P068.04

Topic: B.09. Network interactions

Support: NIH/NIGMS funding: R01 GM109086

Title: Electrophysiological correlates of acute psilocybin in mice

Authors: *Z. W. SULTAN[1], S. M. GRADY[1], H. A. BASTIEN[1], Z. ZAHID[2], C. J. WENTHUR[3], M. I. BANKS[1];
[1]Anesthesiol., [2]Neurosci. Training Program, [3]Sch. of Pharm., Univ. of Wisconsin, Madison, WI

Abstract: Introduction: Emerging evidence indicates that a single dose of the classical psychedelic psilocybin (PSIL) is effective as rapid and prolonged treatment for multiple psychiatric disorders, including depression, anxiety, and substance use disorder, but the neural mechanisms underlying these therapeutic effects are unclear. In human subjects, PSIL's therapeutic effects correlate with the intensity of the psychedelic experience. Effects on brain oscillations and signal complexity also correlate with psychedelic phenomenology, and are blocked by the serotonin 5-HT2A receptor antagonist ketanserin (KTN). Studies in rodents suggest a role as well for 5-HT1A receptors, though results have been inconsistent. We seek to link neural signatures of PSIL to effects on depression-like and anxiety-like phenotypes in mouse models. As a first step, we measured the effects of PSIL, in combination with 5-HT receptor antagonists, on resting-state brain activity in healthy mice.
Methods: We recorded skull-screw EEG, movement, and head-twitch responses in C57BL/6 mice (male & female, aged 2-8 months old) at baseline and up to 4 hours after intraperitoneal injections of vehicle (VEH; n=8 animals), 3mg/kg PSIL (n=13), PSIL + 1mg/kg KTN (n=9), and PSIL + 0.5mg/kg 5-HT1A receptor antagonist WAY100635 (WAY; n=6). EEG band power and Lempel-Ziv complexity (LZcn) were calculated at baseline and treatment. To avoid confounds due to drug-induced changes in movement, which alter EEG power spectra, propensity score matching was used to match distributions of movement magnitude between baseline and treatment periods. Band power was then compared for comparable movement distributions.
Results: PSIL increased delta (0.5-4Hz) and decreased alpha (13-20Hz) band power during the first hour post-injection. After accounting for spectral changes, increased LZcn was observed at 15-30 minutes after PSIL injection. Pretreatment with KTN decreased the peak effects of PSIL on delta, alpha, and LZcn, while WAY shortened the duration of PSIL's effects without altering peak response.

Conclusions: Our analysis of brain activity in mice on PSIL recapitulates previous observations in humans, and suggests contributions of multiple 5-HT receptors. Future work will explore neural effects in models of depression and anxiety.

Disclosures: **Z.W. Sultan:** None. **S.M. Grady:** None. **H.A. Bastien:** None. **Z. Zahid:** None. **C.J. Wenthur:** None. **M.I. Banks:** None.

Digital Abstract Session

P068. Oscillations and Synchrony: EEG Studies

Program #/Poster #: P068.05

Topic: B.09. Network interactions

Support: NIH Grant 1R21MH112019-01A1 to SP and AAB
Elizabeth H. Solomon Center for Neurodevelopmental Research
NSF #SMA-1041755 to the Temporal Dynamics of Learning Center (TDLC), an NSF Science of Learning Center, to AAB

Title: Changing characteristics of infant sleep spindles at 9 months contribute to spectral power in a sex dependent manner

Authors: *S. E. PETERS, A. A. BENASICH;
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Abstract: Sleep spindles (SS) are neurophysiologic components of brain maturation thought to be associated with neuroplasticity, network development and refinement, and information processing. In adults, there is increasing evidence for sexually dimorphic characteristics of sleep spindles, which may correlate with cognitive function. SS are thought to be a better correlate of cognition in women, given that spindle amplitude correlates strongly with white matter tracts, and white matter is a better correlate of intellectual performance in women than in men. SS undergo characteristic changes across the lifespan but at the fastest rate during infancy, a time when rapid myelination is occurring and thus may serve as early biomarkers for neurodevelopmental disorders, which are more prevalent in males. To explore the potential role of SS as cognitive biomarkers and examine possible sex differences, we conducted a dEEG study of daytime naps in typically developing infants, concurrent with behavioral measures. The participants were part of a larger study, covering infants across the first year of life, including both cross-sectional and longitudinal groups. The current dataset includes both groups, at the 9-10 month age point. We extracted SS using a Matlab toolbox, allowing us to look at spindle characteristics including spectral power, peak frequency, globality, spindle length (i.e. duration), and spindle incidence (i.e. density). While controlling for age in days, we found strong correlations between some of these characteristics, which differed in a sex dependent manner. In our sample of 9-10 month-olds (n=42, f=26) spindle spectral power is correlated with globality, and spindle length. Spectral power is also correlated with peak frequency, but only in males. Globality is correlated with peak frequency in the combined group. Spindle length is strongly

correlated with peak frequency and globality across groups, with males having higher peak frequency (t=-2.378, p=.027) and longer duration (t=-2.329, p=.025). Hormonal influence, myelination patterns, and maturational spindle topography are all factors that may contribute to these sex differences. In summary, sleep spindles that occur more globally have a higher peak frequency and higher spectral power. Spectral power and frequency are correlated in males at 9 months, but not in females. Spindle duration is a strong correlate of globality, peak frequency, and spectral power at 9 months, with males having higher peak frequency and longer duration spindles. These characteristics may prove to be biomarkers for maturational patterns of myelination and thalamocortical and cortico-cortical networks.

Disclosures: S.E. Peters: None. **A.A. Benasich:** None.

Digital Abstract Session

P068. Oscillations and Synchrony: EEG Studies

Program #/Poster #: P068.06

Topic: B.09. Network interactions

Support: NIH/NIBIB (P41-EB018783, R01-EB026439)
NIH/NINDS (U01-NS108916 and U24-NS109103)
NIH/NIMH (P50-MH109429)
Fondazione Neurone
National Natural Science Foundation of China (51620105002)
Science and Technology Commission of Shanghai Municipality (18JC1410400)
Shanghai Municipal Science and Technology Major Project
(No.2018SHZDZX01) and ZJLab

Title: Propagation of Slow Wave Activity across the Primate Cortex under Propofol-Induced Unconsciousness

Authors: *T. XIE[1,3,4], Z. WU[5], X. ZHU[3], X. SHENG[3], L. CHEN[5], P. BRUNNER[4,6,2]; [2]Dept. of Neurosurg., [1]Washington Univ. Sch. of Med., St. Louis, MO; [3]State Key Lab. of Mechanical Syst. and Vibration, Shanghai Jiao Tong Univ., Shanghai, China; [4]Natl. Ctr. for Adaptive Neurotechnologies, Albany, NY; [5]Dept. of Neurosurg., Huashan Hospital, Fudan Univ., Shanghai, China; [6]Dept. of Neurol., Albany Med. Col., Albany, NY

Abstract: Slow waves are large-scale electrophysiological signals observed throughout the brain during loss of consciousness (LOC) in the course of anesthesia, non-REM sleep, and coma. These waves arise from the synchronization of slow oscillations in the membrane potentials of millions of neurons and have been suggested as the default emergent activity of the underlying networks. The characteristics of slow wave activity can be shaped by variations of physiological parameters and thus provide information about the underlying neural network. Studies suggest that slow wave activity travels across the cortex. However, these studies were limited to single oscillation cycles, i.e., the transition from the downstate to the upstate, and did not consider the

entire oscillation period. Consequently, the spatiotemporal dynamics with which slow waves propagate across the cortex are not fully understood. Our study aims to obtain this understanding by analyzing slow wave activity throughout the transition period between awake and LOC.To achieve this, we analyzed electrocorticographic (ECoG) signals from the lateral surface of two resting monkeys that underwent Propofol-induced LOC. In our analysis, we were interested in determining the spatiotemporal dynamics of the slow wave activity throughout the different stages of the Propofol-induced LOC. To extract the slow wave activity, we calculated the cross-frequency modulation coupling between slow wave oscillations (0.5-4 Hz) and broadband gamma activity (55-145 Hz). Our results show that the slow wave activity exhibits two distinct stages as it travels through the entire hemisphere throughout the Propofol-induced anesthesia. The first stage (STAGE-I) encompasses an approximately one-minute-long period following the Propofol injection. The spatial dynamics of this period resemble those of previously reported default networks, which are known to have vast anatomical connections through the brain, and are considered to relate to higher-order functions. The second stage (STAGE-II) encompasses the approximate 5-20 minutes after Propofol injection. In this period, the spatiotemporal dynamics of the slow wave activity exhibit the propagation of the Propofol's effect on individual brain regions. In this, the slow wave preferentially originated from the lateral sulci and anterior cingulate gyrus, and propagated along the anterior-posterior direction. In summary, these results shed some light on the propagation of slow wave activity throughout the cortex. This understanding could lead to new diagnostic methods that characterize pathologies directly from the brain's slow wave activity.

Disclosures: **T. Xie:** None. **P. Brunner:** None. **Z. Wu:** None. **X. Zhu:** None. **X. Sheng:** None. **L. Chen:** None.

Digital Abstract Session

P069. Oscillations and Synchrony: LFP Studies

Program #/Poster #: P069.01

Topic: B.09. Network interactions

Title: Eeg phenotyping as a tool to characterize preclinical rodent models of brain disorders

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Abstract: The development of new neurotherapeutics has been facing a tremendous challenge for over a decade. Several promising drug candidates for brain disorders indeed fail too late in the drug development process, most of the time for lacking effectiveness. Finding the most relevant pathological model as well as translational read-outs very early on, count among the biggest hurdles to overcome in CNS drug development. In addition, behavior, or imaging assays remain preferred techniques to investigate drug candidates' efficacy on preclinical animal models, despite limitations for translational validity assessment. In this work, we took advantage

of electroencephalography (EEG) to give a direct access to brain function with high time resolution and a great sensitivity. Indeed, neuronal network oscillations are highly conserved across mammals, which makes EEG a translational brain monitoring technique that bridges the gap between preclinical research and clinical outcomes when it comes to the development of new neurotherapeutics. The aim of this communication is to show how EEG and its related methodologies can be used to reveal or at least improve the translational value of rodent models of brain disorders. We have identified and validated translational EEG biomarkers for several brain disorders in relevant rodent models with the help of our proprietary Cue® platform. These biomarkers are being routinely used to support our predictive drug discovery programs. Epilepsies : Based on the detection of epileptic discharges by EEG, we have characterized non-convulsive models of mesio-temporal lobe and general absence epilepsies and developed solutions ranging from the screening of small libraries of compounds to the selection and validation of lead compounds. Essential tremor: In a pharmacological induced mouse model of essential tremor, we have identified a specific EEG biomarker that relates to the tremor and shows a pharmacosensitivity to drug of reference and useful for drug development. Parkinson's disease (PD): We have identified specific EEG signatures in two models of Parkinson's disease, mimicking either the evolution of the disease, or the late stage of PD and dyskinesia. These new biomarkers allowed the development of drug discovery programs designed for evaluating new neurotherapeutics and neuroprotective agents against PD. Conclusion: When "augmented" by EEG Biomarkers, rodent models of brain disorders can improve the predictivity of preclinical research, accelerating therefore the discovery of new innovative treatments for patients.

Disclosures: **V. Duveau:** A. Employment/Salary (full or part-time):; SynapCell SAS. **A. Evrard:** A. Employment/Salary (full or part-time):; SynapCell SAS. **H. Gharbi:** A. Employment/Salary (full or part-time):; SynapCell SAS. **C. Roucard:** A. Employment/Salary (full or part-time):; SynapCell SAS. **Y. Roche:** A. Employment/Salary (full or part-time):; SynapCell SAS. **C. Ruggiero:** A. Employment/Salary (full or part-time):; SynapCell SAS.

Digital Abstract Session

P069. Oscillations and Synchrony: LFP Studies

Program #/Poster #: P069.02

Topic: B.09. Network interactions

Support: NIH Grant R01MH104638

Title: Bidirectional effects of cholinergic signaling on amygdala network oscillations

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Abstract: In the brain, the neurotransmitter acetylcholine (ACh) is implicated in a wide variety of functions including emotional and social behaviors. Alterations or deficiencies in these

behaviors are major features of anxiety disorders. Individuals with anxiety disorders show deficits in cholinergic receptors and basal forebrain cholinergic signaling, and in rodent models lesions of cholinergic cells in the brain can induce anxiety-related behaviors. The amygdala receives some of the densest cholinergic projections in the brain, is important for emotional and social behaviors, and shows aberrant activity in anxiety disorders. How ACh modulates network activity, measured in network oscillations, in the amygdala is not clear but can offer insight into how ACh deficits may alter emotional and social behaviors. This study explores the effects of ACh signaling on amygdala network oscillations. Brain slice recordings of the local field potential (LFP) in the basolateral nucleus of the amygdala (BL) show a bidirectional effect in response to optogenetically released ACh: an early onset desynchronization followed by a robust theta frequency synchronization. At the single cell level, the same released ACh in pyramidal neuron recordings showed a similar biphasic response that matched in time with changes in the LFP: an early onset hyperpolarization followed by a depolarizing response and theta membrane potential oscillation. Recording inhibitory currents in these same neurons in response to ACh showed an early barrage of events followed by large theta frequency inhibition, which could synchronize pyramidal neuron firing. These early and late events were mediated by nicotinic and muscarinic ACh receptors, respectively. This indicates combined ACh action on pyramidal and interneurons in the BL drives changes in the LFP. Interestingly, while low amounts of ACh stimulation could produce the early nicotinic network events underlying LFP desynchronization, increasing amounts of ACh were required for the later muscarinic-mediated theta oscillations. Collectively, these studies indicate that differing levels of cholinergic stimulation have profoundly different effects on amygdala network oscillations. Thus, deficits in cholinergic functioning in anxiety disorders could result in aberrant amygdalar activity and contribute to deficits in emotional and social behaviors in these individuals.

Disclosures: **J.X. Bratsch-Prince:** None. **J.W. Warren:** None. **G.C. Jones:** None. **A.J. McDonald:** None. **D.D. Mott:** None.

Digital Abstract Session

P069. Oscillations and Synchrony: LFP Studies

Program #/Poster #: P069.03

Topic: B.09. Network interactions

Support: Tiny Blue Dot foundation
NIH grant K24-NS088568
NIH grant R01-NS062092
MGH ECOR Fund for Medical Discovery Postdoctoral Fellowship Award

Title: Not all unconscious states are equal - differences in response to electrical stimulation during sleep and under general anesthesia in humans

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Abstract: Even though being asleep or under general anesthesia are considered states of unconsciousness, they are separable physiological conditions. Under the assumption that the brain's response to perturbations is different during sleep or general anesthesia in contrast to a conscious state, we used multi-region single-pulse direct electrical stimulation (SPES) to probe the human brain in each of these states.
Twelve patients with semi-chronic depth-electrodes implanted for clinical reasons to localize the focus of their seizures voluntarily participated in this study after fully informed consent. We performed SPES (biphasic pulse: 7uA, inter-stimulation interval 2+/-0.25s) sequentially across 4-5 brain regions while simultaneously recording intracranial EEG (iEEG) in the epilepsy monitoring unit (EMU) during wakeful periods and sleep (N=8) and in the operating room (OR) while awake and under propofol induced general anesthesia (N=9).
Focusing on the cortico-cortical evoke potentials (CCEP) in response to SPES, the iEEG was normalized per trial and low-passed filter below 10Hz, after removal of stimulation artifact. We computed the percentage of recorded channels responsive to SPES. We compared anterior (frontal and anterior cingulate cortex) vs. temporal and posterior cortex. We also measured complexity with the perturbational complexity index (PCI) in the first 600ms in the raw iEEG. Wilcoxon test was used for paired comparisons.
We found that the percentage of responsive channels in the brain was significantly smaller under anesthesia than during wakeful periods ($p<0.01$; N=44 channels). When dividing per region, this difference held when stimulation occurred in anterior channels ($p<0.01$; N=24), but not in temporal/posterior channels. In contrast, wake vs. sleep were statistically different only when considering the percentage of responsive channels with stimulation in temporal/posterior regions ($p=0.04$; N=13). Importantly the number of responsive channels was not significantly different between wake in the EMU vs. wake in the OR. Even after removing channels considered epileptogenic (as indicated by the clinical considerations), all these comparisons prevailed.
Complexity was smaller during anesthesia ($p<0.01$; median PCI= 52.3) compared to wake OR (PCI= 107.3) and during sleep ($p<0.01$; PCI=45.8) compared to wake EMU (PCI=86.4).
The brain's distinct response to stimulation during different states not only improves our understanding of the mechanisms of unconsciousness, but has also therapeutic implications for disorders of consciousness, and could inform assessment tools to ensure loss of consciousness during general anesthesia.

Disclosures: R. Zelmann: None. **P. Kahali:** None. **A.C. Paulk:** None. **F. Tian:** None. **B. Crocker:** None. **G. Cosgrove:** None. **Z. Williams:** None. **P.L. Purdon:** None. **S.S. Cash:** None.

Digital Abstract Session

P069. Oscillations and Synchrony: LFP Studies

Program #/Poster #: P069.04

Topic: B.09. Network interactions

Support: NIH Grant EB026955

Title: The hippocampal theta rhythm is present and informative during stillness

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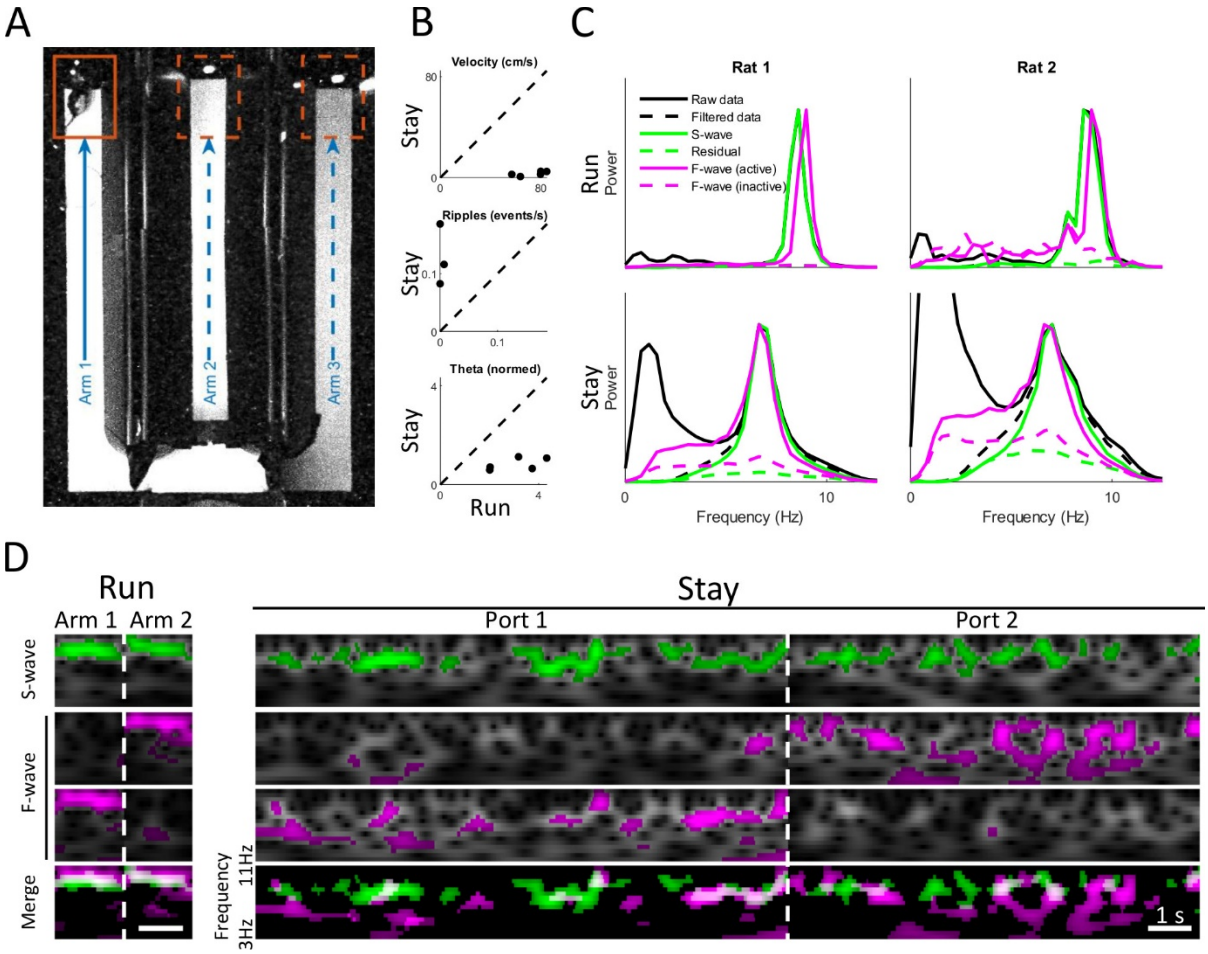
Abstract: Oscillations in the local field potential reflect the synchronized activity of large neuronal populations. Among the most studied is the ~8 Hz theta rhythm, found in the hippocampus during locomotion. The theta rhythm is thought to group temporal sequences during navigation. However, it is unclear whether this rhythm is present or functional during periods of immobility. We use two complementary signal extraction approaches to directly compare theta during movement and stillness in rats navigating a maze. The first is structural: PCA reveals a single oscillatory component ("S-wave") that dominates the multi-electrode signal for the duration of the recording. The second is functional: we developed a novel supervised learning method to isolate oscillatory features ("F-waves") that activate selectively depending on the animal's position. During running, both S- and F-waves are narrow-band and high-amplitude. However, F-waves consistently oscillate at a higher frequency than the S-wave. This frequency shift is a hallmark of the phenomenon of hippocampal phase precession and suggests that S- and F-waves respectively correspond to pacemaker inputs and place cells, as proposed by O'Keefe and Recce's dual-oscillator interference model. We find that during immobility, both oscillations are weaker but clearly present, exhibiting substantial and independent fluctuations in their amplitudes and frequency. Unlike during running, the relative phase of the S- and F-waves is unlikely to carry useful information during stillness, since they are uncoupled in both time and frequency. Our work challenges the common view in the literature that the theta rhythm is absent during stillness, showing that not only is theta present, it also carries information about the animal's position.



A) Alternating maze task used to compare theta during run (blue) and stay (red). B) Stay periods contain biomarkers associated with quiet immobility. C) Power spectra comparing raw LFP, F-, and S-waves. D) Spectrograms show F- and S- waves are uncoupled during stillness. Significant increases in power are colored.

Disclosures: G. Agarwal: None. **B.R. Lustig:** None. **F. Sommer:** None. **E. Pastalkova:** None. **A. Lee:** None.

Digital Abstract Session

P069. Oscillations and Synchrony: LFP Studies

Program #/Poster #: P069.05

Topic: B.09. Network interactions

Support: ERC CoG 647954

Title: What makes a neuron fire? Spike-LFP relationship in human medial temporal lobe

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Abstract: What makes a neuron fire? Previous studies have identified single neurons in the human medial temporal lobe (MTL) that selectively fire to complex, high-level stimuli such as faces and landmarks. These studies have primarily focused on characterising the response profiles of these neurons, for example, the latency of spiking onset and selectivity of spiking response and sensory modality. However, apart from the investigations of Rey et al. (2014), the role of intrinsic oscillatory dynamics in temporally coordinating the firing of such human single neurons has largely been neglected. In the present study, we investigate the role of the local field potential (LFP) in orchestrating single unit activity. To achieve this, 11 patients with intractable epilepsy were implanted bilaterally with microwire hybrid (Behnke-Fried) electrodes in the MTL. We took recordings as patients viewed images presented on a screen. Images were of famous people, landmarks and family relations. A single unit was said to be 'tuned' to an image if its post-stimulus firing rate increased significantly relative to its baseline firing rate, computed across all stimuli. Units could be tuned to one image or several, and each image could have multiple units tuned to it. For analysis, trials were separated into tuning trials where a tuned unit responded and non-tuning trials where there was no response. We found an increase in theta/alpha (4-14Hz) induced power on tuning trials relative to a pre-stimulus baseline, beginning ~100ms after stimulus onset. We also found a broad increase in pairwise phase-consistency of the LFP (1-8Hz) on all trials relative to pre-stimulus baseline, beginning ~200ms after stimulus onset. Both oscillatory measures preceded the average response latency of tuned units, which was ~300ms after stimulus presentation. Our results show that a modulation of oscillatory power in the MTL is concomitant with a spiking rate increase in selectively tuned units. Our results also reveal that phase reorganisation in the MTL following stimulus presentation is a global phenomena that occurs regardless of the presence of selectively spiking tuned units. Further work will involve investigating the spike-LFP relationship through spike-field coupling and multivariate pattern analysis.

Disclosures: D.J. Collins: None. **L. Kolibius:** None. **G. Parish:** None. **R. Chelvarajah:** None. **D. Rollings:** None. **V. Sawlani:** None. **H. Hamer:** None. **S. Gollwitzer:** None. **J. Lang:** None. **G. Kreiselmeyer:** None. **B. Staresina:** None. **M. Wimber:** None. **S. Hanslmayr:** None.

Digital Abstract Session

P069. Oscillations and Synchrony: LFP Studies

Program #/Poster #: P069.06

Topic: B.09. Network interactions

Support: NIH R01-MH115697

NIH R01-DC017687
NSF IOS-1150292
NSF BCS-1439267
Whitehall 2010-05-84

Title: Theta-associated nonspatial sequence coding in hippocampus

Authors: *K. W. COOPER[1], L. LI[2], F. AGOSTINELLI[3], M. SARAF[1], G. A. ELIAS[1], P. BALDI[3], A. BORNSTEIN[4], B. SHAHBABA[2], N. FORTIN[1];
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Abstract: The hippocampus is critical for our memory of sequential experiences. While this capacity is conserved across species and modalities, the underlying neural mechanisms remain unclear. Although considerable evidence indicates hippocampal activity provides strong temporal coding within task events, it remains to be determined how it supports the integration of events in a sequence. Of particular interest here is the finding that theta oscillations capture information about sequences of past, present, and future locations within a single theta cycle. To determine whether this function of theta extends to the processing of nonspatial information, we recorded hippocampal activity as rats performed an odor sequence memory task. Using novel statistical methods, we focused on decoding the information contained in the activity of simultaneously recorded hippocampal ensembles during odor presentations (when strong theta oscillations are observed). We discovered that, within individual odor presentations, hippocampal ensembles exhibited theta-associated replay of the sequential relationships among stimuli. In addition, when focusing the decoding on a specific theta cycle during each odor presentation, we found that ensembles represented information about past, present and future stimuli within individual theta cycles. These findings suggest theta oscillations play a key role in supporting the hippocampus's ability to encode, preserve, and predict the order of nonspatial experiences.

Disclosures: K.W. Cooper: None. **L. Li:** None. **F. Agostinelli:** None. **M. Saraf:** None. **G.A. Elias:** None. **P. Baldi:** None. **A. Bornstein:** None. **B. Shahbaba:** None. **N. Fortin:** None.

Digital Abstract Session

P069. Oscillations and Synchrony: LFP Studies

Program #/Poster #: P069.07

Topic: B.09. Network interactions

Support: NIH/NIGMS Grant: R01 GM109086

Title: Changes in cortical functional geometry across states of awareness under anesthesia

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Abstract: Intro: Leading theories of consciousness predict that loss of consciousness (LOC) under anesthesia is marked by decreased functional integration and differentiation in the brain. Previous work has demonstrated disrupted global integration across several canonical resting-state networks as well as loss of differentiation of brain responses to external stimuli, but how specific networks reorganize upon LOC is unclear. Diffusion Map Embedding (DME) maps data into a space whose geometry represents the functional connectivity structure of the underlying networks. DME can elucidate hierarchical structure in high dimensional cortical networks and how this structure changes with aging and in psychiatric disorders. We applied DME to test the hypothesis that integration between and differentiation of sensory and higher-order networks is disrupted upon LOC.
Methods: Resting-state intracranial recordings were obtained during induction of propofol anesthesia from neurosurgical patients implanted with electrodes in temporal, parietal, and frontal cortex to identify epileptic foci. Three brain states were compared: pre-drug wake (W), sedated/responsive (S) and unresponsive (U). Channel x channel adjacency matrices were computed as pairwise, thresholded, gamma (30-70Hz) power envelope correlations; affinity matrices derived by cosine similarity were subjected to DME analysis focused on cortical regions of interest (ROIs): primary sensorimotor, auditory core, higher-order auditory, and prefrontal cortex (PFC). Integration was assessed as the Euclidean distance between ROI centroids in the embedding space, and differentiation assessed via the silhouette score of each ROI. Centroid distances and silhouette scores were then compared across all states.
Results: Distance between ROI centroids systematically increases from W to U, suggesting that cortical regions become less functionally integrated upon LOC. Shifts from W to U are especially pronounced between auditory core and PFC and between sensorimotor and PFC (n=9/10 subjects). Furthermore, silhouette scores of PFC clusters increase following LOC in PFC (n=9/10), suggesting an increase in intra-ROI functional differentiation in higher-order cortex.
Conclusions: The observation that the functional integration between PFC and sensory ROIs is disrupted upon LOC is consistent with predictions made by both information integration and global neuronal workspace theories. The increased silhouette score in PFC reflects increased homogeneity in functional connectivity which decreases the repertoire of the PFC network overall.

Disclosures: H. Kawasaki: None. **K.V. Nourski:** None. **D.I. Campbell:** None. **M.I. Banks:** None.

Digital Abstract Session

P070. Network Interactions in Oscillations

Program #/Poster #: P070.01

Topic: B.09. Network interactions

Support: EMBO Installation Grant to GU

Title: Basal Forebrain GABAergic Projections to the Amygdala and the BNST in Wistar Rats: A Retrograde Tract-Tracing Study

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Abstract: Basal forebrain (BF) GABAergic neurons from the medial septum selectively target GABAergic interneurons of the hippocampus, forming a disinhibitory circuit that indirectly contributes to the excitation of principal cells. This GABAergic projection is key for the generation of hippocampal theta oscillations (4-12 Hz), which correlate with hippocampal forms of learning and memory including spatial navigation. We test if BF GABAergic projections carry out a similar modulatory function in all the limbic system structures they target. The basolateral amygdala complex and the bed nucleus of the stria terminalis (BNST), also known as the extended amygdala, respectively underlie phasic and sustained fear. BF GABAergic projections innervating these structures may form a similar disinhibitory circuit contributing to local network activity underlying fear and anxiety. We revealed BF GABAergic projections to the amygdala and the BNST by using retrograde tract-tracing and fluorescence immunohistochemistry in adult Wistar rats. In stereotaxic surgeries, red and green Retrobeads were injected unilaterally to different subnuclei of the amygdala and the BNST. The brains were obtained and sliced following 3 days of survival for axonal transport of the tracer. Immunohistochemistry was done for different molecules that may be cell-type or sub-group specific biomarkers. Retrogradely-labeled neurons in different basal forebrain nuclei were counted and mapped. Local connections of the BNST were also examined, as this structure is itself located within the basal forebrain. Injections confined to the lateral and basolateral nuclei of the amygdala revealed the largest number of labeled neurons in the ventral pallidum (VP) and substantia innominata (SI). Fewer labeled neurons were observed in the horizontal limb of the diagonal band of Broca. Remaining BF nuclei were mostly spared, occasionally containing a few labeled neurons. We identified a group of labeled calbindin (CB)-immunopositive putative GABAergic neuron in the VP and SI. Another potential GABAergic subgroup contains fewer neurons that were parvalbumin (PV)-immunopositive. Injections confined to the medial division of the BNST revealed relatively few labeled neurons in the aforementioned BF nuclei. The majority of the labeled neurons were located in the preoptic nuclei of the hypothalamus, especially the medial preoptic nucleus. Several labeled cells were located in other BNST nuclei, some of which were CB- or PV-immunopositive. Current efforts aim to differentiate GABAergic interneurons and long-range GABAergic cells that resemble septo-hippocampal neurons within these populations.

Disclosures: **T. Tuna:** None. **G. Unal:** None.

Digital Abstract Session

P070. Network Interactions in Oscillations

Program #/Poster #: P070.02

Topic: B.09. Network interactions

Support: Okinawa Institute of Science and Technology

Title: Imaging subthreshold voltage oscillation with cellular resolution in the inferior olive in vitro

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[1]Neuronal Rythms in Movment, [2]Optical Neuroimaging Unit, Okinawa Inst. of Sci. and Technol. Grad. Univ., Okinawa, Japan

Abstract: Imaging neuronal activity from inferior olivary nuclei is still considered as a challenge. Despite its crucial role within motor learning, and key role inside the cerebellar system, physiology and function of inferior olive (IO) are poorly understood. Because of the atypical properties of IO neurons as well as the localization of IO itself (on the ventral part of brainstem) already-existing tools for imaging neuronal activity are barely applicable and require proper refinement. Subthreshold activity of IO neurons is a central phenomenon emerging from electrical coupling among IO neurons and give a tempo for network suprathreshold (firing) synchronicity. Here, we developed a method for delivering voltage sensitive dye ANNINE6-plus (A6+) in IO for imaging subthreshold activity of IO with higher signal-to-noise ratio (SNR) than conventional bath application methods. Using stereotactic surgery, we delivered a non-fully dissolved form of ANNINE6-plus that progressively and durably stains stained IO neuron membranes 7 days after injection. After such an extended incubation period, A6+ labelling is homogenized and spreads within IO allowing wide-field imaging *in-vitro* with an excellent coverage of the structure. Also, we performed high-magnification voltage imaging with CMOS camera (1.2x1.2 um^2 resolution) and could monitor subcellular oscillations of membrane voltage on single cell level. Importantly, A6+ is an ultrafast, pure and electrochromic sensor that offers an alternative to genetically-encoded sensors. A6+ is an excellent tool for visualizing subthreshold oscillations (STO) of IO neurons that allowed identifying clusters of neuronal activity from spontaneously oscillating neurons without trial averaging, deconvolution or any machine learning-based image processing. Also, we could study IO network properties by triggering STO resonance after electrical activation of excitatory inputs. The long-lasting staining demonstrated here could be combined with deep brain imaging using implantable GRIN lenses.

Disclosures: K. Dorgans: None. **B. Kuhn:** None. **M. Uusisaari:** None.

Digital Abstract Session

P070. Network Interactions in Oscillations

Program #/Poster #: P070.03

Topic: B.09. Network interactions

Support: National Institute of Neurological Disorders and Stroke (NINDS) of the National Institutes of Health (NIH) R01NS99457 (to I.S)
U19NS104590 (to I.S and A.L)
K99NS117795 (to B.D)
5F31NS110316-03 (to J.C.B.)
R01NS081297 (to G.F.)
5P01NS074972 (to G.F.)
R01MH111529 (to J.D.)

Title: Alternating sources of inhibition during behavior: complementary recruitment of hippocampal cholecystokinin- and parvalbumin-expressing perisomatic interneurons on the time scale of seconds

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Abstract: Basket cells (BCs) are perisomatic GABAergic interneurons (INs) targeting the somata and proximal dendrites of pyramidal cells (PCs), efficiently controlling the spiking output of the network. Two types of basket cells are identified by the expression of either parvalbumin (PV) or cholecystokinin (CCK). PV- and CCK BCs display a striking complementarity in their physiological properties. While fast-spiking PV BCs receive strong glutamatergic input and evoke fast and temporally precise postsynaptic responses, regular spiking CCK BCs receive fewer glutamatergic inputs and their postsynaptic responses are more variable in timing and amplitude. The role of PV BCs in regulating the precise timing of PC firing during synchronous network states (such as theta, gamma and ripple oscillations) is well established. However, the circuit function of CCK BCs remains poorly understood. We have used single cell RNA sequencing, *in vitro* and *in vivo* electrophysiology, as well as *in vivo* calcium imaging and optogenetics to characterize a transgenic mouse line (Sncg-Flp) that labels CCK BCs with high specificity in the CA1 region of the hippocampus in both sexes. In head-fixed mice running voluntarily on a linear treadmill, we found that CCK BC activity is suppressed during the on-line brain state associated with locomotion and theta oscillations, as well as during sharp wave-ripple (SWR) episodes, associated with immobility and the off-line replay neuronal sequences. In contrast, CCK BCs were maximally recruited during a brain state characterized by immobility, a lack of synchronous network oscillations, and suppressed PC

activity. By simultaneous calcium imaging from CCK and PV INs during spontaneous behavior, we found a striking negative correlation between their activities on the time scale of seconds. Optogenetic driving of PV INs triggered strong suppression of CCK IN activity compared to no-opsin and no-light controls *in vivo*, suggesting a disinhibitory push-pull mechanism contributing to the temporal segregation of PV and CCK IN activity. Simultaneous large-scale calcium imaging from hundreds of PCs and either PV- or CCK INs revealed that while PV IN activity is strongly and positively correlated to average network activity on the time scale of seconds, CCK IN activity scales negatively with network activity. Taken together, efficient inhibition of CCK by PV INs inversely couples the activity of two complementary perisomatic inhibitory systems on the behaviorally relevant time scale of seconds, resulting in a "hand-off" of inhibition during brain state transitions.

Disclosures: **B. Dudok:** None. **P.M. Klein:** None. **E. Hwaun:** None. **B. Lee:** None. **Z. Yao:** None. **O. Fong:** None. **J.C. Bowler:** None. **S. Terada:** None. **F.T. Sparks:** None. **G.G. Szabo:** None. **J.S. Farrell:** None. **J. Berg:** None. **T.L. Daigle:** None. **B. Tasic:** None. **J. Dimidschstein:** None. **G. Fishell:** None. **A. Losonczy:** None. **H. Zeng:** None. **I. Soltesz:** None.

Digital Abstract Session

P070. Network Interactions in Oscillations

Program #/Poster #: P070.04

Topic: I.08. Methods to Modulate Neural Activity

Support: ESRC Grant ES/R010072/1
China Scholarship Council

Title: Using fast visual rhythmic stimulation to control inter-hemispheric phase offsets in visual areas

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Abstract: Spike timing dependent plasticity (STDP) is believed to be important for neural communication and plasticity in human episodic memory, but causal evidence is lacking due to technical challenges. Rhythmic sensory stimulation that has been used to investigate causal relations between oscillations and cognition may be able to address this question. The challenge, however, is that the frequency corresponding to the critical time window for STDP is gamma (~40 Hz), yet the application of rhythmic sensory stimulation has been limited primarily to lower frequencies (<30 Hz). It remains unknown whether this method can be applied to precisely control the activation time delay between distant groups of neurons at the millisecond scale. To answer this question and examine the role of STDP in human episodic memory, we simulated the STDP function by controlling the activation time delay between the left and right visual cortices during memory encoding. This was achieved by presenting flickering (37.5 Hz) movie pairs in

the left and right visual fields with a phase lag of either 0, 90, 180 or 270 degrees. Participants were asked to memorize the two movies within each pair and the association was later tested. Behavioral results revealed no significant difference in memory performance across conditions with different degrees of gamma phase synchrony. Yet importantly, our study showed for the first time, that oscillatory activity can be driven with a precision of 6.67 ms delay between neuronal groups. Our method hereby provides an approach to investigate relations between precise neuronal timing and cognitive functions.

Disclosures: Q. Chen: None. **D. Wang:** None. **K. Shapiro:** None. **S. Hanslmayr:** None.

Digital Abstract Session

P070. Network Interactions in Oscillations

Program #/Poster #: P070.05

Topic: C.10. Brain Injury and Trauma

Support: NINDS, NS094655

Title: Robust alternative to the righting reflex to assess arousal in rodents

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Abstract: Abstract: The righting reflex (RR) is frequently used to assess level of arousal in animal models across multiple neurological disorders. RR produces a single binary result to infer awakening, often without further behavioral corroboration. We found that RR is an unreliable metric for arousal/recovery of consciousness. We hypothesized that the combined analysis of cortical activity and motor behavior that accompany RR is a superior criterion to properly calibrate and establish level of arousal in rodents. To test our hypothesis, we simultaneously recorded local field potentials (LFPs) and movement in mice while decreasing anesthetic concentrations. We delineated cortical dynamics during emergence from anesthesia by applying a smoothed-Z score to extract dominant frequencies from spectrogram. Then, we implemented KMeans to obtain cortical sub-states. Finally, we used density estimation and an abrupt change detection algorithm to segment cortical activity into periods together with examining motor behavior in videos. We established a cortico-motor regimen that calibrated levels of arousal that consisted of five cortical periods with progressive restored motor behavior during emergence from isoflurane anesthetic. Results were further validated in sevoflurane and the hypoglycemic coma model. Our data demonstrated that initial spontaneous RR events occur at a low arousal state, and that a single RR does not predict recovery stage. We also observed multiple RR events and they are clearly distinguishable depending on cortical activity and motor behavior context. We demonstrated that RR alone is an imprecise measurement of arousal level.

Disclosures: S. Gao: None. **D. Calderon:** None.

Digital Abstract Session

P071. Computational Modelling of Synaptic Networks

Program #/Poster #: P071.01

Topic: B.09. Network interactions

Support: NIH/NINDS grant NS097185 to Charles Wilson

Title: Local synapses alter spike responses in models of globus pallidus pars externa

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Abstract: The globus pallidus pars externa (GPe) is usually considered a relay nucleus that inverts the inhibitory signal arriving from the striatum and passes it to the basal ganglia output neurons. GPe neurons are also interconnected by inhibitory interconnections, but their function is unknown. GPe neurons are fast autonomous oscillators whose local axon collaterals create an active local inhibitory network, but spike timing correlations among GPe neurons have been elusive. To determine how local connectivity might affect GPe steady-state firing and responses to stimuli, we constructed network models of GPe. As GPe neurons are intrinsic oscillators, we simulated them using phase models that allow us to predict the time of the next spike considering all the synaptic inputs arriving during the inter-spike interval (ISI). We experimentally measured the firing rates, and phase resetting curves of a sample of GPe neurons (n=19) in mouse brain slices. Using the experimental dataset, we generated artificial neurons with a diversity similar to that of the recorded neurons. As the connectivity pattern in GPe is unknown, we placed the cells in two different neural architectures (small-world and random). Each network was created using the same set of 1000 neurons and the same total number of connections (10000). Both networks reduced the GPe neurons' firing rates and caused substantial variation of ISIs. The local networks did not generate global synchrony, and strong pairwise correlations were mostly limited to monosynaptically connected pairs. When stimulated by a shared input, the post-stimulus time histogram (PSTH) in both networks shown larger peaks and shortened responses when compared to disconnected neurons. The increase in the transient response was associated with the perturbation in the stationary density of phases generated by the local inhibitory synaptic barrage which induce the neurons to spent more time at late phases, i.e. near spiking. Our results were robust across the network architectures and show that in sparsely coupled networks of autonomous oscillator neurons like GPe, the local inhibitory activity does not generate global synchrony, however, it increases the network sensitivity to external inputs by changing the steady-state phase distribution.

Disclosures: **E. Olivares:** A. Employment/Salary (full or part-time):; UTSA. **M.H. Higgs:** A. Employment/Salary (full or part-time):; UTSA. **C.J. Wilson:** A. Employment/Salary (full or part-time):; UTSA.

Digital Abstract Session

P071. Computational Modelling of Synaptic Networks

Program #/Poster #: P071.02

Topic: B.09. Network interactions

Support: NIH Grant R01NS054281

Title: Kinetics and spatial profile of fast-firing-based inhibition in layer 2/3 medial entorhinal cortex and its implications for theta-nested gamma oscillations

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Abstract: Medial entorhinal cortex (mEC) contains spatially tuned grid cells that may play a significant role in spatial navigation. mEC also generates theta-nested gamma oscillations that synchronize grid cell spike activity to specific phases of network-wide theta oscillations. The mechanisms underlying network activity in mEC are often accounted for with a canonical cortical circuit model that includes negative feedback from inhibitory cells that is crucial to both theta-nested gamma oscillations and the spatial tuning of grid cells. In mEC, inhibitory interneurons provide the sole synaptic communication path between stellate cells that constitute a substantial fraction of the grid cell population. Key to all these roles are the synaptic kinetics and spatial profile of inhibition originating from fast-firing interneurons. To address the nature of inhibition in mEC, we carried out intracellular paired recordings of layer 2/3 PV-PV and stellate-PV cells at distances between 20 to 400 μm in horizontal brain slices from mice. The likelihood of synaptic connection from PV cells dropped off precipitously at intercellular distances greater than 150 μm. Further, the inhibitory decay time constant between PV cells was much smaller (~2.3 ms) than that between PV and stellate cells (~9.8 ms). PV-PV synapses also depressed more rapidly and recovered more slowly than those targeting stellate cells. Finally, gap junctions between PV cells were somewhat rare, with only 33 out of 122 pairs expressing a gap junction connection. These connections, however, were more likely in mutually connected (~0.53) pairs. Using the above data, we implemented an interneuronal network model with sparse inhibitory connections and a network-wide sinusoidal theta drive. We used the interneuron model of Golomb et al. (2007) to match the type II excitability we have previously demonstrated in the PV+ interneurons. In the presence of heterogeneity and noise, nested gamma was not evoked by the theta drive. However, an excitatory pulse delivered to the population midway through the rising phase of theta, when the stellate cells preferentially fire experimentally, did evoke theta-nested gamma oscillations. Unlike classic PING mechanisms of gamma generation in which the excitatory population fires on every gamma cycle, theta-nested gamma was initiated by excitation with subsequent gamma cycles maintained by mutual connections within the inhibitory population. Since these interneurons require excitatory input for nested gamma, but stellate cells do not to fire throughout the peak of theta drive, our proposed mechanism is consistent with experimental observations.

Disclosures: **F.R. Fernandez:** None. **G.R. Via:** None. **C.C. Canavier:** None. **J.A. White:** None.

Digital Abstract Session

P071. Computational Modelling of Synaptic Networks

Program #/Poster #: P071.03

Topic: B.09. Network interactions

Support: ANR-18-CE37-0014-02

Title: Effective tuning of inter-regional coupling strength and latency through changes in local oscillatory dynamics

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Abstract: Neuronal populations within local regions frequently undergo oscillatory modulations of their activity. The oscillations of distant populations coupled by long-range connections can phase lock at different stable phase differences and the flexible change of the phase locking patterns between different brain regions has been hypothesized to modulate communication and functional interactions between them. It is therefore important to understand which factors can affect and control the established inter-regional phase relations.A tool which has proved useful to model and predict the behavior of coupled oscillating populations is the phase response curve (PRC). The PRC is a local transformation function that determines the phase-dependent response of an oscillator to any given input, provided by external stimulation or by other oscillating regions. For this reason, the PRC plays an instrumental role in determining how coupled regions can form robust phase-locked modes.Importantly, the PRC strongly depends on parameters of the local regional microcircuit such as the relative strengths of recurrent excitation and inhibition. It is on the contrary not affected by parameters of the distal couplings, such as the coupling strength or delay of propagation. Here we investigate how equivalent changes in phase-locking between regions can be induced by either modifying local connectivity parameters and thereby the PRC, or by modifying features of the long-range inter-regional connectivity. In particular, we show that modifying the strength of connectivity between excitatory and inhibitory neurons within populations can emulate the effects of varying the strength of inter-regional tracts. Or analogously, that modulating the strength of recurrent inhibition within a region can emulate the effects of varying the delay of propagation along inter-regional tracts. These are all examples of what we may call an “effective tuning” of long-range coupling, obtained via the reshaping of local PRCs and oscillations.We thus propose that modifications of oscillatory dynamics within regions may lead to distributed changes in the functional connectivity between regions which are mediated by phase-locking. Such changes would be achieved without the need for plastic changes in long-range structural tracts but just through modulations of local

connectivity efficacy, as the ones that could be induced by neuro-modulation or by the recruitment of alternative types of inhibitory interneurons.

Disclosures: **S. Benitez Stulz:** None. **G. Dumont:** None. **B.S. Gutkin:** None. **D. Battaglia:** None.

Digital Abstract Session

P071. Computational Modelling of Synaptic Networks

Program #/Poster #: P071.04

Topic: B.09. Network interactions

Support: FRQNT B2X Scholarship (EVG)
CIHR 142420 (JCB)
NSERC 05830 (JCB)
NSERC Discovery 210977 (JPT)

Title: Paradoxical effects of parvalbumin activation on excitatory and inhibitory neurons in balanced networks

Authors: *E. GIRAUD, M. B. LYNN, J.-C. BEIQUE, J.-P. THIVIERGE;
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Abstract: The prefrontal cortex (PFC) plays an important role in the representation and execution of goal-directed action. The PFC forms a structured recurrent network of excitatory (E) and inhibitory (I) cells whose dynamical interactions remain poorly understood. Recent studies are outlining inhibitory-excitatory dynamics that do not naturally emerge from common connectivity motifs of E/I connectivity. Theoretical work has attempted to explain these emergent dynamics with generic implementations of inhibition-stabilized networks (ISNs) that are characterized by strong recurrent excitation balanced by strong inhibition. Current implementations of ISNs do not, however, capture the diversity and connectivity patterns of interneuron cell types found in cortex. To begin examining the contribution of different interneuron subtypes to cortical dynamics, we employed in vitro recordings of PFC circuits and computational modeling of ISN networks with a diversity of cell types. We recorded and stimulated spiking activity in acute slices of PFC by combining electrophysiological and optogenetic methods on high-density multi-electrode arrays containing 4,096 closely spaced electrodes. To parse out the contribution of distinct cell types, we developed a spike sorting technique with spline interpolation and principal components analysis to distinguish putative regular-spiking excitatory neurons from fast-spiking inhibitory interneurons. Our sorting algorithm was validated using a targeted combination of viral and optogenetic strategies to activate parvalbumin (PV) interneurons. When probing different subsamples of cortical ensembles, we found that optogenetically activating PV neurons led to a paradoxical increase in excitatory pyramidal activity and a decrease in overall inhibition. We developed an integrate-and-fire model of somatostatin, parvalbumin and pyramidal cell populations with balanced E/I

that captured these effects. Crucially, the model relied on a strong tonic activation of PV cells. With lower tonic PV activation, the paradoxical effect was reversed: excitatory cells reduced their activation upon PV stimulation. The model also relied on precise balanced E/I inputs to individual excitatory cells, whereby the sum of incoming E/I contributions to subthreshold membrane potential cancelled out within a rapid time window. These results offer evidence for ISN regimes, and support the role of balanced E/I activity and strong tonic PV firing rates to generate paradoxical effects of excitatory activation and inhibitory deactivation upon optogenetic stimulation of prefrontal PV neurons.

Disclosures: **E. Giraud:** None. **M.B. Lynn:** None. **J. Beique:** None. **J. Thivierge:** None.

Digital Abstract Session

P072. Neuronal and Synaptic Mechanisms Underlying Seizure Pathology

Program #/Poster #: P072.01

Topic: B.10. Epilepsy

Support: Department of Biotechnology, Ministry of Science & Technology, Government of India [Centre of Excellence for Epilepsy/Magnetoencephalography Resource Facility Grant: BT/MED/122/SP24580/2018].

Title: Differential spontaneous synaptic transmission in resected brain specimens obtained from patients with cortical dysplasia (CD) and non-CD drug resistant epilepsy pathologies.

Authors: ***J. BANERJEE**[1], S. DEY[2], A. B. DIXIT[3], M. TRIPATHI[2], M. C. SHARMA[2], S. P. CHANDRA[2];
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Abstract: Altered synaptic transmission was associated with drug-resistant epilepsy (DRE) pathologies like cortical dysplasia (CD) and non-CD pathologies like mesial temporal lobe epilepsy (MTLE). Here we have analyzed the spontaneous glutamatergic and GABAergic activity from resected brain samples obtained from patients with CD and MTLE. CD is a developmental malformation of the cerebral cortex and clinical manifestations in CD varies with age at epilepsy onset. In this study brain specimens were obtained from the maximal spiking region (MAX) and minimal spiking region (MIN) of the epileptic foci of early onset (EO) and late onset (LO) patients undergoing electrocorticography (ECoG) guided surgery. Whole-cell patch clamp technique was used to record spontaneous glutamatergic and GABAergic currents from normal-looking pyramidal neurons in slice preparations of resected brain samples. We detect increased frequency and amplitude of GABAergic events in high and low spiking region samples of EO patients, but only in high spiking region samples in LO patients. We did not observe any alteration in the glutamatergic synaptic transmission in the pyramidal neurons of resected samples obtained from patients with CD. Similar experiments were also carried out on resected hippocampal tissues obtained from patients with MTLE. We observed that frequency of

spontaneous glutamatergic activity was higher in the hippocampal samples obtained from patients with MTLE compared to that in case of non-epileptic controls. Further we observed that glutamatergic activity in the hippocampal samples obtained from patients with MTLE were suppressed by α7 nAChR antagonist, methyllycaconitine, suggesting α7 nAChRs may regulate excitatory transmission in MTLE. We did not observe any alteration in the GABAergic activity on to the pyramidal neurons of resected hippocampal samples obtained from patients with MTLE. Our findings indicate that in patients with CD GABAergic activity was altered and in LO patients with CD, $GABA_A$ receptor-mediated epileptogenicity is confined only to the high spiking area, but in EO patients with CD affects low spiking regions as well. In case of hippocampal samples obtained from patients with MTLE increased glutamatergic activity was observed without affecting the GABAergic synaptic transmission. These findings altogether support the concept that mechanism of hyper excitability varies in patients with CD and non-CD DRE pathologies.

Disclosures: J. Banerjee: None. **S. Dey:** None. **A.B. Dixit:** None. **M. Tripathi:** None. **M.C. Sharma:** None. **S.P. Chandra:** None.

Digital Abstract Session

P072. Neuronal and Synaptic Mechanisms Underlying Seizure Pathology

Program #/Poster #: P072.02

Topic: B.10. Epilepsy

Support: American Epilepsy Society Postdoctoral Fellowship (JHC)
Dravet Syndrome Foundation Postdoctoral Fellowship (JHC)
R01 NS112500-01

Title: Dendritic morphology in the Scn1b knockout mouse model of Dravet syndrome

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Abstract: Dravet syndrome (DS) is a genetic epileptic encephalopathy characterized by prolonged seizures and severe cognitive and developmental deficits. Mutations in *SCN1B*, which encodes the β1 protein, have been linked to DS. *Scn1b* knockout (KO) mice model DS, exhibiting spontaneous seizures, ataxia, developmental delays, and early death. β1 is critical in regulating neurite outgrowth and various ion channels important for neuronal excitability. Previous work in an *Scn1b* DS model found subicular pyramidal neurons exhibit increased excitability and decreased dendritic arborization. Our goal is to determine how loss of β1 affects one of the primary neuron types involved in learning and memory: hippocampal CA1 pyramidal neurons (PNs). We have found that CA1 KO PNs have enhanced intrinsic excitability and increased synaptic temporal integration, leading to higher firing rates in response to physiological synaptic stimuli. Here, we examined dendritic structure of CA1 PNs from *Scn1b* KO mice to investigate if changes in morphology have a role in the physiological underpinnings

of DS. Because we found hyperexcitability and reduced capacitance in *Scn1b* KO PNs, we hypothesized that CA1 KO PNs would exhibit reduced dendritic arborization. PNs from KO mice and wild-type (WT) littermates, age p15-20, were filled with Neurobiotin during whole cell recordings, stained using Vectastain ABC-HRP and DAB kits (Vector Labs), and reconstructed using Neurolucida. Dendrite length and arborization of dendrites were quantified with Sholl analyses. Both WT and KO PNs exhibited a large amount of heterogeneity in their dendritic morphology. Unlike previous reports in subiculum, we found no overall differences in dendritic arborization or length between WT and KO CA1 PNs. Our synaptic physiology experiments also show changes in synaptic integration in KO PNs. We quantified spine density of proximal and distal apical dendrites of PNs by filling neurons with fluorescent dye via the whole cell recording pipette and using two-photon imaging. Our preliminary data suggest that spine density is unchanged in KO PNs, reducing the likelihood that changes in synapse number underlie altered synaptic integration from loss of β1. Our findings illustrate that dendritic structure is unexpectedly preserved in *Scn1b* KO PNs in CA1 compared to WT PNs, despite changes in excitability and synaptic function. This suggests that dendritic structure does not underlie the hyperexcitability of CA1 KO PNs, nor does the hyperexcitability inherent in this epileptic model induce marked structural plasticity.

Disclosures: **A.A. Ahmed:** None. **J. Chancey:** None. **M.A. Howard:** None.

Digital Abstract Session

P072. Neuronal and Synaptic Mechanisms Underlying Seizure Pathology

Program #/Poster #: P072.03

Topic: B.10. Epilepsy

Support: CIHR
Transforming Autism Care Consortium
Savoy Foundation
FRQS
Brain Canada Foundation
Health Canada
CURE Epilepsy

Title: Impaired GABAergic interneuron migration and reduced actin remodeling in Trio-associated epilepsy and autism

Authors: *L. EID[1,2], L. LOKMANE[3], P. K. RAJU[1,2], S. TENE-TADOUM[1,2], X. JIANG[1], A. TOUMI[1,2], K. TOULOUSE[1,2], E. DOUET[1], M. LACHANCE[1], J.-C. LACAILLE[2], E. BLOCH-GALLEGO[3], E. ROSSIGNOL[1,2];
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Abstract: Mutations in the *TRIO* gene are associated with intellectual disability, autism spectrum disorder (ASD) and/or epileptic encephalopathies (EE). TRIO is a dual guanine nucleotide exchange factor (GEF) that activates Rac1 and RhoA, two Rho-GTPases that regulate actin remodeling. In mice, *Trio* is known to regulate the dendritic development of excitatory neurons, the pathfinding of thalamocortical and spinal cord axons as well as the migration and morphogenesis of developing cerebellar granule cells. However, its roles in the development of GABAergic interneurons (INs) are unknown. Given the central role of Rac1 and RhoA in cytoskeletal remodeling during neuronal migration and the implication of IN pathologies in specific forms of ASD and EE, we hypothesized that *Trio* might be a central regulator of IN migration. In $Trio^{-/-}$ mice, we observed a reduction of tangentially migrating INs. To bypass the embryonic lethality of $Trio^{-/-}$ mice and to further study the underlying mechanisms, we generated $Dlx5/6^{Cre};Trio^{c/c}$ mice, carrying a targeted deletion of *Trio* in INs. These mutant mice display cognitive deficits and reduced anxiety, as well as spontaneous tonic-clonic seizures. Further, patch-clamp recordings reveal a reduction in IPSC frequency, consistent with a reduction of cortical inhibition. Cell imaging reveals a delay in IN migration at e13.5 and e15.5, along with an increase in neurite complexity and length at e13.5. Live-imaging of e13.5 acute organotypic slice cultures reveals slower and less frequent nucleokinesis along with impaired orientation, resulting in reduced net displacement. Live-imaging of medial ganglionic eminence (MGE) explants electroporated with *Lifeact* reveals a more diffuse distribution of F-actin in the soma of migrating INs, with a decreased compaction at the rear of the soma, compared to controls. Further, we observe an increase in the G-actin to F-actin ratio in the soma of mutant INs, together with an overall decrease in G-actin and F-actin, suggesting that the migration deficits induced by *Trio* deletion in INs reflect a reduction in actin turnover. Finally, the electroporation of a mutated version of *Trio* cDNA, lacking either the GEFD1 or the GEFD2 domain, in *Trio* mutant MGE explants reveals the requirement of the GEFD2-RhoA pathway in the morphological development and the migration dynamics of GABAergic INs, with some contributions from the GEFD1-Rac1 pathway. Altogether, our data suggest that *TRIO* loss-of-function mutations impact the migration of cortical INs through impaired actin remodeling resulting from imbalanced RhoA and Rac1 activity, disrupting cortical inhibitory networks and leading to EE/ASD.

Disclosures: **L. Eid:** None. **L. Lokmane:** None. **P.K. Raju:** None. **S. Tene-Tadoum:** None. **X. Jiang:** None. **A. Toumi:** None. **K. Toulouse:** None. **E. Douet:** None. **M. Lachance:** None. **J. Lacaille:** None. **E. Bloch-Gallego:** None. **E. Rossignol:** None.

Digital Abstract Session

P072. Neuronal and Synaptic Mechanisms Underlying Seizure Pathology

Program #/Poster #: P072.04

Topic: B.10. Epilepsy

Support: NIH NS083009
NIH NS096012
Chao Family Comprehensive Cancer Center -P30CA062203

Title: Interneuron dysfunction in a new knock-in mouse model of scn1a epilepsy

Authors: *A. DAS, B. ZHU, Y. XIE, L. ZENG, A. T. PHAM, J. C. NEUMANN, O. SAFRINA, D. R. BENAVIDES, G. R. MACGREGOR, S. SCHUTTE, R. HUNT, D. O'DOWD;
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Abstract: Epilepsy is a common neurological disorder that affects 1% of the world population. Advanced genome sequencing has identified over 1300 mutations in the human SCN1A, a gene that encodes the alpha subunit of a sodium ion channel Nav1.1. About 20% of SCN1A missense mutations result in an epilepsy disorder, Genetic Epilepsy with Febrile Seizures Plus (GEFS+). GEFS+ is a childhood onset disorder, characterized by febrile (or fever associated) seizures, which can persist beyond 6-7 years of age. A fundamental challenge in understanding the etiology and developing treatments for genetic epilepsies is the heterogeneity of the phenotypes. Previous studies in the lab have shown that the K1270T ("KT") mutation, linked to GEFS+ in humans, causes heat-induced seizure activity associated with a temperature-dependent decrease in GABAergic neuron excitability in a Drosophila knock-in model and impaired inhibitory neuronal firing in patient-derived human-iPSC cell lines. To examine the behavioral and cellular effects of this mutation in mammalian circuits, we introduced the equivalent KT mutation into the mouse Scn1a gene using CRISPR/Cas9. Mutant mouse lines were generated in two widely used genetic backgrounds: C57BL6NJ and 129X1SvJ. In both backgrounds, mice homozygous for the KT mutation had spontaneous seizures and died by postnatal day 23. There was no difference in mortality of heterozygous KT mice compared to wild-type littermates, monitored up to 6 months. Heterozygous mutants exhibited heat-induced seizures at about 42ºC, a temperature that did not induce seizures in wild-type littermates. In acute brain slices at permissive temperatures, current-clamp recordings revealed a significantly depolarized shift in action potential threshold and reduced action potential amplitude in hippocampal parvalbumin-expressing inhibitory interneurons in heterozygous mice. There was no change in the firing properties of excitatory CA1 pyramidal neurons. These results suggest that a constitutive decrease in inhibitory interneuron excitability contributes to the seizure phenotype in the mouse model- lending support to the 'Interneuron disinhibition' hypothesis for seizure generation. The SCN1A KT mouse model represents an important tool for identifying mechanisms of seizure generation in relation to other epilepsy models, and for development of mutation-specific therapies.

Disclosures: A. Das: None. **D. O'Dowd:** None. **R. Hunt:** None. **Y. Xie:** None. **B. Zhu:** None. **L. Zeng:** None. **A.T. Pham:** None. **J.C. Neumann:** None. **O. Safrina:** None. **D.R. Benavides:** None. **G.R. MacGregor:** None. **S. Schutte:** None.

Digital Abstract Session

P073. Animal Models in Epilepsy Research

Program #/Poster #: P073.01

Topic: B.10. Epilepsy

Support: NIH (R01AA025368, R03DA045897)
Purdue Drug Institute for Drug Discovery

Title: B-arrestin recruitment correlated with delta opioid receptor agonist seizure severity

Authors: *A. T. BLAINE, S. PALANT, R. VAN RIJN;
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Abstract: The δ-opioid receptor (DOR), is a promising target for the treatment of a variety of neurological disorders, including chronic pain disorders, alcohol use disorder and mood disorders. However, a major side effect that occurs when activating the DOR is the induction of seizures. The arrestin proteins, a family of proteins involved in regulation of DOR signaling as well as other G-protein coupled receptors (GPCRs), have been heavily implicated in this mechanism. Within the arrestin family, the β-arrestin 1 (β-arr1) and β-arrestin 2 (βarr2) isoforms are ubiquitously expressed throughout the body, including the central nervous system, and function by uncoupling phosphorylated GPCRs from their corresponding G-protein, terminating GPCR signaling in a process known as desensitization. Notably, mice with a genetic knockout of β-arr 1 (β-arr 1 KO) show a markedly increased sensitivity to DOR agonist induced convulsions compared to wild type or β-arr 2 knockout (β-arr 2 KO) mice. This observation generated several research questions. 1) What is the cellular mechanism of action for β-arr's involvement in the seizurogenic effects of DOR activation? 2) Are DOR agonists with lower β-arr recruitment efficacy less seizurogenic? 3) Are β-arr 1 KO mice a novel model of status epilepticus? To address these questions, we measured seizure activity (assessed by Racine score), in male and female wild-type, β-arr 1 KO and β-arr 2 KO C57BL/6 mice ($n \geq 6$ per treatment). We utilized three different DOR agonists, which we determined differed in their β-arr recruitment efficacy using cellular signaling assays. When we injected these three agonists, SNC80, ADL5859 and ARM390 at equianalgesic doses, we noted a strong correlation between β-arr 2 recruitment efficacy and seizure intensity. We did not observe any sex differences. In the hippocampus of our mice we also noted a strong increase in pERK following DOR activation. The ERK activation was more pronounced when mice were treated with a DOR agonist with strong β-arr recruitment efficacy, and also was more pronounced in β-arr 1 KO mice. Blocking ERK activation, with the MEK inhibitor SL327, however, did not reduce DOR induced seizure, suggesting that the ERK activation comes as a result of the seizure and is not part of the mechanism of action of DOR-induced seizures. Thus far, our results suggest that the likelihood of seizures induced by DOR activation can be mitigated using a DOR agonist with low β-arr 2 recruitment efficacy. The β-arr 1 KO mice provide an interesting and new model system in which to study seizures and status epilepticus. This work was supported by NIH (R01AA025368, R03DA045897) and the Purdue Drug Institute for Drug Discovery.

Disclosures: A.T. Blaine: None. **S. Palant:** None. **R. van Rijn:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Carmot Therapeutics.

Digital Abstract Session

P073. Animal Models in Epilepsy Research

Program #/Poster #: P073.02

Topic: B.10. Epilepsy

Title: Localized elevation of excitation over inhibition is specific to regions of initiation but not propagation in a larval zebrafish seizure model

Authors: *J. NIEMEYER[1], P. GADAMSETTY[1], C. CHUN[2], S. SYLVESTER[2], J. LUCAS[1], H. MA[1], T. SCHWARTZ[1], E. AKSAY[2];
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Abstract: Epilepsy is a debilitating neurological disorder afflicting more than 1% of the US population, characterized by recurring seizures and associated cognitive and memory disturbances. Seizures are believed to be caused by an imbalance in coordinated excitatory and inhibitory (E/I) neuronal activity. Previous studies have examined E/I differences but have focused on small subregions of the brain in anesthetized seizure models, yielding seemingly contradictory findings regarding the roles of these cell types. We aimed to reconcile previous findings by measuring excitatory and inhibitory neuron activity during whole-brain imaging to capture seizure initiation and propagation zones simultaneously in a new seizure model: awake larval zebrafish treated with a chemoconvulsant pentylenetetrazol (PTZ). Unlike other models, these small animals permit whole-brain cellular-resolution calcium imaging of multiple cell types. Our specific goals were to 1) thoroughly characterize seizure development in this model and 2) determine the role of E/I balance during seizure initiation and propagation. We find that generalized seizures in this model most often initiate in midbrain and thalamic structures (N= 167 seizures), spreading rapidly to other brain sites. By developing and applying a novel method of detecting initiation and propagation zones we then analyzed E/I balance at these sites, finding significantly higher E/I cell count ratios and activation ratios in initiation vs propagation zones. Further, network analyses of excitatory and inhibitory cell correlations and activity similarity (by PCA) uncovered a prominent role for excitatory cell activity during seizures. Our results suggest that seizure development can be shaped by local E/I cell ratios and biased by excitatory cell population activity. These results imply that E/I activity differs in small regions throughout the brain and that E/I imbalance in specific brain regions may serve as a novel target in epilepsy treatments.

Disclosures: J. Niemeyer: None. **P. Gadamsetty:** None. **C. Chun:** None. **S. Sylvester:** None. **J. Lucas:** None. **H. Ma:** None. **T. Schwartz:** None. **E. Aksay:** None.

Digital Abstract Session

P073. Animal Models in Epilepsy Research

Program #/Poster #: P073.03

Topic: B.10. Epilepsy

Title: Circumscribing laser cuts attenuate propagation of cortical seizures in a mouse model of focal epilepsy

Authors: *S. LIEBERMAN[1], D. A. RIVERA[2], R. MORTON[2], M. ZHAO[3], N. NISHIMURA[2], T. H. SCHWARTZ[3], C. B. SCHAFFER[2];
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Abstract: One out of twenty-six people will develop epilepsy within their lifetime. Focal epilepsy is characterized by seizures that initiate at one location and propagate through the brain. Medical management fails in about 45% of focal epilepsy cases and resective surgery remains the only alternative, but often leaves patients with severe neurologic deficits. *In-vivo* two-photon imaging of acute seizure propagation has shown that seizures primarily propagate along lateral connection in cortical layers II-III. Therefore, it has been suggested that making minimally invasive incisions to severe lateral connections that seizures propagate along while maintaining vertical connections in the cortex would preserve much of normal brain function while blocking seizure propagation. Tissue ablation by tightly-focused femtosecond laser pulses provides a "laser scalpel" that can make subsurface microincisions in the cortex without damaging the overlying tissue. Previously, we showed that such laser incisions targeted to layers II-IV of the cortex in rats reduced the propagation of acutely-induced seizures. Here we test the long term efficacy of laser cuts in the supragranular layers of the neocortex in interfering with the propagation of seizures in a chronic model of focal epilepsy, and we examine the impact of the cuts on normal cortical function. Chronic focal seizures were induced by microinjection of iron chloride. In mice with laser cuts in layers II-IV of the cortex that encircled the seizure focus, we observed an 80% reduction in the propagation of seizures, as compared with controls. Seizures were also shorter in duration, lower in power, and delayed when they did propagate in animals with cuts. In a preliminary assessment of the impact of these cuts on normal function, we detected no performance difference in a pellet reaching task in mice with a cut centered on the forelimb motor region of the cortex, as compared to control mice. Mice that received not only the encircling, vertical cuts in the cortex, but also a "flat bottom" cut at the bottom of the cylinder showed a reduction in pellet retrieval success that was similar to that seen with a focal stroke. In conclusion, our results suggest cutting lateral connections in the supragranular cortical layers surrounding a seizure focus could be a promising neurosurgical approach that is efficient in blocking seizure propagation while maintaining most normal brain activity.

Disclosures: S. Lieberman: None. **D.A. Rivera:** None. **R. Morton:** None. **M. Zhao:** None. **N. Nishimura:** None. **T.H. Schwartz:** None. **C.B. Schaffer:** None.

Digital Abstract Session

P074. Biomarkers and Therapeutic Approaches in Animal Models of Seizures

Program #/Poster #: P074.01

Topic: B.10. Epilepsy

Title: Variability of Anti-Epileptic Drug Efficacy in the Pentylenetetrazol (PTZ) Zebrafish Behavior Model

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Abstract: In order to evaluate antiepileptic drug (AED) efficacy, pentelyentetrazol (PTZ) has been used to induce seizures within behavioral zebrafish epilepsy models. The PTZ zebrafish epilepsy model has produced conflicting results for the efficacy of three AEDs: Carbamazepine, Lamotrigine, and Topiramate. The goal of our study was to optimize the PTZ assay and then investigate potential causes behind the divergent results across the literature for zebrafish PTZ models. The PTZ assay was optimized using 10 mM PTZ which allowed for consistent seizure activity in 7 days post fertilization (dpf) larvae over a 90-minute period. The 90-minute assay allowed for a broader view of AED efficacy. Seizures were quantified using the large count value and collected in 15-minute time increments using the Zebrabox™ viewpoint software. The data was then examined using the total number of large counts over the 90-minute assay to evaluate the level of seizure activity. We used our optimized assay to test two hypotheses: the efficacy of AEDs will vary based on the circadian effects of light-dark conditions during development and the efficacy of AEDs will depend on whether AEDs are administered acutely with PTZ or whether the zebrafish are pretreated with the AEDs before PTZ exposure. The light exposure during development resulted in differences of AED efficacy in Carbamazepine, Lamotrigine and Topiramate. There were differences in the behavior of pretreated vs acute treated zebrafish in Lamotrigine. Inconsistencies seen within the behavioral assays is due to variability in methodology. The outcomes of our experiments provide a more consistent and effective model that will facilitate comparisons of various drugs for efficacy. We have shown that the zebrafish PTZ behavioral assay can be used to accurately and consistently evaluate AED efficacy.

Disclosures: P. Milder: None. **T. Cummins:** None. **J. Marrs:** None.

Digital Abstract Session

P074. Biomarkers and Therapeutic Approaches in Animal Models of Seizures

Program #/Poster #: P074.02

Topic: B.10. Epilepsy

Title: Mechanisms of neurological protection and death related with transcranial magnetic stimulation using kindled rats.

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Abstract: Mechanisms of neurological protection and death related with transcranial magnetic stimulation using kindled rats. Abstract Epilepsy is an extensively studied neurological disorder, research has been made from animal models to human experimentation, as a result there are several medications and some surgical treatments aim to control this disorder, despite this 30% of patients are hard to control. Transcranial magnetic stimulation (TMS) has been used as an anti-epileptic treatment and neuro-protective barrier, even though this is used as a treatment there is no cellular, molecular or physiological evidence to sustain this. The way we are evaluating cell death is with BCL-2, BAX, citocrom-c and caspase 3 and 8, all of this markers will tell us the pathway of cells death or protection. We are using 25 male Wistar rats, equally divided in five groups, the experimental and sham groups will get an electrode implant by a stereotaxic surgery at the amygdala, the implant will be use for the electrical stimuli using the Kindling model in the experimental group, once the subjects present the conduct associated to Racine 5 they will be randomly included to the TMS or kindling group, TMS group was stimulated before the electrical stimulus, each rat had its own time for the TMS which depended on the time of the last seizure, every EEG was analyzed in both groups, measuring amygdala and cortex response, after ten sessions in Racine 5 the rat will be decapitated immediately after the seizure, the brain will be extracted, dividing it in half, the right hemisphere will be fixated with paraffin for immunohistochemistry, the other half will be dissected for the bulbus olfactorius, motor cerebral cortex, hippocampus, amygdala and cerebellum. So far we have analyzed the EEG and the recordings for both experimental groups, the recording were also examined by a third party to compere the results, showing a difference between groups in a conduct stand of view, the time of seizures decreased as well in the TMS group. Based on the information above our preliminary results shows that there is a behavioral difference during the seizure, with the TMS group having less a lower seizure in time, only with this results we can say that TMS has an effect in epilepsy, the mechanisms in with the effect is made is still unclear, it can either be from the neuro protection created, thus, limiting the recruitment of fibers and structures produced by the recruitment provoked by epilepsy, on the other hand cell death can also be the cause of this behavioral changes, having fewer neurons to work within the structures affected will also reduce the time.

Disclosures: W. Moreno Roman: None. **L.F. Hernandez:** None. **M. Rubio-Osornio:** None. **C.R. Osornio:** None. **P. Rodriguez:** None.

Digital Abstract Session

P074. Biomarkers and Therapeutic Approaches in Animal Models of Seizures

Program #/Poster #: P074.03

Topic: B.10. Epilepsy

Support: NIH/ NINDS grant number NS115049 awarded to Dr. Isgor

Title: Sleep-seizure EEG associations predictive of severity and death risk in epilepsy

Authors: *A. TYULMENKOVA, D. KEITH, S. NICOLAS, G. GARCIA, C. ISGOR;
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Abstract: Mechanisms behind seizure-induced death are not well understood. Sudden Unexpected Death in Epilepsy (SUDEP) seems to rise with seizure severity. Postictal generalized electroencephalography suppression (PGES) is a major risk factor in cardiorespiratory failure, hence an indicator of seizure severity. Findings from EEG monitoring during SUDEP are consistent with a seizure-induced suppression of the brainstem arousal circuits. Majority of SUDEP cases occur during sleep implying that sleep processes may be associated with these fatal effects. In this study, we used a transgenic mouse model of adult-onset spontaneous epilepsy. The mice overexpress brain derived neurotrophic factor (BDNF) in the forebrain under the CaM kinase II alpha promoter (TgBDNF). TgBDNF mice develop convulsive seizures at ~4 months of age which worsen with each seizure episode. Seizures are elicited via tail lifts and cage agitation. We used this model to examine how seizures progress from mild to severe and if this progression is associated with distinct changes in sleep structure. We surgically implanted subdural electrodes on the TgBDNF mice skulls prior to the first seizure episode (3-channel EEG system, Pinnacle Technology, KS) to record seizure and sleep EEG for 12 weeks. We used age- and litter-matched wild-type mice as controls. We hypothesized that as seizure severity progresses, alterations in sleep structure may be used as biomarkers to identify life threatening potential of upcoming seizures. Seizures were recorded once weekly and induced six times at each recording session. We measured duration of total seizure, ictal phase, PGES and post-ictal spiking waves during recovery. Twenty-four hours following seizure induction, mice were connected to EEG apparatus for sleep recording during inactive phase. We analyzed early and late sleep separately to define the dynamic changes in rapid eye movement (REM) and slow-wave sleep (SWS) phases. Data was assessed by simple regression analyses between seizure and sleep parameters. Our data showed a positive association between the amount of time mice spent asleep in early sleep to longer periods of total motor seizure duration. As expected, PGES duration increased as did the seizure episodes. PGES duration showed a positive association with increased time spent sleeping in early sleep. Severe seizures had increased spiking wave discharge abnormalities in the recovery phase of EEG. The duration of these abnormalities showed a positive association with SWS phase during early sleep. Monitoring sleep changes in epileptic patients and finding critical sleep-seizure EEG associations could lead to quicker identification of death risk.

Disclosures: A. Tyulmenkova: None. **D. Keith:** None. **S. Nicolas:** None. **G. Garcia:** None. **C. Isgor:** None.

Digital Abstract Session

P074. Biomarkers and Therapeutic Approaches in Animal Models of Seizures

Program #/Poster #: P074.04

Topic: B.10. Epilepsy

Support: NIH Grant NS111122

Iowa Neuroscience Institute Fellowship

Title: Diverse spiking signatures of excitability mutations emerge from a stereotypic seizure-flight sequence in Drosophila

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Abstract: High-frequency electrical stimulation across the brain triggers seizures in humans, mice and flies. In *Drosophila* such stimulation induces stereotypic electroconvulsive seizure (ECS) discharges which manifest across the nervous system and can be monitored through spiking in the large indirect flight muscles (DLMs) in conjunction with microphone recordings of wing beats. During flight, the DLM motor neuron spikes rhythmically (~5 Hz) providing Ca^{2+}influx for stretch-activated myogenic contractions which powers wing beats (~200 Hz). In contrast, during ECS discharges, a distinctive sequence of firing modes have been reported: an initial discharge (ID, ~50 Hz), a quiescent period, and a delayed seizure discharge (~30 Hz peak). Although widely in studies of circuit excitability, most studies employing ECS in *Drosophila* examine the induction threshold, with considerably less attention on alterations in firing patterns during the discharges. Using an isolated stimulation configuration, we discovered that wild-type (WT) flies would reliably display sustained flight activity following DD (DD-flight). Compared to air-puff triggered flight, DLM spiking during DD-flight was higher (~10 - 20 Hz) while the wing-beat frequency was lower (~170 Hz). We observed a similar motor sequence in other WT strains and in other *Drosophila* species. Furthermore, we observed DD-flight after the surgical removal of sensory structures required for air-puff triggered flight, including the halteres, antennae and wings. Across broad ranges of excitability mutants, we found that spiking activity during the DD can serve as a 'signature' of the specific manifestation hyperexcitability. Non-linear dynamical systems analyses revealed clear distinctions between two classes of hyperexcitable mutants: 'leg-shakers'(e.g. *Sh*, *qvr*) that twitch under ether anesthesia, and 'bang-sensitives' (e.g. *eas*, *sda*) which display mechanical shock-induced seizures. In leg-shaker mutants, with disrupted I_AK^+currents, the DD-evoked flight sequence was largely intact, but with a declining wing beat frequency, presumably reflecting alterations direct flight muscle biophysical properties. In contrast, bang-sensitive mutants displayed mutant-specific DLM ECS discharge patterns, with varying duration, firing frequency and regularity. However, a common feature across the bang-sensitive mutants was the complete absence of DD-evoked flight. This work demonstrates that our quantitative treatments of spike patterning can provide succinct signatures for a variety of mutations-specific vulnerabilities in motor circuits.

Disclosures: A. Iyengar: None. **C. Wu:** None.

Digital Abstract Session

P075. Molecular Pathways in Seizures: Animal Models

Program #/Poster #: P075.01

Topic: B.10. Epilepsy

Title: Expression of the wnt-b catenin pathway in the limbic system in seizure-induced models subjected to caloric restriction

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Abstract: The Wnt/β-catenin pathway increases glucose absorption at the cortical level, increasing neuronal excitability, translating epileptic seizures. By conditioning caloric restriction in rats with epileptic seizures induced by the kindling model, the intention was to decrease the cerebral metabolic substrate and observe changes in the expression of the Wnt pathway in the hippocampus and amygdala, the most susceptible zones to nutritional changes, as well as to epileptic activity. We quantify the expression of the proteins Wnt3A, β-catenin, P-β-catenin, cyclin D, and c-Myc in the brain tissue of the amygdala and hippocampus of the rat with generalized crisis and caloric restriction. We used male Wistar rats; we implanted electrodes in the basolateral nucleus of the amygdala and underwent stimulation to develop the kindling model. After the experimentation phase, we performed immunohistochemistry and Western blot techniques with specific antibodies of the Wnt / β-catenin pathway. The caloric restriction showed an increase of c-Myc, cyclin D, β-catenin, P-β-catenin in the amygdala and hippocampus comparing with the kindled group; and a decrease in the Wnt/β-catenin pathway in the dentate gyrus, in relation to the animals that only had kindling, resulting in a decrease in the duration of amygdalin epileptic activity in our caloric restriction group. Restrictive diets provide a neuroprotective effect in epilepsy. CR has anticonvulsant effects in murine and other epilepsy models. CR in rats with induced-seizures using the kindling model increased the threshold after a stimulus applied to the amygdala as well as decreased the duration of the convulsive activity.

Disclosures: R. Luna: None. **D. Rojas:** None. **E. Taddei:** None. **M. Rubio Osornio:** None. **L.F. Hernandez:** None. **M.C. Rubio Osornio*:** None.

Digital Abstract Session

P075. Molecular Pathways in Seizures: Animal Models

Program #/Poster #: P075.02

Topic: B.10. Epilepsy

Title: Participation of the Wnt / β-catenin pathway in the cerebello-thalamo-cortical pathway of rats with epileptic seizures induced by kindling model subjected to caloric restriction.

Authors: *D. A. ROJAS HERNÁNDEZ[1], R. LUNA[1], M. RUBIO OSORNIO[2], L. F. HERNÁNDEZ LÓPEZ[1], E. TADEI[1], J. BRITO[1], M. C. RUBIO OSORNIO[1];
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Abstract: The cerebello-thalamo-cortical pathway participates in epileptic activity, this activity can be modified by glucose metabolism and by ketone products derived from glycolytic metabolism and fatty acids; the *Wnt / β-catenin* pathway has shown to induce metabolic changes in neuronal glucose; these changes have shown to have a stabilizing effect on the neuronal membrane with respect to its excitability, producing an anticonvulsant effect. We infer that caloric restriction (CR) generates a decrease in the metabolic substrate, then in rats with epileptic seizures induced by the kindling model, a modification in the expression of the *Wnt* pathway will be observed. We quantified the expression of the *Wnt*3a, β-catenin, Pβ-catenin, c-Myc and cyclin-D proteins in the cerebellum, ventrolateral nucleus of the thalamus (VL) and cortex of rats with generalized seizures and CR; male Wistar rats were used which were divided into 3 groups: a) control group; b) kindled group fed ad libitum; c) kindled group with CR, fed with controlled diets according to their weight. Once the experimentation phase was finished, the rats were sacrificed, the cerebellum, VL and cortex were extracted, immunohistochemistry and Western Blot was performed with specific *Wnt*3a, β-catenin, Pβ-catenin, c-Myc and cyclin-D antibodies. We found that the animals subjected to CR decrease the epileptic activity, in addition to restoring the quantification of the *Wnt* / β-catenin pathway proteins to control values, compared to the group that had epileptic seizures with *ad libitum* food. We conclude that restrictive diets provide a neuroprotective effect in epileptic seizures. According to the immunohistochemical analysis, we showed changes in the amount and location of the Wnt / β-catenin pathway in the cerebello-thalamo-cortical pathway, a structure that has shown its participation in epileptic activity. These results suggest that the changes induced by the kindling epilepsy model generate modifications in the expression and activation of proteins of the *Wnt* / β-catenin pathway and that this is modified depending on CR.

Disclosures: D.A. Rojas Hernández: None. **M.C. Rubio Osornio:** None. **R. Luna:** None. **J. Brito:** None. **E. Tadei:** None. **M. Rubio Osornio:** None. **L.F. Hernández López:** None.

Digital Abstract Session

P075. Molecular Pathways in Seizures: Animal Models

Program #/Poster #: P075.03

Topic: B.10. Epilepsy

Support: NIGMS T32 GM008541
NINDS R01 NS051710
NINDS R56 NS117141

Title: Knockout of canonical STAT3 signaling in excitatory neurons of a temporal lobe epilepsy model attenuates the development of chronic seizures and fear learning deficits and rescues inflammatory and synaptic signaling at a transcriptomic level.

Authors: *A. E. TIPTON[1,2], K. M. HIXSON[1,2], Y. CRUZ DEL ANGEL[3], J. CARLSEN[3], A. BROOKS-KAYAL[3], S. J. RUSSEK[1,2];
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Abstract: Epilepsy is a common neurological disorder characterized by presence of unprovoked, spontaneous seizures (SS) on at least two occasions, affecting 65 million people worldwide. Currently available treatments only work in 2/3 of patients, and often exacerbate neuropsychiatric comorbidities commonly seen with epilepsy, including mood disorders and cognitive dysfunction. The design of novel therapeutics, including those with potential for disease modification, requires a deeper understanding of the molecular cascades responsible for generating and maintaining chronic, spontaneous seizures. This process of epileptogenesis can be studied using the intrahippocampal kainic acid (IHKA) model of temporal lobe epilepsy (TLE). Previous work from our labs has identified the JAK2/STAT3 pathway, traditionally associated with inflammatory responses in immune cells, as relevant in the response of neurons to excess stimulation such as elevated BDNF levels, or status epilepticus (SE). *In vitro* studies of primary hippocampal neuronal cultures demonstrated that application of JAK2/STAT3 inhibitors rescues the dysregulated BDNF-induced changes in expression levels of key ion channels and neurotransmitter receptors, as well as genes implicated in neuroinflammation. Moreover, it appears this is a therapeutically relevant target in epilepsy, as rats treated with one dose of the JAK/STAT inhibitor WP1066 developed significantly fewer SS than controls. The current study utilized a tamoxifen-inducible, cell-type specific STAT3 KO mouse driven by a CAMKIIA promoter, such that STAT3 KO is specific to excitatory neurons (NSTAT3KO). Remarkably, NSTAT3KO mice display both decreased frequency of SS as well as rescue of deficits in hippocampal-dependent fear conditioning, suggesting inhibition of STAT3 in excitatory neurons may help address both seizures and epilepsy comorbidities. Analysis of bulk RNA-sequencing data obtained from hippocampi harvested 24 hours post-SE suggests neuronal STAT3 is an important regulator of both neuronal excitability and its inhibition. Moreover, NSTAT3KO leads to reversal in the upregulation of numerous inflammatory pathways activated in epilepsy via microglia and astrocytes. This finding suggests that *neurons themselves may play an important role in the induction and maintenance of inflammation after SE.* Future studies will utilize single nuclei transcriptomic approaches to directly determine how STAT3 impacts gene expression and DNA accessibility across all brain cell types, with the ultimate goal of uncovering the function of JAK2/STAT3 in excitatory neurons and the viability of targeting this novel pathway in epilepsy.

Disclosures: A.E. Tipton: None. **K.M. Hixson:** None. **Y. Cruz Del Angel:** None. **J. Carlsen:** None. **A. Brooks-Kayal:** None. **S.J. Russek:** None.

Digital Abstract Session

P075. Molecular Pathways in Seizures: Animal Models

Program #/Poster #: P075.04

Topic: B.10. Epilepsy

Support: Canadian Institutes of Health Research
Savoy Foundation Canada

Epilepsy Canada
Fonds de recherche Santé Quebec
Fondation CHU Sainte-Justine

Title: The role PIGB in neurodevelopment and in the pathogenesis of early epileptic encephalopathy

Authors: *P. K. PEDABALIYARASIMHUNI[1,2], K. TOULOUSE[1,2], T. T. M. NGUYEN[1], A. LUPIEN-MEILLEUR[1,2], L. EID[1,2], M. LACHANCE[1], P. CAMPEAU[1,3], E. ROSSIGNOL[1,4]; [1]CHU Ste-Justine Res. Ctr., Montréal, QC, Canada; [2]Dept. of Neurosciences, [3]Dept. of Pediatrics, [4]Departments of Neurosciences and Pediatrics, Univ. de Montréal, Montréal, QC, Canada

Abstract: BACKGROUND. Epileptic encephalopathies (EE) are severe early-onset epilepsies with cognitive deficits and early lethality. We recently identified recessive mutations in the *PIGB* gene, encoding the mannosyltransferase 3 protein, acting in the glycosylphosphatidylinositol (GPI)-anchor biosynthesis pathway, in patients with EE. GPI anchors localize various receptors, ion channels and cell adhesion proteins on cell membranes. However, the mechanisms by which deficits in GPI anchors result in such a striking neurodevelopmental phenotype are unknown. Recent data from our group suggest that impairments in the early development or function of inhibitory GABAergic interneurons (IN) result in epilepsy and cognitive deficits in mice, thus contributing to specific genetic forms of EE. **HYPOTHESIS**. We postulate that GPI anchor deficit results in EE by impairing IN development. **METHODS**. We generated different mouse models carrying targeted *Pigb* mutations in distinct neuronal populations. We combined high-resolution cell imaging, immunohistochemistry, neuronal quantification, and morphological reconstitution, together with video-EEG recordings and behavioral analysis to characterize these models and investigate the underlying mechanisms. **RESULTS**. While constitutive knock-out and conditional mutant mice in cortical excitatory neurons ($Emx1^{Cre}$) are not viable, mice with GPI deficiency carrying a targeted deletion of *Pigb* in MGE-derived INs ($Nkx2.1^{Cre}$) are viable and develop spontaneous seizures as well as behavioral deficits reminiscent of those observed in patients. Furthermore, neuronal quantification at different key developmental stages (e13.5, e15.5, P0) revealed a delay in IN migration, with reduced number of IN at the migratory front embryonically (e13.5 and e15.5) and in the postnatal cortex (P14 and P21). Notably, 3D reconstruction of e13.5 migrating INs revealed striking perturbations in IN morphology, including increased neurite length, number and complexity of leading and trailing processes. Moreover, live imaging of MGE explants suggests perturbations of migration kinetics in INs. **CONCLUSION**. Our results reveal that the loss of GPI anchors, through *Pigb* recessive mutations, impairs IN migration, resulting in epilepsy and cognitive deficits. Subsequent studies will help unveil the specific candidate GPI-anchored proteins required to sustain IN migration, further clarifying the underlying disease mechanisms.

Disclosures: P.K. Pedabaliyarasimhuni: None. **K. Toulouse:** None. **T.T.M. Nguyen:** None. **A. Lupien-Meilleur:** None. **L. Eid:** None. **M. Lachance:** None. **P. Campeau:** None. **E. Rossignol:** None.

Digital Abstract Session

P075. Molecular Pathways in Seizures: Animal Models

Program #/Poster #: P075.05

Topic: B.10. Epilepsy

Support: NHMRC grant APP1161571
The Lambert Initiative

Title: Trpv1 deletion reduced the severity but not the frequency of spontaneous seizures in a Scn1a+/- model of Dravet syndrome.

Authors: *V. S. JANVE, D. BAHCECI, L. ANDERSON, D. MORGAN, J. ARNOLD; Pharmacol., The Univ. of Sydney, Sydney, Australia

Abstract: Rationale: Dravet syndrome (DS) is an early onset epileptic encephalopathy that begins with febrile seizures and progresses to spontaneous afebrile seizures that are poorly managed by anti-seizure drugs. Majority of the DS patients have loss of function *Scn1a* mutations. The Scn1a$^{+/-}$ mouse model recapitulates features of DS with increased sensitivity to febrile seizures and early onset spontaneous seizures. Cannabidiol (CBD) has been FDA-approved for treatment of DS. While the anti-seizure mechanism of CBD is unknown, it activates and rapidly desensitizes transient receptor potential vanilloid type 1 (Trpv1) cation channels. Trpv1 channel inhibition has been anticonvulsant against PTZ, 6-Hz, and MES-induced seizures in mice. Thus, we tested whether heterozygous deletion of *Trpv1* or inhibition of Trpv1 channels is anticonvulsant in the *Scn1a*$^{+/-}$ model of DS. **Methods:** Scn1a$^{+/-}$ mice were generated as in Miller AR, 2014 and bred with *Trpv1*$^{+/-}$ mice to generate *Scn1a*$^{+/-}$*,Trpv1*$^{+/-}$ mice. Hyperthermia was induced in postnatal day (P) 14-16 mice with a heat lamp and measured with a rectal probe. Onset of the 1st generalised tonic-clonic seizure (GTCS) was noted as the seizure threshold temperature. Mice that reached 42.5 °C without a GTCS for 3 min were considered seizure free. To mimic clinical progression, P18 mice were subjected to a single hyperthermia-induced GTCS followed by rapid cooling (priming). After recovery spontaneous seizures were video monitored for 60 hours. *Scn1a*$^{+/-}$ mice were challenged with hyperthermia-induced GTCS 15 minutes (T_{max}) following i.p. injection of 10 and 20 mg/kg of SB-705498. **Results:** *Scn1a*$^{+/-}$*,Trpv1*$^{+/-}$ had similar hyperthermia-induced GTCS threshold compared to that of Scn1a$^{+/-}$ mice at P14-16 (n=13, p=0.15). Survival and frequency of spontaneous seizures were also similar between *Scn1a*$^{+/-}$*,Trpv1*$^{+/-}$ mice and Scn1a$^{+/-}$ mice (n=16-18, p≥0.05). However, severity of spontaneous seizures, i.e. the percent of seizures progressing to full hindlimb extension, was significantly reduced in Scn1a$^{+/-}$,Trpv1$^{+/-}$ mice compared to Scn1a$^{+/-}$ mice (n=16-18, p=0.0003). Interestingly, priming P18 significantly reduced the hyperthermia-induced GTCS threshold in *Scn1a*$^{+/-}$*,Trpv1*$^{+/-}$ compared to Scn1a$^{+/-}$ mice (n=18-20, p=0.007). Further, acute administration of Trpv1 selective antagonist SB-705498 in Scn1a$^{+/-}$ mice did not affect hyperthermia-induced GTCS threshold compared to vehicle-treated mice, and its effects on spontaneous seizures are being tested. While Trpv1 channel inhibition has been anticonvulsant in several seizure models,

genetically or pharmacologically targeting Trpv1 is not anticonvulsant in the *Scn1a*$^{+/-}$ model of DS.

Disclosures: **V.S. Janve:** None. **D. Bahceci:** None. **L. Anderson:** None. **D. Morgan:** None. **J. Arnold:** None.

Digital Abstract Session

P075. Molecular Pathways in Seizures: Animal Models

Program #/Poster #: P075.06

Topic: B.10. Epilepsy

Support: Canada Research Chairs program
Natural Sciences and Engineering Research Council of Canada

Title: Panx1 and seizures

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Abstract: Pannexin1 Knockout Zebrafish as a Model for Seizure Investigation.
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1. Department of Biology, York University, Toronto2. Krembil Research Institute, University Health Network, Toronto, Canada.
Recurrent seizures are a hallmark of epilepsy. An imbalance of excitation-inhibition is known to promote seizure generation, and molecular determinants of these alterations remain to be elucidated. Experimental evidence suggests that Pannexin1, an ATP release channel, is a candidate for modifying neuronal excitability that underlies seizure activity. Here, genetically modified zebrafish lines and pharmacological inhibition of Panx1 channels with the FDA-approved drug Probenecid was used to evaluate the efficacy of targeting Panx1 for improving seizures. The pentylenetetrazol (PTZ) model was used to chemically induce seizures during *in vivo* electrophysiological recordings from tectal brain regions of zebrafish larvae. We paired these results with behavioral phenotyping in age-matched larvae. Further, molecular biological techniques were used to determine stress responses and to investigate a mechanistic explanation for the involvement of Panx1 in seizures. Results demonstrate that loss-of-function mutations in Panx1 genes, or pharmacologically blocking the channels, significantly reduces ictal-like events during electrophysiological recordings without altering inter-ictal like activity. A decrease in seizure-related locomotor behavior is accompanied by an improvement in survival rates and reduced molecular fingerprints of cellular stress responses. Our research suggests that Panx1 channels contribute to distorting neurons' electrochemical balance, likely via an ATP release mechanism, and aid in propagating or maintaining seizures. With almost 40% of people with epilepsy having recurrent seizures uncontrolled by medication, we propose that Panx1 channel inhibition could open a window of opportunity to treat drug-resistant epilepsies.

Disclosures: **G. Zoidl:** None. **P. Whyte-Fagundes:** None. **D. Taskina:** None. **N. Safarian:** None. **C. Zoidl:** None.

Digital Abstract Session

P076. Networks

Program #/Poster #: P076.01

Topic: B.10. Epilepsy

Support: NIH NINDS K08NS105929

Title: Marked Insomnia in a Mouse Model of Medial Temporal Lobe Epilepsy

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Abstract: In many patients with epilepsy, seizures have a predilection for a particular sleep-wake state or sleep-wake state transition. Further suggestive of a strong relationship between sleep-wake and seizures is the commonality of sleep disturbances in epilepsy and the improvement of seizures with the treatment of sleep disorders. While sleep-wake has been studied in patients with epilepsy, there are relatively few animal studies. We sought to examine the sleep effects of developing seizures in a mouse model of medial temporal lobe epilepsy. These studies will form the basis for cell-selective manipulations of sleep-wake networks to develop a mechanistic understanding of how sleep-wake circuits contribute to seizures. Mice were instrumented using a custom-designed head plate to permit reproducible recordings of the bilateral hippocampus, electrocorticogram, and electromyogram of neck muscles, with the later capacity for optogenetics and fiber photometry. Mice were habituated and underwent chronic video-EEG recording with intra-amygdala kainic acid microinjection by cannula one week later, and recording continuing for a further three weeks. Sleep was significantly disrupted: We found increased wake in mice with seizures (~ 15%, ANOVA $P = 0.019$) as well as increased frequency ($P < 0.05$) and duration of seizures (ANOVA $P = 0.03$) during sleep in this model. Seizures were rarely observed in rapid eye movement (REM) sleep. These findings recapitulate observations from patients with MTLE and form a basis of further work that will cell- selectively manipulate components of sleep-wake networks to modify seizure risk and help understand underlying mechanisms.

Disclosures: **N.P. Pedersen:** F. Consulting Fees (e.g., advisory boards); Dixie Medical USA. **L.M. Aiani:** None. **L. Rosenberg:** None.

Digital Abstract Session

P076. Networks

Program #/Poster #: P076.02

Topic: A.07. Developmental Disorders

Support: 2019 CDKL5 PROGRAM OF EXCELLENCE GRANT PROGRAM/ODC
University of Pennsylvania & Loulou Foundation
KAKENHI 18K07784

Title: In vivo wide-field Ca^{2+} imaging of the cortical spontaneous activity in the Cdkl5 mutant mice reveals altered functional connectivity upon the loss-of-function of CDKL5

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Abstract: Loss-of-function (LOF) mutations in the Cyclin-dependent kinase-like 5 (CDKL5) gene cause severe neurodevelopmental disorders accompanied by early-onset intractable epilepsies, i.e. CDKL5 deficiency disorder (CDD). We have previously generated the *Cdkl5* knock-out (KO) mice and demonstrated postsynaptic overaccumulation of GluN2B-containing NMDA receptors in the hippocampus, significantly enhanced anxiety-like behaviors, and impaired acquisition and retention of spatial reference memory. However, mechanisms underlying these phenotypes remain unknown, and identifying the brain-wide, synapse-to-circuit functional alteration upon the LOF of CDKL5 is necessary to understand the mechanisms. In order to determine how the LOF of CDKL5 has an impact on the cortical neurocircuits, we have adopted the high-resolution wide-field Ca^{2+} imaging of the cortical spontaneous activity in the genetically encoded calcium indicator (GECI)-expressing *Cdkl5* mutant mice. We generated the GCaMP6f-expressing *Cdkl5* KO and kinase-dead knock-in (KI) mice in which GCaMP6f was neuronally expressed by the Thy1 promoter. We performed the *in vivo* wide-field Ca^{2+} imaging of GCaMP6f-expressing *Cdkl5* hemizygous mutant and control mice under anesthesia, awake, and with visual stimulation, at postnatal day (P) 21 and P60. We set 18 regions of interest (ROIs) in each hemicortex, and analyzed spontaneous activity patterns by functional correlation analysis and principal component analysis (PCA). We then evaluated differences in the functional connectivity across different genetic groups. We have so far acquired data from four *Cdkl5* KO, four *Cdkl5* kinase-dead KI, and eight control mice at P60. Functional correlations of ROIs between right and left hemispheres (correlation inside the red square) are analyzed by one-way analysis of variance (ANOVA) and multiple comparison test. The average inter-hemispheric correlation was higher in *Cdkl5* KO mice than that in the control mice. There was no statistically significant difference in inter-hemispheric correlation between *Cdkl5* kinase-dead KI mice and control mice, but hypersynchronization appeared in several areas such as V1 and V2 in the KI mice. These data suggest that a loss-of-function of CDKL5 causes the inter-hemispheric hypersynchronization of cortical spontaneous activity. Since the inter-animal variation was still high in the present set of data, we need to acquire more data to reach reliable outcomes. We will compare the altered neurocircuit activity patterns with behavioral endophenotypes of the *Cdkl5* mutant mice and verify the site-specific neurocircuits impaired upon the loss-of-function of CDKL5.

Disclosures: **T. Tanaka:** None. **T. Nomura:** None. **T. Murakami:** None. **K. Saitou:** None. **K. Ohki:** None.

Digital Abstract Session

P077. Epilepsy: Anticonvulsant and Antiepileptic Therapies

Program #/Poster #: P077.01

Topic: B.10. Epilepsy

Support: 2019 Neurological IAA AOD19020-001-00000

Title: Investigation of retigabine as a potential treatment for nerve agent-induced status epilepticus

Authors: *P. SHEU[1], B. S. BARKER[1], J. SPAMPANATO[2], K. BERGER[1], C. E. JACKSON PIERCY[1], H. S. MCCARREN[1], J. H. MCDONOUGH, Jr[1];
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Abstract: Nerve agents can induce seizures that rapidly progress to status epilepticus (SE). These seizures are clinically treated with benzodiazepines. However, studies in delayed treatment models have established that benzodiazepines lose the ability to stop seizures. Retigabine is a K_v7 (KCNQ) potassium channel opener originally used chronically as an adjunctive treatment for partial seizures. Retigabine promotes K^+ efflux, which can counteract Na^+ influx during depolarization. To investigate the effectiveness of retigabine in controlling nerve agent-induced SE, male Sprague Dawley rats were exposed to the nerve agent soman to elicit SE and continuously observed through electroencephalogram recording. Retigabine was administered at 30 mg/kg as an adjunct to midazolam at 20 or 40 min after SE initiation to evaluate its anticonvulsant effects against benzodiazepine-resistant SE. Controls received PEG200. In the 12 subjects that received 30 mg/kg at 20 min post-initiation of SE, SE stopped in 8 and stayed off for 4 hr. Their gamma power and mean spike frequency were significantly lower for these 4 hr compared to controls (n=17). Animals that received retigabine at 30 mg/kg administered 40 min after SE initiation (n=15) showed similar results compared to controls (n=11), where 9 had seizure termination. To evaluate retigabine as a monotherapy, it was administered without midazolam at 30 mg/kg or 60 mg/kg at 20 min after SE initiation. At 30 mg/kg retigabine alone (n=8) showed minimal anticonvulsant effects with only one animal showing seizure termination and no overall significant difference found versus controls. However, when the dose was increased to 60 mg/kg (n=14), 10 animals showed seizure termination, and of those 10, 8 were seizure free for 24 hr. This group showed significant differences in gamma power and mean spike frequency when compared to both controls and the 30 mg/kg retigabine only group. These results indicate retigabine is effective as not only an adjunctive anticonvulsant with midazolam at both time points but also a stand-alone SE treatment when administered at a higher dose. Additionally, these results prompt increased interest in K_v7 channels as possible targets for attenuating benzodiazepine-resistant SE.

Disclosures: P. Sheu: None. **K. Berger:** None. **H.S. McCarren:** None. **J.H. McDonough:** None. **B.S. Barker:** None. **C.E. Jackson Piercy:** None. **J. Spampanato:** None.

Digital Abstract Session

P077. Epilepsy: Anticonvulsant and Antiepileptic Therapies

Program #/Poster #: P077.02

Topic: B.10. Epilepsy

Support: AOD18013-001-00000
AOD19020-001-00000

Title: Super refractory status epilepticus in rats following multiple therapeutic treatments for nerve agent exposure

Authors: *B. J. TRAVIS, H. M. BELSKI, K. M. BOUNADER, J. M. COPPOLA, E. N. HORNUNG, K. A. HUNDERTMARK, C. E. JACKSON-PIERCY, K. T. PAGARIGAN, S. C. WILSON, J. E. MORGAN, H. S. MCCARREN;
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Abstract: Organophosphate nerve agent (NA) exposure poses risk to both military and civilians, producing many toxic symptoms including status epilepticus (SE). SE can quickly progress if not treated promptly and can be fatal if left untreated. Emergency treatments for NA poisoning are often ineffective if delayed and provide no lasting seizure control. Thus, in-hospital care would be required to definitively treat NA-induced SE; yet, no standard of care exists. Clinical care for SE of other etiologies utilizes benzodiazepines as first-line treatment, anti-epileptics as second-line, and general anesthetics as third-line. SE is considered established if first-line treatment fails, refractory status epilepticus (RSE) if second-line fails, and super refractory status epilepticus (SRSE) if third-line fails. Given the severity and generalized nature of the cholinergic crisis produced, it's expected NA-induced SE cases would advance to RSE or SRSE; though, progression to SRSE following third-line treatment has not been researched. We demonstrated the presence of NA-induced SRSE after third-line drug administration. Adult male rats were challenged with a seizure-inducing dose of soman. Following seizure onset, determined by electroencephalography, standard medical countermeasures (atropine sulfate, 2-PAM, and midazolam) were administered, and the rat was placed in an oxygen-rich, temperature-controlled chamber. If seizure wasn't controlled, up to 2 boluses of midazolam or lorazepam were administered intravenously, followed by up to 2 boluses of phenobarbital or valproate, and finally repeated bolus administration of propofol or ketamine until seizure termination, with continuous infusion for 18 hr upon termination. If seizure returned, it was considered to be SRSE. All rats that received third-line treatment (n=67) had seizure termination following intravenous administration of propofol (n=31) or ketamine (n=36). Fifty rats died during continuous infusion. The incidence of mortality was the same regardless of treatment (p=0.57). SRSE was observed in 17 rats (propofol n=10; ketamine n=7). One rat was weaned off ketamine without seizure recurrence and showed promising markers of functional recovery, including a return to baseline weight by day 3 post-exposure and absence of brain damage in 5 critical regions with histopathologic scores of 0. Standard treatment protocols and clinical

recommendations failed to prevent SRSE, highlighting both the necessity for a standardized clinical intensive care regimen for treatment of SE and justification for further research into alternative pharmacological agents for effective termination of NA-induced SE.

Disclosures: B.J. Travis: None. **H.M. Belski:** None. **K.M. Bounader:** None. **J.M. Coppola:** None. **E.N. Hornung:** None. **K.A. Hundertmark:** None. **C.E. Jackson-Piercy:** None. **K.T. Pagarigan:** None. **S.C. Wilson:** None. **J.E. Morgan:** None. **H.S. McCarren:** None.

Digital Abstract Session

P077. Epilepsy: Anticonvulsant and Antiepileptic Therapies

Program #/Poster #: P077.03

Topic: B.10. Epilepsy

Support: NIH AOD20020-001-00000

Title: Therapeutic regimens for complete resolution of nerve agent-induced status epilepticus in rats

Authors: *S. C. WILSON, B. J. TRAVIS, K. T. PAGARIGAN, K. A. HUNDERTMARK, C. E. JACKSON PIERCY, E. N. HORNUNG, K. M. BOUNADER, H. M. BELSKI, J. M. COPPOLA, J. E. MORGAN, H. S. MCCARREN;
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Abstract: Soman is a highly potent organophosphorus nerve agent that inhibits the activity of acetylcholinesterase. The resulting excess of acetylcholine at neuromuscular junctions, glands, and within central nervous system synapses results in miosis, hypersecretion, fasciculation, respiratory distress, and seizures that rapidly progress to status epilepticus (SE). Currently no clinical protocol exists for the definitive treatment of nerve agent-induced SE. Pre-hospital treatment alone in the form of benzodiazepine auto-injectors likely would not be sufficient to stop SE if treatment were delayed. A severely poisoned casualty is highly likely to need intensive in-hospital care to completely terminate SE. This work investigated the utility of commonly used SE treatment protocols in a simulated hospital setting to assess their efficacy against soman-induced SE.Adult male rats were challenged with soman, and 20 min after SE onset, as confirmed by electroencephalographic recording, they were administered standard pre-hospital medical countermeasures (atropine sulfate, pralidoxime, and midazolam). They were then moved to intensive care units (ICU) containing 100% oxygen and thermal support. Ten minutes after arrival in the ICUs, rats were administered intravenous (IV) first-line treatment of either midazolam or lorazepam. If this treatment failed to control SE they were administered a second-line treatment of either phenobarbital or valproate. Midazolam (n = 72) and lorazepam (n = 64) terminated SE in 10% and 9% of rats respectively, but in all cases SE returned or the rat died. Phenobarbital (n = 78) was significantly better at terminating SE than was valproate (n = 43) (56% vs. 35%, $p > 0.0360$). SE returned within 24 hours for all rats treated with valproate, while 21% of phenobarbital-treated rats had lasting seizure control ($p > 0.0006$). These rats with

definitive SE resolution returned to baseline weight 3-7 days after exposure, while controls that only received pre-hospital countermeasures took at least 8 days to regain weight. There was also a significant reduction in the severity of histopathology, based on H&E staining, for successfully treated rats compared to controls ($p < 0.0001$).In conclusion, while first-line administration of benzodiazepines was ineffective at permanently terminating SE, second-line treatment with phenobarbital was effective in a subset of cases and led to positive indications of recovery. Future work will investigate the effectiveness of first-line treatment with phenobarbital and other less established SE therapies.

Disclosures: S.C. Wilson: None. **B.J. Travis:** None. **K.T. Pagarigan:** None. **K.A. Hundertmark:** None. **C.E. Jackson Piercy:** None. **E.N. Hornung:** None. **K.M. Bounader:** None. **H.M. Belski:** None. **J.M. Coppola:** None. **J.E. Morgan:** None. **H.S. McCarren:** None.

Digital Abstract Session

P077. Epilepsy: Anticonvulsant and Antiepileptic Therapies

Program #/Poster #: P077.04

Topic: B.10. Epilepsy

Support: AOD20020-001-00000

Title: Evaluating anti-epileptic drugs in an intensive care setting for the treatment of nerve agent-induced status epilepticus in rats

Authors: J. E. MORGAN, S. C. WILSON, B. J. TRAVIS, K. PAGARIGAN, P. SHEU, K. A. HUNDERTMARK, H. S. MCCARREN;
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Abstract: Nerve agent (NA) poisoning initiates a catastrophic cholinergic crisis that can induce *status epilepticus* (SE) if left untreated. Survivors of NA exposure will likely require treatment in a hospital intensive care setting. Guidelines currently exist for pre-hospital management of NA-induced SE, which includes up to two doses of a benzodiazepine. However, previous research in rodent models has indicated that doses of diazepam equivalent to 3-12 convulsive antidote for nerve agent (CANA) auto-injectors are insufficient to stop SE. There is currently no standard therapy regimen for definitive termination of NA-induced SE. Recent work in our lab has attempted to determine a treatment strategy to completely resolve NA-induced SE in a hospital setting that is guided by clinical strategies for treating SE of other etiologies. Male Sprague Dawley rats were surgically implanted with jugular vein catheters and tethered EEG headpieces. Twenty minutes after soman exposure they received human equivalent doses of standard pre-hospital countermeasures for NA exposure (atropine, 2-PAM, and midazolam), followed ten minutes later by initiation of in-hospital treatments. The typical first-line treatments midazolam and lorazepam were equally ineffective at terminating SE ($p > 0.999$), with respective seizure termination rates of 10% ($n = 72$) and 9% ($n = 64$). When administered after the failure of first-line benzodiazepines, valproic acid and phenobarbital showed significantly different efficacy (p

= 0.036), with respective seizure termination rates of 34% (n = 43) and 56% (n = 78). However, seizures returned in all of the valproate-treated rats, and only a small subset of phenobarbital-treated rats (n = 16) remained seizure free 24 hours later. We hypothesized that treating with antiepileptic drugs first, rather than waiting until after benzodiazepine refractoriness was established, would lead to greater seizure control. When administered immediately upon arrival in the ICU, phenobarbital (n=17) was more effective at terminating seizures than was valproic acid (n= 10, p = 0.015). While both valproic acid and phenobarbital had good initial seizure termination (80% and 100%, respectively), phenobarbital was more effective at permanently terminating seizures (59%) than was valproic acid (0%). This suggests that benzodiazepines may not be a necessary precedent for termination of NA-induced seizures with anti-epileptic drugs. Additionally, brain tissue of rats that received phenobarbital displayed minimal damage in key areas such as the amygdala, thalamus, and piriform cortex. Evaluation of other anti-epileptic drugs in this model is ongoing.

Disclosures: J.E. Morgan: None. **H.S. McCarren:** None. **S.C. Wilson:** None. **B.J. Travis:** None. **K. Pagarigan:** None. **P. Sheu:** None. **K.A. Hundertmark:** None.

Digital Abstract Session

P077. Epilepsy: Anticonvulsant and Antiepileptic Therapies

Program #/Poster #: P077.05

Topic: B.10. Epilepsy

Support: NINDS CounterACT program grant 1U54NSO79202

Title: Intramuscular dose of midazolam in rats producing exposure equivalent to recommended dose of Seizalam® (midazolam injection) in humans

Authors: *A. DHIR, C. YI-WU, M. A. ROGAWSKI;
Neurol., Univ. of California, Davis, Sacramento, CA

Abstract: Seizalam® is a midazolam HCl (MDZ) formulation approved by the FDA as a first-line treatment for status epilepticus (SE) in adults. The recommended dose is 10 mg, administered by intramuscular (IM) injection. According to its label, Seizalam® at the recommended dose yields a Cmax of 113.9 ± 30.9 (SD) ng/ml. Here we sought to estimate the dose of MDZ which when administered intramuscularly in rats would produce a plasma exposure equal to this value. Our objective was to define the appropriate dose of MDZ to be used in studies in rats of novel SE treatments that may be administered in conjunction with Seizalam®. We provide pharmacokinetic (PK) parameters based on noncompartmental analysis (NCA) of plasma level data and we used two-compartment PK modeling to estimate the rat-equivalent dose. Our analysis assumes that Cmax is the appropriate PK parameter to match. Male Sprague-Dawley rats were implanted with a permanent cannula in the right jugular vein. After 7-10 days of post-operative care, animals were injected with a 0.9 mg/kg IM dose of MDZ. Blood was withdrawn at 0, 2, 5, 15, 30, 60, 120, 240, 300, 360, 480 and 1440 min after the MDZ

injection. The plasma was separated and MDZ levels were measured using LC-MS/MS (<LLOQ=50 pg/ml). A NCA was performed on the plasma level data from each animal to estimate the Tmax, Cmax and AUC. The plasma level data was also fit with a 2-compartment PK model to obtain the clearance parameters for each individual and for the entire group. Simulations were performed of 0.5, 0.7, 0.9, 1, 1.1 mg/kg doses using the PK parameters either from group model fitting or mean of individual model fitting to estimate the dose of MDZ. The dose required to achieve the targeted Cmax was interpolated assuming the Cmax is linearly proportional to the input dose. NCA of the plasma level measurements in each animal yielded a mean Cmax value of 187.7 ± 64.5 (SD) with Tmax values in the range of 2-15 min (mean, 7.2 ± 5.6 min). The mean AUC0-480min was 8840.3 ± 1623.2 min*ng/ml. The levels remained greater than 115 ng/ml for at least 15 min after dosing in 10 of 13 animals tested. Using the PK parameters from the group model fitting, the dose of MDZ required to reach the Cmax of 113.9 ng/ml is 0.647 mg/kg (simulated Cmax is 158.5 ng/ml at 0.9 mg/kg dose). When using the mean of the individual PK parameters, a dose of 0.655 mg/kg MDZ is required to reach the targeted Cmax (simulated Cmax is 156.61 ng/ml at 0.9 mg/kg dose). In conclusion, a MDZ dose of 0.65 mg/kg, IM, in rats is estimated to yield the same plasma exposure (Cmax) as obtained with the recommended Seizalam® dose in humans and is proposed as the appropriate dose to be administered in rat models to mimic the human dosing.

Disclosures: **A. Dhir:** None. **M.A. Rogawski:** None. **C. Yi-Wu:** None.

Digital Abstract Session

P077. Epilepsy: Anticonvulsant and Antiepileptic Therapies

Program #/Poster #: P077.06

Topic: B.10. Epilepsy

Support: AOD20020-001-00000

Title: Dexmedetomidine is an effective anticonvulsant via intramuscular, intranasal and intravenous routes of administration in a rat model of acute organophosphate intoxication

Authors: *K. T. PAGARIGAN, B. J. TRAVIS, P. SHEU, J. M. COPPOLA, C. E. JACKSON PIERCY, J. E. MORGAN, P. B. DUBEE, H. S. MCCARREN;
USAMRICD, Gunpowder, MD

Abstract: Exposure to nerve agents (NA) induces seizures that can progress to status epilepticus (SE). NA-induced SE is primarily due to the irreversible inhibition of acetylcholinesterase (AChE) and the subsequent overstimulation of central muscarinic acetylcholine receptors. Currently, benzodiazepines such as diazepam, midazolam or lorazepam are the first line of treatment for controlling SE of any etiology. However, NA-induced SE is notoriously difficult to treat due to the time-dependent development of benzodiazepine-refractory SE. As such, effective therapeutics that control SE when benzodiazepines fail need to be identified. One such candidate is the FDA-approved, highly selective α2-adrenoceptor agonist dexmedetomidine (DEX). DEX

provides anxiolytic, analgesic, and sedative effects without causing respiratory depression like other similar drugs. We have previously shown that co-administration of DEX and midazolam 20 or 40 minutes after NA-induced SE terminated seizures and returned electroencephalographic (EEG) measurements to baseline, suggesting that DEX could enhance neuroprotection alongside midazolam auto-injectors in response to acute NA exposure. However, more research is needed to evaluate the effective dosing range and efficient routes of administration of DEX in the context of NA exposure. Here we determined the anticonvulsant ED90 of DEX when delivered intravenously (IV), intranasally (IN) and intramuscularly (IM) in a rat survival model of soman exposure. Male and female Sprague Dawley rats implanted with cortical electrodes were exposed subcutaneously to 150-180 μg/kg of soman to induce SE, as confirmed by EEG. To model pre-hospital DEX use, IM or IN DEX was administered concurrently with IM midazolam, atropine, and 2-PAM at 20 min after SE onset. To model in-hospital DEX use, IM midazolam, atropine, and 2-PAM were administered 20 min after onset, and then animals were transported to a heated, oxygen-enriched chamber and administered DEX IV via a jugular vein catheter 10 minutes later. The ED90 for females (n = 46 per route of administration) given DEX IN was 0.57 mg/kg (95% CI: 0.16, 61.4), 0.44 mg/kg (95% CI: 0.11, 78) for IM and 0.59 mg/kg (95% CI: 0.13, 39) for IV. Work to determine the anticonvulsant ED90 for males and evaluate the degree to which DEX prevents the long-term consequences of NA-induced SE is ongoing.

Disclosures: K.T. Pagarigan: None. **B.J. Travis:** None. **P. Sheu:** None. **J.M. Coppola:** None. **C.E. Jackson Piercy:** None. **J.E. Morgan:** None. **P.B. Dubee:** None. **H.S. McCarren:** None.

Digital Abstract Session

P077. Epilepsy: Anticonvulsant and Antiepileptic Therapies

Program #/Poster #: P077.07

Topic: B.10. Epilepsy

Support: NINDS grant #1U54NS079202

Title: Adjunctive high dose intramuscular allopregnanolone in the treatment of tetramethylenedisulfotetramine (TETS)-induced status epilepticus in mice

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Abstract: Benzodiazepines, the standard of care for initial treatment of status epilepticus (SE) in a chemical exposure emergency, are often ineffective in managing SE, especially when therapy is delayed. Intoxication with tetramethylenedisulfotetramine (TETS), a highly lethal GABA-A receptor antagonist, causes convulsive SE that is refractory to treatment. We have previously shown that allopregnanolone (5α,3α-P), a neurosteroid positive modulator of synaptic and

extrasynaptic GABA-A receptors, may provide an effective treatment option in benzodiazepine refractory SE including that caused by TETS. In the present study, we determined whether high dose of intramuscular (IM) 5α,3α-P (24 mg/kg) administered adjunctively to IM midazolam (MDZ, 1.8 mg/kg) was more effective than IM midazolam alone in terminating SE in mice exposed to TETS. To create a model mimicking the SE produced by TETS in humans, mice implanted with EEG electrodes were pretreated with a single dose of riluzole (10 mg/kg, IP) and 10 min later received a lethal dose of TETS (0.2 mg/kg, IP). Riluzole does not inhibit TETS-induced SE but does protect against the rapidly lethal effects of TETS in mice, providing a model of persistent seizure activity. Video EEG recording was carried out for at least 4 h after exposure to TETS. Animals were monitored for survival for 72 h after treatment. MDZ at a dose of 1.8 mg/kg (IM) was administered once at 40 min after the first myoclonic twitch either alone or in combination with 5α,3α-P (24 mg/kg) that was injected at 40 min and 100 min. Separate group of animals received 2 injections of 5α,3α-P alone. 5α,3α-P alone and in combination with midazolam led to rapid termination of the behavioral and electrographic SE as assessed by EEG power. 5α,3α-P combined with MDZ reduced the time of SE termination from 30.84 ± 12.12 min (MDZ alone) to 3.17 ± 0.58 min and increased 72 h survival from 50% (MDZ alone) to 100%. 5α,3α-P alone terminated SE (mean time to termination 3.04 ± 0.4 min) and protected 87.5% (7 of 8) of animals from mortality. Although all animals receiving the dual therapy and 5α,3α-P alone experienced sedation with loss of righting reflex for 5.72 ± 0.54 h and 3.85 ± 0.31 h, respectively, every animal fully recovered. Our results demonstrate that addition of high dose of 5α,3α-P to standard of care MDZ more rapidly and effectively terminates TETS-induced behavioral and EEG seizures than does MDZ alone. Sedation is a risk to the combination therapy but it is encouraging there is full recovery with no apparent untoward long term effects.

Disclosures: **D. Zolkowska:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); UC Davis. **R. Jung:** None. **A. Kiriaki:** None. **D.J. Tancredi:** None. **M.A. Rogawski:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); UC Davis.

Digital Abstract Session

P077. Epilepsy: Anticonvulsant and Antiepileptic Therapies

Program #/Poster #: P077.08

Topic: B.10. Epilepsy

Title: Idh-mutated gliomas promote epileptogenesis via d-2-hydroxyglutarate dependent mtor hyperactivation

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Abstract: Introduction:Epileptic seizures in patients with low-grade, isocitrate dehydrogenase (IDH) mutated gliomas reach 90%, a major source of morbidity for these patients. Albeit there are multiple features that contribute to tumor related epileptogenesis, IDH mutations are determined to be an independent factor, although the pathogenesis remains poorly understood. We demonstrate IDH-mutated tumors promote epileptogenesis through D-2-hydroxyglutarate (D-2-HG) dependent mTOR hyperactivation and metabolic reprogramming.
Methods:Human epileptic and nonepileptic cortex were identified via subdural electrodes in patients with IDH-mutated gliomas (n=5). An *in vitro* rat cortical neuronal model on microelectrode arrays were utilized to investigate the role of D-2-HG on neuronal excitability. mTOR and lysine demethylase (KDM) modulators were applied to elucidate the epileptogenic mechanism. Tetrodotoxin was utilized to evaluate the contribution of neuronal activity to mTOR signaling and metabolism. mTOR signaling was evaluated through western blot analysis and multiplex immunofluorescence. Metabolic function were analyzed via Seahorse assays and metabolomic analysis.
Results:
D-2-HG increased normalized bursting rate in the neuronal cultures ($p<0.0001$). Inhibition of mTOR with rapamycin corrected bursting levels to control levels. Furthermore, D-2-HG induced mTOR hyperactivation, independent of bursting activity, which correlated with upregulation of mTOR signaling in human epileptic tissue. KDM inhibition resulted in mTOR hyperactivation and neuronal hyperexcitability, which we demonstrated with D-2-HG, succinate, and PFI-90, a small molecule KDM inhibitor. Epileptic cortex and D-2-HG-treated neurons, have distinct metabolisms independent of neuronal activity compared to peritumoral nonepileptic cortex and control, respectively.
Conclusion:We demonstrate IDH-mutated gliomas promote epileptogenesis through a D-2-HG dependent mTOR hyperactivation via KDM inhibition, a putative mechanism and potential therapeutic targets. Furthermore, we argue mTOR hyperactivation results in metabolic reprogramming, independent of neuronal firing, which may contribute to epileptogenesis, a heretofore unrecognized aspect of pathologic mTOR signaling in neurological diseases.

Disclosures: A. Mortazavi: None. **I. Fayed:** None. **M. Bachani:** None. **A. Ksendzovsky:** None. **D. Maric:** None. **C. Yang:** None. **T. Dowdy:** None. **M. Larion:** None. **K.A. Zaghloul:** None.

Digital Abstract Session

P078. Epilepsy: Seizure Classification and Localization

Program #/Poster #: P078.01

Topic: B.10. Epilepsy

Support: NIH/NIBIB Grant No. R01EB026899-01
NIH/NINDS Grant No. R01NS10436
ONR GrantNo. N0014-15-1-2328
NIH/NINDS Grant Nos. NS062092 & NS088568

Mission funding from the U.S. Army Research Laboratory

Title: Uncovering mechanistic differences across epileptic seizure types with graph network analyses

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Abstract: A fundamental question that remains unsolved in clinical neuroscience and epilepsy research is how the brain generates unprovoked hypersynchronous electrical discharges. Notably, these spontaneous activities are highly variable across individual seizures and across patients. Specifically, seizures can be classified into different types based on the localization of onset regions and the accompanying behavioral manifestations. However, the underlying mechanisms that give rise to these different seizure types are not understood. The present study hypothesized that the mechanisms inherent to specific seizure types can be observed through graph network features. We analyzed intracranial electroencephalography (iEEG) data recorded from patients with four epilepsy types: 1) simple focal (onset regions in one hemisphere; intact awareness); 2) complex focal (onset regions in one hemisphere; impaired awareness often accompanied by automatisms); 3) secondary generalized (focal seizures that spread to both hemispheres; impaired awareness); and 4) generalized seizures (onset regions in both hemispheres of the brain; various clinical manifestations). Specifically, we modelled each patient's brain as a graph network where each node and edge represent a cortical region under a recording electrode and its connectivity weight to other electrodes, respectively. Graph network features were then computed separately for each seizure type. We found that the neural dynamics associated with different seizure types can be assessed and identified through a combination of graph network features such as degree, synchronizability, clustering coefficient, and spectral radius. Specifically, we showed that generalized seizures can be identified through a significantly higher spectral radius prior to seizure onsets as compared to the other seizures. After the onsets, network behavior of the three focal seizure types become more similar to that of generalized seizures, whereas the distributed nature of secondary generalized seizures can be captured by the increased degree per node and clustering coefficients as compared to the other two focal seizure types. To summarize, the present study illustrates graph network features as a novel biomarker for determining epileptic seizure types, which can subsequentially be used to guide a personalized patient- and type-specific seizure treatment.

Disclosures: N. Rungratsameetaweemana: None. **C. Lainscsek:** None. **S.S. Cash:** None. **T.J. Sejnowski:** None. **J.O. Garcia:** None. **K. Bansal:** None.

Digital Abstract Session

P078. Epilepsy: Seizure Classification and Localization

Program #/Poster #: P078.02

Topic: B.10. Epilepsy

Support: Stanford University Wu Tsai Neurosciences Institute
Stanford Bio-X Seed Grant Award IIP9-104

Title: Seizure detection and localization using human intracranial electrophysiology via information theoretic methods

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Abstract: Of the 1% of the world population with epilepsy, one-third have refractory epilepsy, in which their only option to manage seizures is a high-risk surgery to remove seizure onset zones (SOZs, brain regions that are most likely to cause seizures). The current state of epilepsy treatment heavily relies on manual evaluation of EEGs by epileptologists and lacks interventions that leverage their rich information. To combat this limitation, we introduce an information theoretic estimate of joint entropy called the inverse compression ratio (ICR), which utilizes compression algorithms, as a potential quantitative EEG (qEEG) method. With our data repository of continuous, 10kHz intracranial EEGs acquired from clinical neuromonitoring studies of adult and pediatric participants, we study the relationship between ICR and seizure activity. When comparing ICR across time, we observed a sharp peak at seizure onset, followed by a dip, before returning to baseline. Furthermore, when analyzing characteristics of ICR peaks that occurred at seizure onsets (e.g. peak amplitude) across intracranial channels, we observed prominent changes that distinguished channels located in SOZs. When using ICR to perform seizure detection, we found an average sensitivity/specificity (SE/SP) of 81%/98% across 5 participants with a total of 30 seizures. In comparison, the average SE/SP of sample entropy, approximate entropy, and variance were 29%/97%, 36%/97%, and 45%/96%, respectively. Our results demonstrate that our information theoretic measure of ICR performs comparably to - if not better than - other qEEG measures, suggesting their potential in seizure detection and localization. Previous studies on the entropy of epileptic EEGs may not have detected the brief spike in information content at seizure onset due to signal quality or limitations in sampling rate. Implementing robust qEEG techniques to clinical practice may offload labor-intensive tasks from clinicians and uncover EEG features that cannot be detected by eye, broadening our understanding of epilepsy and improving therapy.

Disclosures: L. Yamada: None. **T. Oskotsky:** None. **P. Nuyujukian:** None.

Digital Abstract Session

P079. Astrocytes: Biology

Program #/Poster #: P079.01

Topic: B.11. Glial Mechanisms

Support: AFOSR ASTRONIR to VB

AFOSR 3DNeuroglia to VB
AFOSR AstroDyn to WL VB GPN KMO
AFOSR MURI to WL

Title: Actin as a sensor of the chemophysical environment for primary rodent neocortical astrocytes

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Abstract: The importance of astrocytes - non-neuronal glial cells - to cognitive function has been increasingly highlighted in recent work. Indeed, astrocytes have physiological roles ranging from maintaining brain homeostasis to modulating neuronal communication, and much of these functions occur at the endfeet of astrocytes. In this work, we have considered the crucial role of the cytoskeleton, both in fixed and live astrocytes, to understand the functional responses that result from extracellular environmental changes. To accomplish this goal, we culture primary neocortical rat astrocytes on a variety of surfaces, including poly-D-lysine (PDL) coated glass as a control and hydrotalcite nanoparticle films (HTlc), the latter of which causes astrocytes to become stellate.
For imaging cytoskeletal organization, astrocytes are grown on either PDL or HTlc, fixed, and stained with Phalloidin-488. The fixed cells are then imaged using high resolution stimulated emission depletion (STED) microscopy to obtain detailed images of the actin structure. To determine how actin structure differs for astrocytes grown on PDL versus HTlc, we use image processing methods to extract the actin filaments and quantify their relative angle to the cell boundary. We find significant differences in relative angles of astrocytic actin for cells grown on PDL versus HTlc. These results illustrate the importance of actin as a sensor of mechanical influences in the environment.
For imaging cytoskeletal dynamics, astrocytes are transduced with actin-GFP at 48 hr prior to imaging, and we then use live confocal microscopy to capture changes in actin for astrocytes cultured on PDL and HTlc. We also introduce a change to the chemical environment by replacing the standard, control media with saline containing either high potassium (40 mM KCl) or hypotonic challenge (ΔOsm=60 mOsm) to further understand cytoskeletal adaptations. We then employ optical flow to analyze actin dynamics and to study their persistence. We find that the chemophysical environment greatly influences the localization of actin dynamics and propose that these dynamics are a local manifestation of astrocytes' global homeostatic response to changes in the extracellular microenvironment.
* O'Neill & Saracino co-first; # Benfenati & Losert co-corresponding.

Disclosures: **K.M. O'Neill:** None. **E. Saracino:** None. **B. Barile:** None. **N.J. Mennona:** None. **S. Pathak:** None. **M.G. Mola:** None. **G.P. Nicchia:** None. **V. Benfenati:** None. **W. Losert:** None.

Digital Abstract Session

P079. Astrocytes: Biology

Program #/Poster #: P079.02

Topic: B.11. Glial Mechanisms

Support: NIH 1F31MH118822-01A1
NIH R01MH107886
Alzheimer's Association SAGA-17-419092

Title: Chemogenetic activation of Gi signaling in dorsal hippocampal astrocytes prevents object memory consolidation in ovariectomized female mice

Authors: *L. TAXIER, S. PHILIPPI, K. M. FRICK;
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Abstract: Astrocytes, despite being the most abundant cell type in the brain, have historically been relegated to a supportive role in the central nervous system. However, recent evidence suggests a more active role for astrocytes in synaptic activity in brain regions like the hippocampus. To test whether dorsal hippocampal (DH) astrocytic activity impacts memory consolidation, we used Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to manipulate Gi signaling in DH astrocytes. Nine week-old ovariectomized female C57BL/6 mice were infused into the DH with control viral construct (AAV-GFAP-eGFP) or hM4Di-containing viral construct (pAAV-GFAP-HAhM4D(Gi)-IRES-mCitrine) (n=10-12/group). Two weeks following viral delivery, mice were trained and tested in the object recognition (OR) and object placement (OP) tasks to assess object recognition and spatial memory consolidation, respectively. Immediately after training in each task, mice received an intraperitoneal (i.p.) injection of the DREADD actuator Clozapine-N-Oxide (CNO), and were then tested 24 h (OR) or 4 h (OP) later. Here, we tested effects of two doses of CNO, 1 and 5 mg/kg. hM4Di-infused mice injected post-training with 5 mg/kg CNO exhibited impaired OR and OP memory relative to mice expressing control virus, suggesting that activation of Gi signaling in DH astrocytes blocks object recognition and spatial memory consolidation. Two weeks later, mice were retrained with novel objects, received a 1 mg/kg post-training i.p. injection of CNO, and were tested at the same delays as before. The 1 mg/kg dose of CNO did not impair OR and OP memory in mice expressing hM4Di virus relative to chance levels of performance or to GFP-expressing controls. This dose will therefore be used in future studies to determine if activation of astocytic Gi signaling interferes with the memory-enhancing effects of neuromodulators. Combined, these data suggest that activating Gi signaling in DH astrocytes with a sufficiently high dose of CNO impairs object memory consolidation.

Disclosures: L. Taxier: None. **S. Philippi:** None. **K.M. Frick:** None.

Digital Abstract Session

P079. Astrocytes: Biology

Program #/Poster #: P079.03

Topic: B.11. Glial Mechanisms

Support: UND VPRED Postdoctoral support
Host-pathogen interactions CoBRE 25012
UND Histology Core
UND Imaging Core
UND Flow-cytometry Core

Title: Brain sub-region-specific gliosis during subcutaneous Group A Streptococcal infection in humanized mice

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Abstract: Group A Streptococcus (GAS, *Streptococcus pyogenes*) is an important human pathogen that can cause mild self-limiting pharyngitis to life-threatening streptococcal toxic shock and necrotizing fasciitis (NF) that manifests as rapidly spreading progressive necrosis of the subcutaneous tissue and deep fascia. Recurrent GAS infections are not uncommon, and GAS mimics of host proteins can prime individuals with HLA-II predilection for increased risk of severity, outcomes, and post-streptococcal sequelae, that include a spectrum of neurological manifestations and neuropsychiatric disorders. However, the pathological changes in the brain that are in tandem with or latently following GAS NF are unknown. To study the concomitant neuroinflammatory changes during subcutaneous GAS infection and study the effect of clindamycin (CLN) treatment, the standard care for invasive GAS infections, we used clinically relevant HLA-II transgenic mice expressing a human HLA-II DQ8 allele as a preclinical model of GAS NF with no apparent lethal systemic toxicity. Mice were subcutaneously infected with a clinical isolate of GAS 5448 and were either untreated or treated with CLN (10mg/kg, initiated at 6 hours post-infection intraperitoneally and continued for 4 days) and monitored for 15 days. Most strikingly, our data showed a GAS burden in the cerebral cortex (CC) and the hippocampus (HC) lysates from infected mice, that was more profound in male compared to female mice. Treatment with CLN was significantly effective in reducing skin lesion areas, GAS burden, and levels of the pro-inflammatory mediators TNF-α, IL-6, IL-17F, and IL-22 in the skin. However, a sex-dependent difference was seen in the effect of clindamycin treatment against GAS that disseminated into the CC or the HC. The existence of a viable GAS reservoir in the brain was concomitant with a high intensity of staining with anti-glial fibrillary acidic protein (GFAP) and anti-Iba-1 antibodies, suggestive of reactive gliosis. In addition, we observed a spatial selective and region-specific gliosis that was significantly higher in the dentate gyrus and CA1 subfields despite CLN treatment. This was in stark contrast to the decreased reactive gliosis seen in the amygdala and CA3 subfields in male and female mice. Western blot analysis of the CC lysates confirmed significant changes in GFAP and Iba-1. Interestingly, CLN treatment increased

protein levels of occludin, a marker of BBB integrity. Our data provide critical information on the neuropathological changes that occur during or latently following subcutaneous GAS infections.

Disclosures: **A. Satpati:** None. **S. Mukundan:** None. **C. Combs:** None. **S. Nookala:** None.

Digital Abstract Session

P079. Astrocytes: Biology

Program #/Poster #: P079.04

Topic: B.11. Glial Mechanisms

Support: Brain & Behavior Research Foundation (NARSAD)
Quinnipiac University Faculty Scholarship Grants

Title: Morphological differences in astrocyte populations of the basolateral amygdala and anterior cingulate cortex

Authors: *J. KOCUR[1], K. JONES[2], A. BETZ[3], M. MIRRIONE[4];
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Abstract: Astrocytes are an omnipresent and seemingly omnipotent subset of the glial cell population found in the brain. They perform a variety of functions critical to CNS homeostasis, including the maintenance and formation of the blood brain barrier, migration of developing neurons, metabolic regulation, neurotransmission, and synaptogenesis. Dysregulation of normal astrocyte function has been implicated in the etiology and progression of numerous neurologic and psychiatric disorders. As such, there is a great demand for advancements with respect to better understanding astrocyte development and heterogeneity. Morphological classification of astrocytes has largely stagnated, with the majority of astrocytes broadly classified as protoplasmic or fibrous. In the present study, we aim to compare the astrocyte populations of the basolateral amygdala (BLA) and anterior cingulate cortex (ACC) of the medial prefrontal cortex, regions responsible for the processing of external stimuli and emotional regulation. Five adolescent female Sprague Dawley rats (postnatal day 40) were tested in the elevated plus maze to confirm non-anxious behavior, and two days later, brain tissue was collected and processed for microscopy. Astrocytes expressing glial fibrillary acidic protein (GFAP), an intermediate filament protein found in mature astrocytes, were evaluated using immunofluorescence and confocal microscopy. Astrocytes in two ACC subdivisions, area 1 and area 2, across three Bregma coordinates (2.28 mm, 0.00 mm, -1.44 mm) as well as the BLA (-1.56 mm, -2.40 mm, and -3.24 mm) bilaterally were quantified and analyzed. An open source software ImageJ, along with the plug-in Simple Neurite Tracer, was employed to count and trace astrocytes. A Sholl analysis was used to quantify the complexity of astrocyte process arbors. We found the total number of astrocytes counted per square mm in the BLA was significantly higher compared to the ACC. Furthermore, we found astrocytes in the BLA had significantly more complex process arbors with a significantly higher average number of intersections, as well as longer process

lengths when compared to the astrocytes in the ACC. While our sample is limited, this data supports further investigation into the characteristics of astrocyte populations in cortical and subcortical regions across sex, age, and behavioral state, of both male and female subjects. Further experimentation will examine the functional significance of these differences in morphology and may provide in-roads to modernizing the broad astrocyte classification system.

Disclosures: J. Kocur: None. **A. Betz:** None. **M. Mirrione:** None. **K. Jones:** None.

Digital Abstract Session

P080. Glia-Neuron Interactions In Physiology

Program #/Poster #: P080.01

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01 EY028219
NIH Grant R01 DA049005

Title: Astrocytes contribute to motor learning, neuronal correlations and movement encoding by motor cortex neurons

Authors: *C. DELEPINE, K. LI, J. SHIH, P. GAUDEAUX, M. SUR;
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Abstract: While motor cortex is crucial for learning precise and reliable movements, whether and how astrocytes contribute to its plasticity and function during motor learning is unknown. Here we report that primary motor cortex (M1) astrocytes in mice show in vivo plasticity during learning of a lever push task, as revealed by transcriptomic and functional modifications, particularly changes in expression of glutamate transporter genes and increased coincidence of microdomain calcium events. Astrocyte-specific manipulations of M1 are sufficient to alter motor learning and execution, and neuronal population coding, in the same task. Specifically, mice expressing decreased levels of the astrocyte glutamate transporter GLT1 show impaired and variable movement trajectories. Mice with increased astrocyte Gq signaling show decreased performance rates, delayed response times and impaired trajectories, along with abnormally high levels of GLT1. In both groups of mice, M1 neurons have altered inter-neuronal correlations and impaired population representations of task parameters, including response time and movement trajectories. Thus, astrocytes have a specific role in coordinating M1 neuronal activity during motor learning, and control learned movement execution and dexterity through mechanisms that include fine regulation of glutamate transport.

Disclosures: C. Delepine: None. **K. Li:** None. **J. Shih:** None. **P. Gaudeaux:** None. **M. Sur:** None.

Digital Abstract Session

P080. Glia-Neuron Interactions In Physiology

Program #/Poster #: P080.02

Topic: B.11. Glial Mechanisms

Support: CNPq
FAPERJ

Title: Establishment of an in vitro model for myelination studies using neonate mice dorsal root ganglia explants

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Abstract: Schwann cells (SC) myelinate neurons in the peripheral nervous system and differentiate to terminal phenotypes in a temporally regulated manner. Some demyelinating diseases compromise in a severe and progressive way the patient's life quality. Therefore, studying myelination in an *in vitro* model that mimics demyelinating pathologies might help important future therapeutic strategies. Our study aims to establish an *in vitro* myelination model using C57BL/6 neonatal mice (P0-P2) dorsal root ganglia (DRG) explants. We evaluated DRG cultures over 4 substrate conditions: poly-L-lysine (control), poly-L-lysine associated with laminin-111 in PBS buffer, poly-laminin (laminin-111, pH 4), and laminin (laminin-111, pH7). Neurite outgrowth was evaluated through phase-contrast and fluorescence microscopy in all DRG explant conditions, while migrating cells were aligned to the neurites and had a SC morphology. The laminin group had the largest neurite extension and migration area, when compared to other substrates. Neuro-glial connections were observed using single cell calcium imaging responses, showing a functional connected system, correlated to the expression of connexin-43. The expression of the transcriptional factors Sox10 and Krox20 was also evaluated through fluorescence microscopy, characterizing the total glial and myelinating cell number, respectively, over the time course of 3-5 days *in vitro* (div). None of the groups showed differences in the percentage of myelinating cells over the observed timepoints, but poly-laminin and laminin groups had an increase in the total number of migrating cells in cultures maintained for 5 div. Immunostaining for myelin basic protein (MBP) confirmed myelin presence, and relative quantification showed that the laminin group had the highest expression of MBP. All results were obtained from at least 3 replication experiments, with individual DRG from different animals as sample. Statistics were performed following a normality test, 1-way ANOVA and Tukey's post hoc. Altogether, our results show that DRG explants from neonatal mice are a suitable model for *in vitro* myelination studies, resulting in functional Schwann cells. Moreover, DRG explants had a better adhesion and stability at polymerized laminin substrate (pH 4), displaying migrated cells with myelinating profile with higher MBP expression. The model stands out for its simplicity, not requiring the addition of many factors and its potential application for many myelination experimental approaches.

Disclosures: G. Sardella-Silva: None. **R.A. de Melo Reis:** None. **V.T. Ribeiro-Resende:** None.

Digital Abstract Session

P080. Glia-Neuron Interactions In Physiology

Program #/Poster #: P080.03

Topic: B.11. Glial Mechanisms

Support: 5R01 NS045630-15

Title: Loss of the planar cell polarity protein CELSR2 delays Schwann cell myelination

Authors: *L. R. FRICK[1], Y. POITELON[2], N. LAHUE[3], L. MARZIALI[4], E. HURLEY[4], K. SHEN[5], J. QU[5], L. WRABETZ[6], F. TISSIR[7], M. FELTRI[8];
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Abstract: The mechanisms that govern peripheral myelination are not completely understood. To identify novel candidates involved in axo-glial interactions we used a Schwann cell pseudopod assay with the addition of laminin to generate polarity. The proteomic analysis of subcellular fractions revealed an enrichment of the Planar Cell Polarity protein CELSR2 (for Cadherin EGF LAG Seven-Pass G-Type Receptor 2) in Schwann cell protrusions extended towards neuronal membranes.Thus, we evaluated whether CELSR2 is necessary for Schwann cell myelination in co-cultures with dorsal root ganglia (DRG) neurons. Indeed, knockdown of CELSR2 in Schwann cells impaired myelination in vitro. In vivo, constitutive knockout of CELSR2 delays peripheral myelination. During development (postnatal days P5-20), sciatic nerves of CELSR2 KO mice have an impairment on radial sorting characterized by higher number of bundles of unsorted axons and more axons per bundle compared to WT littermates. Additionally, these animals have more amyelinated axons and their G-ratios are higher indicating thinner myelin. This delay is transient and is resolved by the time myelination is complete at P30. CELSR2 does not have an apparent role in myelin maintenance since KO and WT aged mice have similar numbers of degenerating axons and demyelinated fibers. The internodal length is also normal in adult CELSR2 KO mice. The family of orthologs of *Drosophila's* protein Flamingo also includes CELSR1 and CELSR3. Therefore, one possibility is that these similar proteins have redundant roles with CELSR2. The mRNA expression levels of CELSR2 are higher than the ones of CELSR3 whereas the expression of CELSR1 is almost negligible in mouse sciatic nerves. It is plausible that CELSR3 may compensate for the loss of CELSR2, thus concomitant ablation of the respective genes might worsen the phenotype. Using isolated Schwann cells and neurons we found that CELSR2 is expressed in both cell types, therefore a role for neuronal CELSR2 cannot be ruled out in the constitutive KO mouse. One way to address

these questions is by generating CELSR2 (and CELSR3) in conditional knockout mice in which the gene is only knocked out in Schwann cells. CELSR2 is a promising candidate to evaluate regarding its role in peripheral myelination and the underlying mechanisms are to be elucidated.

Disclosures: L.R. Frick: None. **N. Lahue:** None. **L. Wrabetz:** None. **M. Feltri:** None. **Y. Poitelon:** None. **L. Marziali:** None. **E. Hurley:** None. **J. Qu:** None. **K. Shen:** None. **F. Tissir:** None.

Digital Abstract Session

P080. Glia-Neuron Interactions In Physiology

Program #/Poster #: P080.04

Topic: B.11. Glial Mechanisms

Support: PAPIIT num. IN221820
UNAM, PI
APEC num. LOP-INV-001

Title: Identification of the VEGF-C isoformin Muller cell cultive cultures from mice retina.

Authors: *Z. C. JIMENEZ[1], E. J. GALVAN[2], H. SANCHEZ-CASTILLO[3], *D. O. DE LA PAZ[4], A. M. ARELLANO[4];
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Abstract: Identification of the VEGF-C isoform in Muller cells cultures from mice retina. Carranza Jiménez Zoe Pamela[1], Medina Arellano Alan[1], Sánchez-Castillo Hugo[3], Galvan Emilio[3], Ochoa-de la Paz Lenin David[1,2]. Laboratorio de neurobiología molecular y celular de la glia, Departamento de Bioquímica, Facultad de Medicina, UNAM[1]. Unidad de investigación UNAM-APEC Asociacion para prevenir la ceguera en México I.A.P. Hospital "Dr. Luis Sánchez Bulnes"[2], Facultad de Psicología[3], UNAM, CINVESTAV Sur[4].Muller cells (MC) are the main macroglia of the retina. They extend from the ganglion cell layer to the internal segments of photoreceptors, facilitates physical contact with many types of cells in the retina. So it plays an important role in various physiological processes in the retina such as regulating the blood-retinal barrier, releasing growth factors, homeostasis of water and ions (K^+) and regulating innate immunity of retina through microglia interaction, among others. In pathological conditions such as diabetic retinopathy or age-related macular degeneration, MC contribute to neurovascular dysfunction by producing inflammatory and angiogenic factors. In the last years, experimental observations, indicate that MC is an important source of VEGF in the retina under pathological conditions. The VEGF family consists of five members (VEGF-A, B, C, D, and PlGF), all of them, structurally related to three different tyrosine kinase receptors (VEGFR1, VEGFR2, and VEGFR3) and two neuropilin choreographers (NRP1 and NRP2) that improve ligand affinity for VEGFR receptors, thus improving their physiological actions. VEGF-C is a specific growth

factor for lymph vessels in a variety of models, also promotes the growth of and also blood vessels. One of the functions of MC is to regulate the angiogenic factors release, such as VEGF-A, however, it is unknown about the presence of VEGF-C in these glial cells. We aimed to determine if the VEGF-C form is present in the MC. Using immunofluorescence assays we observe that VEGF-C is expressed in MC (Fig 1), also the mRNA, according to the rt-PCR analysis of MC in cultures. These results, shown the presence of VEGF-C in MC, so the presence of this growth factor suggests that MC not only regulate the angiogenic process in the retina, also, could interact with microglia, playing a role in the inflammatory mechanism presents in different retinopathies. Nevertheless, more experiments are necessary to confirm this hypothesis.

Disclosures: Z.C. Jimenez: None. **E.J. Galvan:** None. **H. Sanchez-Castillo:** None. **D.O. De la Paz:** None. **A.M. Arellano:** None.

Digital Abstract Session

P080. Glia-Neuron Interactions In Physiology

Program #/Poster #: P080.06

Topic: B.11. Glial Mechanisms

Support: NSF IOS 1755341

Title: An optogenetic approach to understand molecular mechanisms of astrocyte-neuron interactions in the development of neuronal synchrony.

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Abstract: Neurons must fire together in networks to perform a variety of functions but the mechanisms by which they accomplish this synchronization are not entirely understood. In some cases, this phenomenon does not function as intended, leading to states like epileptic seizures. Existing models of synchronous activity assume that it is intrinsic to neurons. Astrocytes have been shown to modulate oscillatory activity in networks of neurons possibly by releasing gliotransmitters like glutamate and ATP. Using the mitotic inhibitor 5-fluorodeoxyuridine (FUdR) we established pure neuron only cultures from chick embryonic day 7 (E7) optic tectum and recorded neuronal network activity on multielectrode arrays (MEAs). Our preliminary results indicate that astrocytes are necessary for synchronous activity of neurons in culture. Mixed neuron and astrocyte cultures on multi-electrode arrays (MEAs) show random spiking activity which synchronizes over time whereas astrocyte-free neurons only show random activity without synchronization. Our results validated that the physical presence of astrocytes is required to properly establish synchronized activity in neuronal networks. We implicate calcium mobilization within astrocytes via metabotropic g-protein coupled receptor (GPCR) pathways leading to gliotransmitter release as a possible mechanism for neuronal synchronization. To test this hypothesis, we used an optogenetic approach - using a lentiviral vector we expressed the

photopigment melanopsin, a Gaq coupled GPCR with an absorption peak of around 470nm, in chick optic tectum astrocytes. Our preliminary optogenetic activation experiments indicate Ca++ flux in astrocytes upon optogenetic stimulation with 470nm blue light. This powerful tool allows us to combine live calcium imaging with multielectrode array experiments to clearly define the role of astrocytic calcium in the development of network activity of neurons, particularly synchronization.

Disclosures: T. Clifford: None. **M. Temburni:** None.

Digital Abstract Session

P080. Glia-Neuron Interactions In Physiology

Program #/Poster #: P080.07

Topic: B.11. Glial Mechanisms

Support: NINDS F32 NS114040
NINDS R01 NS112144
NINDS R01 NS088627
NINDS R01 NS110825
NINDS R01 NS110949

Title: Microglial calcium signaling is attuned to neuronal activity shifts

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Abstract: Microglial calcium signaling underlies a number of key physiological and pathological processes in situ, but has not been studied in vivo in awake mice. Using multiple GCaMP6 variants targeted to microglia, we assessed how microglial calcium signaling responds to alterations in neuronal activity across a wide range. We find that only a small subset of microglial somata and processes exhibited spontaneous calcium transients in a chronic window preparation. However, hyperactive shifts in neuronal activity (kainate status epilepticus and CaMKIIa Gq DREADD activation) triggered increased microglial process calcium signaling, often concomitant with process extension. Additionally, hypoactive shifts in neuronal activity (isoflurane anesthesia and CaMKIIa Gi DREADD activation) also increased microglial process calcium signaling. Under hypoactive neuronal conditions, microglia also exhibited process extension and outgrowth with greater calcium signaling. Our work reveals that microglia have highly distinct microdomain signaling, and that processes specifically respond to bi-directional shifts in neuronal activity through increased calcium signaling.

Disclosures: A.D. Umpierre: None. **L. Wu:** None.

Digital Abstract Session

P080. Glia-Neuron Interactions In Physiology

Program #/Poster #: P080.08

Topic: B.11. Glial Mechanisms

Support: NSF RIA 11401026
NSF IOS 1755341

Title: Role of the Astrocytic mGluR Pathway in the Development of Neuronal Synchrony

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[1]Biol. Sci., [2]OSCAR Imaging Ctr., Delaware State Univ., Dover, DE

Abstract: Synchronous oscillations are necessary for establishing functional neuronal networks in normal vertebrate brain development - however, the mechanisms of neuronal synchronization are not fully understood. Existing models of synchronous activity assume that it is intrinsic to neurons. Astrocytes have been shown to modulate oscillatory activity in networks of neurons possibly by releasing gliotransmitters like glutamate and ATP. We have established pure neuron only and mixed (astrocyte and neurons) cultures from the developing chicken brain (optic tectum) and recorded neuronal network on multi-electrode arrays. Our preliminary results indicate that astrocytes are necessary for synchronous activity of neurons in culture. To further dissect the molecular pathways involved, we targeted the metabotropic glutamate receptor (mGluR) pathway within astrocytes as a mechanism by which astrocytes influence synchronous firing. Astrocytes express mGluRs that consist of the same subunits and stoichiometry as those expressed in neurons. Our model predicts that glutamate sensing at tripartite synapses via mGluRs elevates local calcium within astrocyte processes. With sufficient activation, the localized calcium elevation crosses a threshold causing a global calcium release within the astrocyte leading to the exocytosis of glutamate. To test this model we expressed a truncated mGluR subunit (mGluR DN) which acts as a dominant negative to block downstream signaling along with GCaMP6F for assaying Ca^{++}activity in primary chick astrocytes. Our results demonstrated that astrocytes expressing the mGluR DN reduced the calcium elevation upon stimulation with glutamate. Our next steps involve co-culture of the mGluRDN-GCamP6F astrocytes with neurons on MEAs and measure synchrony index, mean firing rates, spike amplitudes and other network parameters.

Disclosures: M. Temburni: None. **V.A.N. Talabattula:** None. **M. Moore:** None.

Digital Abstract Session

P080. Glia-Neuron Interactions In Physiology

Program #/Poster #: P080.09

Topic: B.11. Glial Mechanisms

Support: NSF RIA 1401026
NSF IOS 1755341

Title: Role of synaptobrevin in glutamate exocytosis by astrocytes

Authors: *T. I. AMANFO[1], V. A. N. TALABATTULA[1], M. TEMBURNI[2];
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Abstract: Synchronous neuronal activity is a hallmark of developing as well as fully developed neuronal networks and is necessary for the functional organization of the brain. However, the mechanisms of the development of these synchronous oscillations are poorly understood. While existing models assume it as a process intrinsic to neurons, recent evidence suggests that astrocytes have an important role in the development of the synchronous activity. We established pure neuron only and mixed (astrocyte and neuron) cultures on multielectrode arrays (MEAs) from the embryonic chick optic tectum. Our preliminary results indicate that astrocytes are necessary for the synchronous activity of neurons in culture. Mixed neuron and astrocyte cultures show random spiking activity which synchronizes over time whereas astrocyte-free neurons only show random activity without synchronization. Our results validated that the physical presence of astrocytes is required to properly establish synchronized activity in neuronal networks. Our model predicts that glutamate sensing at tripartite synapses via mGluRs elevates local calcium within astrocyte processes. With sufficient activation, the localized calcium elevation crosses a threshold causing a global calcium release within the astrocyte leading to glutamate exocytosis. We targeted the SNARE protein Synaptobrevin (Vamp2) within astrocytes as crucial for communication with neurons via the exocytotic release of glutamate. We proposed to test this model by disrupting glutamate exocytosis within astrocytes using a truncated Vamp2 subunit (Vamp2 DN) which acts as a dominant-negative blocking exocytotic release. Astrocytes expressing the Vamp2 DN are expected to not release glutamate causing a problem in the synchronization provided by astrocytes. We have generated astrocyte lines expressing the synaptobrevin dominant-negative (Vamp2 DN) along with the glutamate sensor iGluSnFR. With these tools, a more comprehensive molecular model for astrocyte involvement in the generation of neuronal synchrony can be developed.

Disclosures: T.I. Amanfo: None. **M. Temburni:** None. **V.A.N. Talabattula:** None.

Digital Abstract Session

P080. Glia-Neuron Interactions In Physiology

Program #/Poster #: P080.10

Topic: B.11. Glial Mechanisms

Support: CIHR Foundation Grant (ESR)
CIHR CGS-M (ZC)
MNI–Cambridge Collaborative Grant (RT, ESR)
MNI–Cambridge Douglas Arvith Award (ZC)

McGill IPN Awards (ZC, VL)

Title: Myelin ensheathment and activity-dependence in a developing visual system

Authors: *Z. CHORGHAY[1,3], D. MACFARQUHAR[2], V. J. LI[1,3], S. AUFMKOLK[2], A. SCHOHL[1,3], P. W. WISEMAN[2], R. T. KÁRADÓTTIR[4], E. S. RUTHAZER[1,3];
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Abstract: Myelination is important for the fidelity of information transmission in brain circuits and is increasingly thought to be a substrate of plasticity. The African claw-toed frog, *Xenopus laevis*, is a good model organism for the study of circuit development and function, particularly in response to changes in activity, but a comprehensive characterization of the timecourse of myelin ensheathment during its development has been lacking. Here, we use immunostaining of myelin basic protein (MBP) as a proxy for myelin ensheathment, and cross-validate it with third-harmonic generation microscopy, an emerging label-free technique for imaging myelin in the nervous system that relies on sub-micrometer heterogeneities produced by optical interfaces of neighboring regions. Consistent with other species, we observe a caudal-to-rostral developmental progression of MBP expression. This detailed characterization allowed us to narrow down a developmental window and region in which to systematically study the interplay between myelination and activity. We focus on the visual system at a developmental stage where MBP expression is just beginning to occur, and ask what happens to myelin ensheathment here when we manipulate neuronal activity through visual experience. First, we recorded calcium responses from GCaMP6s animals to systematically characterize the persistent activation and adaptation of the visual system in response to LED flashes ("strobe") presented at different frequencies. Based on these findings, we selected two strobe frequencies: 1/16 Hz to evoke ongoing activity and 1 Hz to habituate the visual response. We reared stage 48 animals at either strobe frequency or under ambient light (control) for 7 days, then collected high-resolution confocal images of the immunostained optic chiasm of each animal. We report a significant difference in the MBP-associated axonal volume at the chiasm between these conditions, with ongoing calcium responses and increased MBP expression elicited by 1/16 Hz stimulation compared to adaptation of calcium responses and correspondingly reduced MBP expression with 1 Hz strobe stimulation. Taken together, we report myelin ensheathment in normal Xenopus development and the effects of sensory experience, highlighting the interaction between neuronal activity and myelination in developing brain circuits.

Disclosures: Z. Chorghay: None. **D. MacFarquhar:** None. **V.J. Li:** None. **S. Aufmkolk:** None. **A. Schohl:** None. **P.W. Wiseman:** None. **R.T. Káradóttir:** None. **E.S. Ruthazer:** None.

Digital Abstract Session

P081. Astrocytes: Disease Mechanisms

Program #/Poster #: P081.01

Topic: B.11. Glial Mechanisms

Support: Philip Morris Products S.A.

Title: Systems approach to neuroinflammation - computable biological network model for reactive astrogliosis

Authors: *M. BARKHUIZEN, V. BELCASTRO, M. C. PEITSCH, J. HOENG, M. TALIKKA;
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Abstract: Astrocytes play a central role in maintaining normal CNS function and the neuroimmune response. Following a CNS insult, astrocytes respond with diverse molecular and morphological changes in a process commonly referred to as reactive astrogliosis. While traditional approaches have unraveled critical mechanisms, the insights are often limited to the production of inflammatory proteins and changes in key astrocyte markers. To complement traditional endpoints, the changes in thousands of molecules (e.g., RNA) triggered by reactive astrogliosis can be captured using high-throughput technologies and interpreted with computational approaches. Causal biological network models, scripted in the Biological Expression Language, facilitate the assembly of available biological knowledge in a structured computable format that facilitates mechanistic interpretation of molecular data in a well-defined biological context. The network model consists of biological entities (nodes) and relationships between the nodes (edges). Information regarding gene expression regulation by some of the nodes in the network backbone is employed to build a second, scorable layer to the network model. This layer is used to infer the activity of the backbone nodes from transcriptomic data, and the impact on the network as a whole can be assessed using the network perturbation amplitude algorithm. Here, we introduce causal biological network models that describes the molecular pathways underlying reactive astrogliosis. The models captures key signaling pathways involved in i) pan-insult astrocyte reactivity, ii) neurotoxic A1 specific reactivity, iii) neurotrophic A2 specific reactivity, iv) loss of homeostatic functions, and v) glial scar formation pathways. Owing to the second layer, the network models can be scored with transcriptomic data from studies of CNS insults to derive a quantitative measure and mechanistic understanding of key molecules driving astrocyte reactivity. This is the first step to comprehensively model reactive astrogliosis in a computable form.

Disclosures: **M. Barkhuizen:** A. Employment/Salary (full or part-time):; Philip Morris Products S.A. **V. Belcastro:** A. Employment/Salary (full or part-time):; Philip Morris Products S.A. **M.C. Peitsch:** A. Employment/Salary (full or part-time):; Philip Morris Products S.A. **J. Hoeng:** A. Employment/Salary (full or part-time):; Philip Morris Products S.A. **M. Talikka:** A. Employment/Salary (full or part-time):; Philip Morris Products S.A..

Digital Abstract Session

P081. Astrocytes: Disease Mechanisms

Program #/Poster #: P081.02

Topic: B.11. Glial Mechanisms

Support: Conacyt No. A1-S-40569

Title: Combination of Albendazole and Melatonin against glioblastoma: in vitro evaluation

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Abstract: Purpose: Glioblastoma (GB) is one of the most aggressive tumors affecting the central nervous system (CNS) due to its rapid progress and resistance to drugs associated with the presence of ATP-binding cassette (ABC) transporters, such as breast cancer resistant protein (BCRP), a drug efflux transporter. The first-line treatment of the disease is surgery, followed by radiotherapy combined with chemotherapy (temozolomide-TMZ), but the prognosis is poor. Recent studies have shown that albendazole (ALB), and their active metabolite albendazole sulfoxide (ALBSO), have anticancer activity attributed to their tubulin binding capacity, resulting in de-polymerization and cell-cycle arrest; whereas melatonin (MLT) with antioxidant properties, has been associated with the inactivation of signaling pathways for the proliferation and survival of tumor cells. In the search of new treatments, we considered that this drug combination could be an alternative approach. Objective: Evaluate the in vitro effect of the ALB-MLT and ALBSO-MLT combinations against glioblastoma cells.Methods: C6 cells were treated with ALB in the range of 0.16 to 1.25 µM; ALBSO in a range of 2.0 to 64.0 µM; MLT in the range of 0.25 to 8.0 mM and TMZ (positive control) at 100 µM, for 72 hours. Cell viability was evaluated with the MTT technique. The mean effect (Dm) was calculated and then the ALB-MLT combination was evaluated in a 1:1 and 1:4 ratios, and also the ALBSO-MLT combination in a 1:1 ratio. The combination index (CI) and dose reduction index (DRI) were determined using CompuSyn software. To determine the type of the cell death (apoptosis or necrosis) the annexin V / 7AAD test was performed using by flow cytometry. Data were expressed as the mean of the standard error (MSE). All experiments were performed in triplicate. The determination of significant differences in the experiments of cell viability and induction of necrosis and apoptosis was performed with a one-way analysis of variance (ANOVA) and a Tukey post hoc test. In all cases, significance was considered when * $p < 0.05$.Results: The Dm for ALB, ALBSO and MLT were 0.6 µM, 20.0 µM and 1.0 mM, respectively. Most combinations of ALB-MLT and ALBSO-MLT exhibited synergism (CI between 0.1 and 0.85) and favorable dose reduction (DRI between 2.0 and 19.0). Cytometric analysis showed a greater effect of cell death with the combination than with the drugs alone, mainly due to apoptosis.Conclusion: The results are promising, mainly for the favorable dose reduction. Other studies in animal models are needed to determine the advantages of the combination.

Disclosures: M.H. Cerón: None. **I.S. Rojas:** None. **C. Rios:** None. **B.P. Olvera:** None. **F.P. Alonso:** None. **H.J. Cook:** None.

Digital Abstract Session

P081. Astrocytes: Disease Mechanisms

Program #/Poster #: P081.03

Topic: B.11. Glial Mechanisms

Support: Audrey Lewis Young Investigator Award, CureSMA
NINDS, NIH

Title: The role of astrocytes in SMA motor neuron synaptic defects

Authors: *E. WELBY, A. D. EBERT;
Cell Biology, Neurobiology, and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Spinal muscular atrophy (SMA) is a neurodegenerative disease primarily characterized by the loss of lower spinal motor neurons. Evidence suggests that survival motor neuron (SMN) protein deficiency in other cell types, including astrocytes, also contributes to SMA pathology. We and others have previously demonstrated that astrocytes derived from patient induced pluripotent stem cells (iPSCs) show SMN-dependent intrinsic defects. In addition to providing neurotrophic support, astrocytes play an important regulatory role in synapse development and function. Motor neuron peripheral and central synaptic defects have been previously described and restoration of SMN expression in motor neurons can improve synaptic integrity, but only to a certain extent. It remains to be fully determined how human glial cells contribute to SMA synapse pathogenesis.
Differential gene expression analysis of our iPSC-derived astrocyte RNA seq data demonstrates a significant down-regulation of genes associated with synaptic transmission and plasma membrane cell projections in patient-derived samples. Using the SurfaceGenie prediction tool, we verified many of these down-regulated genes are likely to encode cell surface proteins. A large number of genes are associated with regulation of ion gradients (K^+ ion channels, Ca^{2+} regulatory channels), neurotransmitter release (glutamate receptors and transporters) and synaptic formation and integrity (ephrins, cell adhesion proteins). We therefore hypothesize that SMA astrocytes may lack important synaptic-related cell surface genes and their encoding proteins, which could contribute towards motor neuron synaptic defects. We will further characterize abnormal cell surface protein expression in SMA astrocytes using cell surface capture mass spectrometry and investigate the downstream molecular and functional implications in our *in vitro* co-culture system using super resolution microscopy and multi-electrode array (MEA) approaches. Preliminary MEA data from SMA iPSC-derived motor neuron cultures already suggests an intrinsic defect in mean firing rate and we will investigate if SMA astrocytes could further compound this intrinsic synaptic deficit.

Disclosures: E. Welby: None. **A.D. Ebert:** None.

Digital Abstract Session

P081. Astrocytes: Disease Mechanisms

Program #/Poster #: P081.04

Topic: B.11. Glial Mechanisms

Support: MOST108-2321-B-010 -012 -MY2

Title: Cerebral cavernous malformation 3 regulates astrocytic plasticity and neurovascular coupling under excitotoxicity

Authors: Y.-T. LIU[1,3], P.-C. HSU[2], P.-K. YU[2], Y.-C. HSIN[2], J.-F. LIRNG[1,4], *Y.-H. LEE[2]; [1]Fac. of Med., [2]Physiol., Natl. Yang-Ming Univ. Sch. of Med., Taipei, Taiwan; [3]Neurol., [4]Radiology, Taipei Veteran Gen. Hosp., Taipei, Taiwan

Abstract: Cerebral vascular malformations (CVMs) are lesions due to abnormal vasculogenesis in the brain, with arteriovenous malformation (AVM) and cerebral cavernous malformation (CCM) as the two commonest types. CCM is the most frequent epileptic CVM. Familial CCM mutations in the three genes are most frequently identified: *CCM1 (KRIT1), CCM2 (MGC4607)* and *CCM3 (PDCD10). CCM3* mutations are responsible for the most aggressive phenotypes and an earlier age of onset, and is predominantly expressed in glial cells. In this study, we used human cortical astrocytes and human brain microvascular endothelial cells (BMEC) to study the role of CCMs in astrocyte functions. Our data show that reduction of CCM1, 2, and 3 expressions by RNA interference (siCCMs) inhibit the expression of glutamate transporter 1 in astrocytes and VCAM1 in BMEC, but only siCCM3 increase reactive astrocyte marker GFAP. Indeed, we found that siCCM3 in astrocytes not only causes hypertrophy but also increase the vulnerability to high glutamate stimulation. Importantly, CCM3 is predominantly expressed in astrocytes, not brain vascular endothelial layers, in mouse brain hippocampus. Astrocyte conditioned medium from siCCM3- astrocytes show damaging effects on BMEC morphology and tight function protein expression For the immune response of astrocytes, siCCM3, not siCCM1 or siCCM2 in human astrocyte-conditioned medium show common feature in cytokine elevation as in CCM patients' serum. Thus, these results suggest that CCM3 loss of function would cause molecular and structural alterations of astrocytes, which may be causal to the neuronal hyperexcitation and vasculopatholgy in CCM.

Disclosures: Y. Liu: None. **P. Hsu:** None. **P. Yu:** None. **Y. Hsin:** None. **J. Lirng:** None. **Y. Lee:** None.

Digital Abstract Session

P081. Astrocytes: Disease Mechanisms

Program #/Poster #: P081.05

Topic: B.11. Glial Mechanisms

Support: FOSSIS-CONACyT 273308
DGAPA-PAPIIT IN210817
FONCICYT-DADC 273553 OBETEEN
CONACyT CB250880

Title: Serum levels of glial fibrillar acidic protein as a peripheral biomarker of cognitive dysfunction due to high-fat/high-fructose diet consumption.

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Abstract: One of the main public health problems in the world is the high prevalence of overweight and obesity. The fundamental cause of these metabolic disorders is an energy imbalance between the expended and the consumed calories, together with the increase of sedentary behaviors from early ages. In consequence, it is evident an increase of the prevalence of cardiovascular problems, dyslipidemias, liver disease, inflammation, metabolic syndrome and type II diabetes mellitus, chronic non-communicable diseases that are expensive for the health sector. Along with the peripheric consequences of such states, there is an increased risk to suffer cognitive impairment, which occurs naturally until advanced ages. Both, aging and metabolic disorders are associated with production of oxidative stress in the brain and neuroinflammation which is considered a main culprit of the development of such cognitive impairment. Astrocytes are the most abundant cells in the brain and respond to any type of brain insult through astrogliosis. One hallmark of astrogliosis is the increase of glial fibrillar acidic protein (GFAP); essential for the growing and proliferation of astrocytes which expression is predominantly in the central nervous system. It has been reported that astrogliosis increase during aging and after exposure to a high-fat diet in young mice, so it is possible that serum GFAP would be a good peripheral biomarker of central injury and aid us to predict the beginning of cognitive impairment. To test this hypothesis, first we analyzed the cognitive performance and astrocyte morphology on dorsal hippocampus (DH) from male and female C57BL/6 mice of 5 (n=17), 10 (n=15), 15 (n=17), and 19 (n=18) months. Then, we analyzed the same parameters from male and female C57BL/6 weaning mice (n=13) exposed during 5 months to a diet composed of 10% fructose and 60% caloric intake from fat. We found a progressive decline of cognitive function in DH dependent memory tasks such as Morris's water maze in normal aging. On the other hand, high fat/fructose diet in adult mice induces increased weight, central adiposity, glucose intolerance and causes cognitive impairment like older mouse. Sholl analysis showed slight morphological changes in DH astrocytes from high fat/fructose group. However, we did not find changes in serum GFAP levels among groups, indicating that, under these conditions, serum GFAP is not a good peripheral biomarker, so we suggest examining serum GFAP levels in advanced stages of central damage.

Disclosures: L. Ayala-Guerrero: None. **F. Bermúdez-Rattoni:** None. **K.R. Guzman-Ramos:** None.

Digital Abstract Session

P081. Astrocytes: Disease Mechanisms

Program #/Poster #: P081.06

Topic: B.11. Glial Mechanisms

Support: R01AI116456 NIAID, NIH
D43TW001140 FIC,NIH

Title: Determination of histological changes in the brain through the astrocytic response in the calcification process in neurocysticercosis

Authors: *L. E. BAQUEDANO SANTANA[1], N. MIRANDA[2], J. BUSTOS[2], H. H. GARCIA[3], M. FORERO[4];
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Abstract: Neurocysticercosis (NCC) is a parasitic disease caused by the Taenia solium larval stage (cyst) in the central nervous system. It is the leading cause of acquired epilepsy in endemic countries. Calcifications produced by this disease are associated with seizures in 20% of patients. This study aimed to characterize and evaluate the neuropathological findings of calcifications in the brain of pigs with NCC. We used 14 pigs naturally infected with T. solium, and they received antiparasitic treatment. Were sacrificed at eight months post-treatment and the brains were perfused, removed, and fixed in paraformaldehyde. Hematoxylin-Eosin and Masson's Trichrome stain were used to describe the response inflammatory. Also, we use Von Kossa and Alizarin red stain to identify and describe the presence of calcium deposits and scanning electron microscopy to quantify calcium phosphate deposits, and the immunostaining studies were evaluated with IBA, and GFAP. We found differences in collagen scarring, microglial reactivity, calcium accumulation, and axonal damage in the classic morphological appearance of traumatic axonal injury in the treated group compared with non-treated group. Our data provide novel insight into the relationship between disrupted blood-brain barrier (BBB), glial reactivity, and the progression of calcification of the neurocysticercosis. Future studies in new drugs on the antiparasitic treatments will be conducted.

Disclosures: L.E. Baquedano Santana: None. **N. Miranda:** None. **J. Bustos:** None. **H.H. Garcia:** None. **M. Forero:** None.

Digital Abstract Session

P081. Astrocytes: Disease Mechanisms

Program #/Poster #: P081.07

Topic: B.11. Glial Mechanisms

Support: NIMH Grant R25 MH101076.

NIH Grant P20GM130452

Title: Peripheral astrocyte protein level characterization after transcranial magnetic stimulation in treatment resistant depression

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Abstract: Background: Transcranial magnetic stimulation (TMS) therapy is effective for treatment resistant depression (MDD) but the therapeutic mechanism of action is still being elucidated. Astrocytic contributions to the pathophysiology of depression has started garnering attention but has not been explored in TMS in depression. A pilot study examining serum levels of three astrocytic proteins: S100 Calcium Binding Protein B (S100B), Glial Fibrillary Acidic Protein (GFAP), and Vascular endothelial growth factor (VEGF), before and after TMS in MDD was carried out to study their potential roles in the mechanism of TMS. **Methods:** Serum was collected from a naturalistic population of 35 patients (40% male, 60% female) with MDD at two timepoints: prior to receiving first session of TMS and after the 30th session. Protein concentrations were determined via Enzyme-linked Immunosorbent Assay (ELISA) and all samples were run in duplicates. Inventory of Depressive Symptomatology Self Report (IDS-SR) was used as a measure of depression symptom severity, clinical response, and remission. TMS was given at standard 10 Hz delivered to the dorsolateral left prefrontal cortex daily at 120% maximum intensity relative to their motor threshold for a minimum of 3000 pulses. **Results:** There was a positive correlation between %change in IDS-SR and %change in GFAP ($r=.561$, $p<0.05$), and VEGF ($r=.358$, $p<0.05$). Amongst those who remitted, VEGF increased from pre to post TMS (%change +12.30%) whereas VEGF decreased in nonremitters (% change -9.24%) ($p=0.054$). This same pattern was observed when comparing VEGF changes between responders (+9.35%) and non-responders (-12.42%) ($p<0.05$). Similarly, GFAP also increased in responders (+151.18%) but decreased in non-responders (-41.10%) ($p<0.05$). S100B did not correlate with depression severity changes, nor did they differ between those with different clinical outcomes. The protein levels did not differ by sex or with age. **Conclusions:** Patients with a successful treatment with TMS had significantly greater increase in VEGF and GFAP from pre to post treatment compared to non-responders and a larger increase was associated with greater improvement in depressive symptoms after TMS. These patterns were not seen in S100B. The functional implications of the differential changes in these astrocytic proteins are yet to be elucidated, but data from preclinical work hint at neuroinflammation, angiogenesis, synaptic remodeling, and blood brain permeability changes. This pilot study provides promising exploratory data showing that GFAP and VEGF is an important mediator in the mechanism behind TMS' antidepressant effects.

Disclosures: A.M. Fukuda: None. **L.E. Hindley:** None. **J.D. Kang:** None. **E. Tirrell:** None. **A.R. Tyrka:** None. **L.L. Carpenter:** None.

Digital Abstract Session

P081. Astrocytes: Disease Mechanisms

Program #/Poster #: P081.08

Topic: B.11. Glial Mechanisms

Support: NIH/NIDA R21DA046600
Skaggs Graduate Research Fellowship
Roy Kuramoto Award

Title: Functional Remodeling of the Tripartite Synapse in Cocaine Abuse and Addiction

Authors: *D. M. GIANGRASSO, J. N. WAGNER, M. M. TIMM, K. S. WILCOX, K. A. KEEFE;
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Abstract: Cocaine is a commonly abused psychostimulant. With repeated drug exposure, there is a shift from drug abuse to addiction that is thought to develop via transition from goal-directed to habitual control over behavior. The transition to habitual control over behavior is associated with potentiated corticostriatal glutamate signaling in dorsolateral striatum (DLS); however, the mechanisms underlying such potentiation, particularly in drug addiction, are largely unknown. Astrocytes critically regulate synaptic transmission. In particular, astrocytes express glutamate transporter-1 (GLT-1), which regulates the concentration and time-course of extracellular glutamate. Rats with a history of cocaine self-administration display decreased GLT-1 protein expression in ventral striatum and DLS. However, the functional impact of the decrease in GLT-1 on glutamate clearance in DLS is unknown, as is the relation to development of habitual control over drug-seeking. The objective of this study therefore was to determine if glutamate clearance is reduced in DLS following repeated cocaine self-administration in rats exhibiting habitual control over cocaine-seeking. We expressed the intensity-based glutamate-sensing fluorescent reporter (iGluSnFr) in astrocytes of DLS of male Long-Evans rats before they underwent training on a chained cocaine self-administration paradigm. At the end of training, rats underwent extinction of the taking lever, followed by testing under devalued and then re-valued conditions. The ratio of cocaine-seeking under devalued vs. valued conditions was used to classify rats as being habitual (resistant to outcome devaluation) or goal-directed (sensitive to outcome devaluation) in their cocaine-seeking. Following testing, coronal brain slices were collected and local stimulation (1mA pulses) was used to evoke glutamate release and iGluSnFr signals. Experimenters were blinded to drug condition and classification during imaging and data analysis. The average decay tau of evoked iGluSnFr signals in DLS was significantly slower in rats whose cocaine-seeking was under habitual control relative to rats whose cocaine-seeking was goal-directed ($p<0.003$) and yoked-saline controls ($p<0.006$). There was no difference between rats whose cocaine-seeking was goal-directed and yoked-saline rats ($p=0.56$). These data suggest that glutamate clearance in DLS is slowed in rats exhibiting habitual, but not goal-directed, cocaine-seeking. Studies are ongoing to examine whether these changes are specific to

DLS and are associated with alterations in GLT-1 expression and excitatory transmission onto striatal efferent neurons.

Disclosures: D.M. Giangrasso: None. **K.A. Keefe:** None. **J.N. Wagner:** None. **M.M. Timm:** None. **K.S. Wilcox:** None.

Digital Abstract Session

P081. Astrocytes: Disease Mechanisms

Program #/Poster #: P081.09

Topic: B.11. Glial Mechanisms

Support: NIH Grant T32 DA007097

Title: Role of CD38 in Spinal Opioid Antinociception

Authors: *R. E. QUINTANA, A. GUEDES;
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Abstract: The number of deaths by opioid overdose has risen fivefold over the past twenty years, yet opioids remain highly prescribed for the treatment of pain. In addition, opioid therapy is frequently associated with tolerance and addiction. Better understanding of the mechanisms that modulate opioid antinociception and tolerance could lead to the development of better pain treatment alternatives. The transmembrane glycoprotein CD38, which is important for intracellular calcium homeostasis, has been linked to supraspinal opioid anti-nociception and tolerance, but these experiments were only on male mice and failed to provide a pain model to study the role of CD38 in opioid antinociception. There is also a lack of information regarding the cellular expression of CD38 in the spinal cord, a site relevant for opioid actions. Our study aims to understand the role of CD38 in opioid antinociception in chronic neuropathic pain and to explore the cellular expression of CD38 in the spinal cord. To investigate the role of CD38 in neuropathic pain, we used the spared nerve injury (SNI) model in male and female, 6-12-month-old wild type (WT) and CD38 knockout (CD38KO) mice. We determined mechanical withdrawal thresholds using von Frey filaments using the up-and-down method. We found that both mice developed similar magnitudes and significant mechanical hypersensitivity as determined using the von Frey test during a 12-week test period. To determine the role of spinal CD38 in opioid antinociception in the SNI model, CD38KO and WT animals received cumulative doses of the mu-opioid receptor agonist morphine (0, 0.125, 0.25, 0.5, 1 and 2 μmol/5μl, I.T.). We found the anti-nociceptive effect of morphine to be significantly decreased in CD38KO compared to WT mice. Together, these results suggest that CD38 is not involved in the development and maintenance of mechanical hypersensitivity due to peripheral nerve injury, but its presence in the spinal cord is required for the pain-relieving action of mu-opioid receptor agonists. To further understand how CD38 might mediate opioid-induced anti-nociception, we performed in-situ hybridization and immunohistochemistry in spinal cord tissues from WT mice and found that CD38 is localized exclusively in astrocytes. The canonical opioid anti-nociceptive

mechanisms have been thought to be exclusively neuronal, but our results suggest that CD38-expressing astrocytes are critical for effective opioid anti-nociception in the SNI model of neuropathic pain. Further exploration of astrocytic mechanisms in opioid signaling at the spinal level could reveal novel anti-nociceptive targets for neuropathic pain.

Disclosures: R.E. Quintana: None. **A. Guedes:** None.

Digital Abstract Session

P082. Microglia: Biology

Program #/Poster #: P082.01

Topic: B.11. Glial Mechanisms

Support: National Key Research and Development Program of China grant 2018YFA0800200
Research Grants Council of Hong Kong grant 16131916
Research Grants Council of Hong Kong grant 16102617
Research Grants Council of Hong Kong grant 16103718
Research Grants Council of Hong Kong grant C6002-17GF
Research Grants Council of Hong Kong grant N_HKUST621/17
Research Grants Council of Hong Kong grant AoE/M-09/12

Title: Two phenotypically and functionally distinct microglial populations in adult zebrafish

Authors: *S. WU[1], L. T. M. NGUYEN[1], H. PAN[1], S. HASSAN[1], Y. DAI[1], J. XU[2], Z. WEN[1]; [1]Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong; [2]South China Univ. of Technol., Guang Zhou, China

Abstract: Microglia are the tissue-resident macrophages in the central nervous system and are critically involved in immune defense, neural development and function, and neuroinflammation. The versatility of microglia has long been attributed to heterogeneity. Recent studies revealed possible heterogeneity in human but not in murine microglia, yet a firm demonstration linking microglial heterogeneity to functional phenotypes remains scarce. Here, we identified two distinct microglial populations in adult zebrafish that differ in morphology, distribution, development, and function. The predominant population - phagocytotic microglia, which expresses *ccl34b.1*, is broadly distributed, amoeboid in shape, highly mobile and phagocytotic. The other white matter-enriched *ccl34b.1*$^{-}$ population, regulatory microglia, has ramified protrusions but has limited mobility and phagocytosis capability. Their functional differences are further supported by distinct transcriptomes and responses to bacterial infection, where *ccl34b.1*$^{+}$ microglia function in tissue clearance and *ccl34b.1*$^{-}$ microglia release immune regulators. Our study sheds light on the heterogeneity and functional diversification of microglia.

Disclosures: S. Wu: None. **L.T.M. Nguyen:** None. **H. Pan:** None. **S. Hassan:** None. **Y. Dai:** None. **J. Xu:** None. **Z. Wen:** None.

Digital Abstract Session

P082. Microglia: Biology

Program #/Poster #: P082.02

Topic: B.11. Glial Mechanisms

Support: NS088627
NS114122
NS110825
NS110949
AG064159

Title: How Microglia Sense and Regulate Neuronal Activity?

Authors: *L.-J. WU;
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Abstract: Microglia are innate immune cells of the central nervous system (CNS) and are sensitive to extracellular cues. Brain injuries, inflammation, and pathology evoke dynamic structural responses in microglia, altering their morphology and motility. In addition, microglia respond to and engage in neuronal activity—a facet of their biology that exists alongside their pathological responses. Using in vivo imaging approaches, we found that microglia increase their process dynamics and surveillance in response to either hyperactive or hypoactive network activity in mouse brain. Interestingly, microglia sense neuronal hyperactivity via P2Y12 receptors while they sense hypoactivity via adrenergic β2 receptors. Using microglia calcium imaging and chemogenetic manipulation in awake mice, we further demonstrate that microglia calcium is attuned to neuronal activity. Specifically, microglia increase calcium signaling in response to bi-directional shifts in neuronal activity. Using genetic ablation of microglia, we found that both acute and chronic seizures are increased after microglial depletion. Consistently, in P2Y12 deficiency mice with impaired microglia-neuron interaction, KA-induced seizures are aggravated. Together, these results demonstrate that microglia can dampen neuronal activity in seizures. Therefore, our studies elucidate how microglia sense neuronal activity and later influence neuronal function following their engagement. The dynamic function of microglia in monitoring and dictating neuronal activity is critical for brain hemostasis and repair in health and disease.

Disclosures: L. Wu: None.

Digital Abstract Session

P082. Microglia: Biology

Program #/Poster #: P082.03

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01MH106553

Title: Quantification of microglia in the dorsal hippocampus following temporary intra-hippocampal microglia depletion during development

Authors: *N. HABASH, M. B. BIELICKI, N. HAAS, J. M. SCHWARZ;
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Abstract: Microglia, the resident immune cells of the brain, interact with neurons to shape cognitive development. Microglial activation during the early postnatal period has been linked to long-term effects on cognition and learning in rats. We seek to examine the role of microglia in hippocampal learning by establishing a method to selectively deplete microglia in the dorsal hippocampus during a unique juvenile period development in rats. Liposome-encapsulated clodronate (LEC) is a drug that is selectively ingested by and results in the death of phagocytic cells. We infused 3 microliters LEC bilaterally into the dorsal hippocampus (dHP) of male and female rats (Sprague-Dawley) at P16 to determine whether LEC selectively depletes microglia during a period of hippocampal development. We utilized immunohistochemical (IHC) staining and RT-PCR to examine changes in microglia density and gene expression in the CA1, CA3, and dentate gyrus (DG) subregions of dorsal and ventral hippocampus (vHP) on P21, P24, P27, P30, and P40. Iba1 stain for microglia revealed that LEC significantly decreased the percent area of microglia density and the number of microglia cells per area of microglia in subregions CA1 and DG of dHP from P21-P30. LEC did not significantly deplete microglia in any vHP subregions, nor the CA3 subregion of dHP, which indicate that the spread of LEC does not go beyond the intended target of dHP CA1 and DG subregions. Future research will use this data to establish a timeline of microglial depletion during the juvenile period in order to investigate the role of microglia in hippocampal learning deficits following immune activation.

Disclosures: M.B. Bielicki: None. **J.M. Schwarz:** None. **N. Haas:** None. **N. Habash:** None.

Digital Abstract Session

P082. Microglia: Biology

Program #/Poster #: P082.04

Topic: B.11. Glial Mechanisms

Support: NARSAD Young Investigator
EIPOD Fellowship
EMBL Intramural Fund

Title: Complement Cascade Mediated Pruning of the Developing Brain

Authors: *S. DEIVASIGAMANI[1], M. T. MITEVA[3], S. NATALE[1], D. GUTIERREZ-BARRAGAN[4], C. PAPE[2], G. BOLASCO[1], A. GALBUSERA[4], A. GOZZI[5], C. GROSS[1];
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Abstract: Cell death, axon elimination, and synaptic pruning are major events during development that dramatically rewire cortical projections, eliminating up to 70% of connections. Defects in this process is hypothesized to result in psychiatric diseases like Schizophrenia. Recently this theory received a boost when a genome-wide association study identified copy number variants in the C4 gene as a risk factor for schizophrenia. The proteolytic cascade of Complement signalling factors (C1q, C3, C4, C5) is thought to serve as an opsonization signal to promote microglial phagocytosis of synapses. However, the role of Complement Cascade in the development of cortical structures in unclear. We discovered that mice lacking Complement 3 receptor (*CR3*), a downstream receptor in complement cascade have increased number of axons in the developing optic nerve but not in cortex. These mice do not show any difference in synapse density in cortex. We also find, using resting-state fMRI, that *CR3* knockout mice have defective functional connectivity selectively in the Prefrontal Cortex and Dorsal Thalamus areas implicated in schizophrenia risk in humans. These data suggest a divergent role for Complement cascade in cortical development.

Disclosures: S. Deivasigamani: None. **M.T. Miteva:** None. **S. Natale:** None. **C. Pape:** None. **G. Bolasco:** None. **A. Gozzi:** None. **C. Gross:** None.

Digital Abstract Session

P083. Microglia: Disease Mechanisms

Program #/Poster #: P083.01

Topic: B.11. Glial Mechanisms

Support: R01NS088627
R01NS112144
R01NS110949
R01NS110825
F32NS114040

Title: Optogenetic activation of spinal microglia triggers chronic pain in mice

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[1]Dept. of Neurol., Mayo Clin., Rochester, MN; [2]Dept. of Neurosci., Mayo Clin., Jacksonville, FL; [3]Dept. of Immunol., Mayo Clin., Rochester, MN; [4]Dept. of Anesthesiol. & Perioperative Med., Xijing Hospital, Fourth Military Med. Univ., Xi'an, China

Abstract: Spinal microglia are highly responsive to peripheral nerve injury and are known to be a key player in neuropathic pain. However, there has not been any direct evidence showing selective microglial activation in vivo is sufficient to induce chronic pain. Here we used optogenetic approaches in microglia to address this question employing $CX3CR1^{creER/+}$: $R26^{LSL-}$

$^{ReaChR/+}$ transgenic mice, in which red-activated channelrhodopsin (ReaChR) is inducibly and specifically expressed in microglia. We found that activation of ReaChR by red light in spinal microglia evoked reliable inward currents and membrane depolarization. In vivo optogenetic activation of microglial ReaChR in the spinal cord triggered chronic pain hypersensitivity lasting for 5-7 days. In addition, activation of microglial ReaChR upregulated neuronal c-fos expression and enhanced C-fiber responses. Mechanistically, ReaChR activation led to a reactive microglial phenotype with increased IL-1β production. IL-1 receptor antagonist was able to reverse the pain hypersensitivity and neuronal hyperactivity induced by microglial ReaChR activation. Therefore, our work demonstrates that optogenetic activation of spinal microglia is sufficient to trigger chronic pain phenotypes by increasing neuronal activity via IL-1 signaling.

Disclosures: **M. Yi:** None. **Y. Liu:** None. **A. Umpierre:** None. **T. Chen:** None. **Y. Ying:** None. **A. Dheer:** None. **D. Bosco:** None. **H. Dong:** None. **L. Wu:** None.

Digital Abstract Session

P083. Microglia: Disease Mechanisms

Program #/Poster #: P083.02

Topic: B.11. Glial Mechanisms

Support: 5R01NS093416-05

Title: Functional Changes in Microglia in a Neonatal Rabbit Model of Cerebral Palsy

Authors: ***A. SHI**, E. KHOURY, K. LIAW, A. FOWLER, A. SHARMA, R. M. KANNAN, S. KANNAN;
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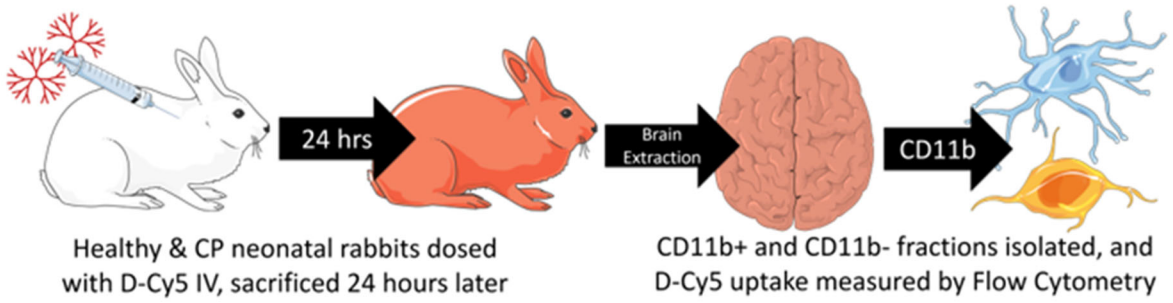
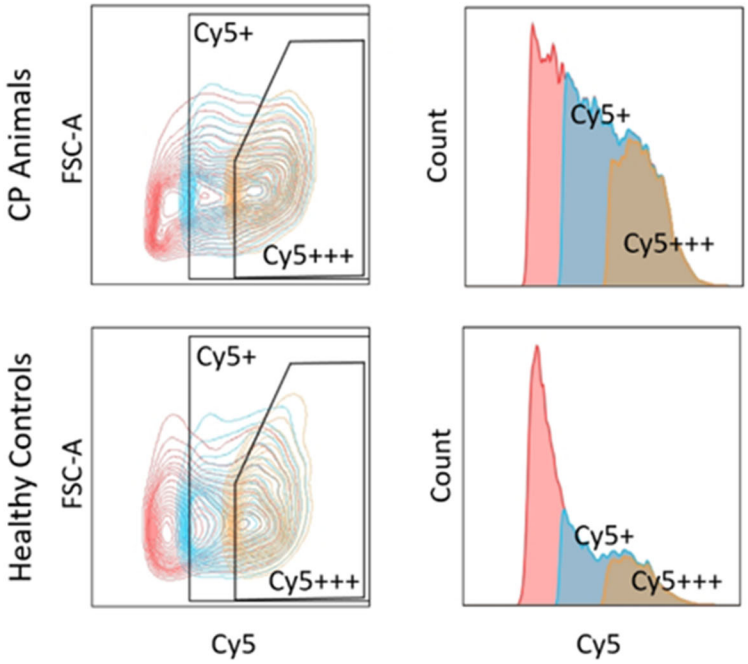
Abstract: Cerebral palsy (CP) is a pediatric neurodevelopmental disorder that manifests in a range of motor and cognitive symptoms with no effective cure. The microglial response to neuroinflammation during fetal and infant brain development is a major contributor to CP pathogenesis; in fact, inducing microglial activation by in utero exposure to lipopolysaccharide in rabbits results in a robust animal model of CP. We have previously demonstrated that microglia of CP rabbits undergo change to a pro-inflammatory phenotype, that these activated microglia uptake dendrimer nanoparticles via endocytosis while healthy microglia do not, and that delivery of anti-inflammatory drugs via dendrimers to activated microglia can reverse motor deficits. However, the functional changes in microglia associated with activation and related mechanisms of dendrimer uptake are not well characterized. We established a method for isolating microglia from rabbit brain and assessed the difference in microglial (1) phagocytic activity and (2) dendrimer uptake from CP and healthy rabbits. Flow cytometry studies showed that surface marker CD11b is reliably expressed and can be used to isolate microglia in rabbit brain tissue. Phagocytic activity was studied by exposing isolated microglia to fluorescent *E. coli*-coated beads and uptake was quantified by flow cytometry. Phagocytic activity was found to be significantly higher in CP animals compared to healthy controls. Another functional measure

studied was difference in microglial dendrimer uptake; to assess this, dendrimer conjugated to fluorescent dye (D-Cy5) was dosed intravenously and microglia were collected 24 hours later. D-Cy5 localized in a subset of microglia but not in CD11b- cells, and to a greater extent in microglia extracted from CP rabbits than from healthy controls. Ongoing studies are focused on further characterization of this subpopulation of microglia. A better understanding of inflammatory microglial function will inform design of microglia-targeted drug delivery platforms for treatment of neuroinflammatory disorders such as CP.





Disclosures: **A. Shi:** None. **E. Khoury:** None. **K. Liaw:** None. **A. Fowler:** None. **A. Sharma:** None. **R.M. Kannan:** None. **S. Kannan:** None.

Digital Abstract Session

P083. Microglia: Disease Mechanisms

Program #/Poster #: P083.03

Topic: B.11. Glial Mechanisms

Support: R15 NIMH MH101700
GM103475 (PRINBRE)
RCMI NIMHD MD007579
RISE GM082406

Title: Icv administration of p2x7 antagonist prevents sps-induced ptsd-related behavior

Authors: *O. I. TORRES-RODRÍGUEZ, Y. RIVERA-ESCOBALES, M. COLÓN-ROMERO, A. HERNÁNDEZ, J. T. PORTER;
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Abstract: Clinical evidence has linked increased peripheral levels of pro-inflammatory cytokines with the severity of PTSD symptoms. Since microglia are known as a major producer of pro-inflammatory cytokines in the brain, the inflammatory manifestation mediated by microglia may contribute to PTSD-related behaviors. We hypothesized that stress exposure induces a microglial-mediated inflammation that precedes the PTSD behavioral manifestations. To test this, we exposed rats to single prolonged stress (SPS) one week before measuring fear discrimination, extinction, and anxiety-like behaviors. Consistent with the literature, our data show that exposing rats to SPS produces a PTSD-like phenotype of impaired fear extinction and increased anxiety in male and female rats. Additionally, SPS-exposed animals showed increased expression of Iba-1, P2X7R, and CD68 by microglia in the ventral hippocampus (VH), a structure that regulates fear extinction and anxiety. Furthermore, VH microglial cells increased their expression of IL-1β and TNF-α genes three days following SPS suggesting an increased inflammatory-state before behavioral testing. Although our data suggest that SPS induces an inflammatory manifestation mediated by microglia, it is not clear whether inhibition of the inflammation will prevent the SPS-induced PTSD-related behaviors. Since P2X7R leads to inflammation, we hypothesized that inhibition of inflammatory P2X7R signaling would prevent the SPS-induced PTSD-related behaviors. To test this hypothesis, we implanted Intracerebroventricular (ICV) cannulas into the animals 2 weeks before SPS exposure for the administration of the P2X7R antagonist, A-438079. Animals were randomly assigned and received one week of daily infusions of the vehicle or A-438079 starting the day of SPS exposure. Then, animals were subjected to a behavioral paradigm to examine cue-associated fear discrimination, fear extinction, and anxiety. Our results indicate that animals that received A-438079 showed improved extinction and less anxiety-like behaviors. Consistent with our hypothesis, inhibition of the P2X7R prevented the SPS-induced impaired fear extinction and increased anxiety-like behaviors in male and female rats. Altogether, these data suggest that inhibiting inflammatory responses, such as P2X7R signaling in microglia, following trauma exposure might prevent the development of trauma-associated neuropsychiatric disorders such as PTSD.

Disclosures: O.I. Torres-Rodríguez: None. **Y. Rivera-Escobales:** None. **M. Colón-Romero:** None. **J.T. Porter:** None. **A. Hernández:** None.

Digital Abstract Session

P083. Microglia: Disease Mechanisms

Program #/Poster #: P083.04

Topic: B.11. Glial Mechanisms

Support: NIH R01NS102583
AHA 16SDG30980031

Title: Analysis of Glucose Metabolism by ^{18}F-FDG-PET Imaging and Glucose Transporter Expression in a Mouse Model of Intracerebral Hemorrhage

Authors: *X. HAN, H. REN, A. NANDI, X. FAN, R. KOEHLER;
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Abstract: The relationship between cerebral glucose metabolism and glucose transporter expression after intracerebral hemorrhage (ICH) is unclear. Few studies have used positron emission tomography (PET) to explore cerebral glucose metabolism after ICH in rodents. In this study, we produced ICH in mice with an intrastriatal injection of collagenase to investigate whether glucose metabolic changes in ^{18}F-fluoro-2-deoxy-D-glucose (FDG)-PET images are associated with expression of glucose transporters (GLUTs) over time. On days 1 and 3 after ICH, the ipsilateral striatum exhibited significant hypometabolism. However, by days 7 and 14, glucose metabolism was significantly higher in the ipsilateral striatum than in the contralateral striatum. The contralateral hemisphere did not show hypermetabolism at any time after ICH. Qualitative immunofluorescence and Western blotting indicated that the expression of GLUT1 in ipsilateral striatum decreased on days 1 and 3 after ICH and gradually returned to baseline by day 21. The ^{18}F-FDG uptake after ICH was associated with expression of GLUT1 but not GLUT3 or GLUT5. Our data suggest that ipsilateral cerebral glucose metabolism decreases in the early stage after ICH and increases progressively in the late stage. Changes in ^{18}F-FDG uptake on PET imaging are associated with the expression of GLUT1 in the ipsilateral striatum.

Disclosures: X. Han: None. **H. Ren:** None. **R. Koehler:** None. **A. Nandi:** None. **X. Fan:** None.

Digital Abstract Session

P083. Microglia: Disease Mechanisms

Program #/Poster #: P083.05

Topic: B.11. Glial Mechanisms

Title: Bpa induced increases in microglia tspo clustering are reduced by emapunil

Authors: *H. N. STRONG, K. I. MCGLOTHEN, R. M. HINES, D. J. HINES;
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Abstract: Bisphenol *A* (BPA) is a common environmental pollutant that has been linked to neurodevelopmental and mitochondrial dysfunction. Alterations in the molecular pathways underlying the effects of BPA on the brain remain unclear. The mitochondrial 18kDa translocator protein (TSPO) is a particular protein that changes expression in response to damaging changes in energy demands. Microglia play a major role in brain energy homeostasis and contain the majority of brain derived TSPO. We show that TSPO forms clusters in microglia and acute BPA exposure increases TSPO clustering in the branches of microglia. Treatment with the TSPO agonist Emapunil attenuates BPA induced increases in branch clustering. Using immunofluorescent staining of TSPO and microglia, we labeled TSPO clustering patterns. Mice not treated with BPA or Emapunil showed somatic TSPO clustering in microglia of the prefrontal cortex. BPA exposure increased branch clustering of TSPO, while treatment with Emapunil reduced branch clustering. These results show BPA alters TSPO clustering patterns in microglia and TSPO function is important in regulating clustering patterns. Further research into the variations and role of TSPO clustering patterns will be important for understanding the progression of neurological diseases.

Disclosures: H.N. Strong: None. **K.I. McGlothen:** None. **R.M. Hines:** None. **D.J. Hines:** None.

Digital Abstract Session

P083. Microglia: Disease Mechanisms

Program #/Poster #: P083.06

Topic: B.11. Glial Mechanisms

Title: Increased TSPO in microglia endfeet aids cell survival and attenuates gross-motor deficits following a blast induced traumatic brain injury.

Authors: *E. A. AQUINO[1], D. J. HINES[2];
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Abstract: Traumatic brain injuries (TBIs) are hallmarked by prolonged cognitive and motor deficits. Neuroinflammation is been implicated in promoting secondary injuries yet its role in the neuropathology of secondary injuries is poorly understood. Microglia are the resident immune cells of the central nervous system responsible for the regulation of neuroinflammatory responses, spine development and removal of waste and debris. Microglia undergo a series of dynamic structural changes called *reactive microgliosis,* following injury. Microgliosis is necessary for a proper immune response and is crucial to recovery, but if it persists, dysregulated microgliosis can become detrimental and result in secondary injury. When activated, microglia express an abundance of translocator protein (TSPO). TSPO is an 18kDA outer mitochondrial protein that is upregulated during neuroinflammatory responses and is implicated in regulating pro-inflammatory and anti-inflammatory processes. Despite its involvement in microglia activation, its precise role remains elusive. To relate changes in microglial TSPO expression to gross motor sequela, TSPO levels and motor behavior will be assessed 24, 72, and 120 hours

following a blast induced TBI (bTBI). To determine how modulation of TSPO levels can affect TBI outcomes, mice were treated with electroconvulsive shock (ECS) prior to bTBI. ECS is a neurostimulation that elicits microglia activation. Using immunohistochemistry and multiple behavior tasks, we found a distinct activity zone (β) containing microglia with elevated TSPO levels in their endfeet proceeding behavioral improvements. Assessing changes in microglial TSPO levels in response to TBI at different time points can help determine the ideal therapeutic window to prevent progression of chronic pathologies.

Disclosures: E.A. Aquino: None. **D.J. Hines:** None.

Digital Abstract Session

P084. Oligodendrocytes

Program #/Poster #: P084.01

Topic: B.11. Glial Mechanisms

Support: NIH/NIAAA R01 AA027269-01
NIH/NIAAA R21 AA026613-01
NIH 2P20GM10365

Title: Detecting myelin-dependent plasticity: therapeutic effect of an aerobic exercise intervention on corpus callosum myelination in a rodent model of Fetal Alcohol Spectrum Disorders

Authors: *K. A. MILBOCKER[1], G. L. LEBLANC[1], E. K. BRENGEL[1], S. K. HEKMATYAR[2], P. P. KULKARNI[3], C. F. FERRIS[3], A. Y. KLINTSOVA[1];
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Abstract: 1 in 20 infants born annually in the U.S. has been exposed to alcohol prenatally, sufficient to produce long-term cognitive deficits that disrupt learning and memory and are clinically categorized as Fetal Alcohol Spectrum Disorders (FASD) (May et al., 2015). Higher-order cognitive processing requires exchange of information between hemispheres and cortical and subcortical structures that is largely achieved via myelinated corpus callosum (CC) axons. Abnormalities in CC, including reduced axon myelination, are well-described in adolescent children with prenatal alcohol exposure (AE) (i.e., Wozniak et al., 2019; Jacobson et al., 2017; Gautam et al., 2014). This study employs diffusion tensor imaging (DTI) scanning to investigate the effects of: 1) developmental AE and 2) an adolescent exercise intervention on CC axonal myelination in a rodent model of FASD. Male and female Long-Evans pups were AE via intragastric intubation (5.25 g/kg/day) on postnatal days (PD) 4-9, during the rodent brain growth spurt (BGS), a period of rapid brain growth. Sham-intubated controls received no liquid during intubation. From PD 30-42, half of all rats had free access to a running wheel for aerobic intervention. DTI scans were acquired twice longitudinally (on PD 30 and 42) in all rats using a 9.4T Bruker scanner to assess alterations to CC axonal myelination noninvasively. CC was

divided into two sub-regions for analysis: the interhemispheric region (ICC) and cortically-projecting region (PCC). Preliminary cross-sectional analyses show that AE during the BGS reduces ICC and PCC volume pre-intervention on PD 30 ($F_{1, 35} = 9.4$, $p = .004$; $F_{1, 32} = 14.1$, $p = .001$, respectively) and PCC volume only post-intervention on PD 42 ($F_{3, 32} = 5.0$, $p = .006$). DTI scan analysis shows that fractional anisotropy, the movement of water molecules, in ICC and PCC is decreased in AE rats on PD 30 ($F_{1, 35} = 16.6$, $p < .001$; $F_{1, 32} = 17.7$, $p < .001$). Fractional anisotropy remains reduced in PCC only in AE rats on PD 42 ($F_{3, 32} = 5.5$, $p = .004$). Radial and axial diffusivity were analyzed to interpret whether these neuroanatomical changes could be attributed to decreased myelination or axonal degeneration, respectively. We found that on PD 30, radial but not axial diffusivity is increased in ICC and PCC of AE rats ($F_{1, 35} = 22.9$, $p < .001$; $F_{1, 32} = 14.9$, $p = .001$). On PD 42, both radial and axial diffusivity are unchanged in ICC, while radial diffusivity only is increased in PCC of AE rats ($F_{3, 32} = 3.8$, $p = .020$). These findings suggest that AE during the BGS reduces CC myelination in adolescence. PCC myelination is particularly vulnerable to the teratogenicity of AE likely due to the lateral-medial development of CC myelination during the BGS.

Disclosures: **K.A. Milbocker:** None. **G.L. LeBlanc:** None. **E.K. Brengel:** None. **S.K. Hekmatyar:** None. **A.Y. Klintsova:** None. **C.F. Ferris:** None. **P.P. Kulkarni:** None.

Digital Abstract Session

P084. Oligodendrocytes

Program #/Poster #: P084.02

Topic: B.11. Glial Mechanisms

Support: R37-MH065635
HHMI Gilliam Fellowship

Title: Oligodendrocyte mechanisms in the anterior cingulate cortex are critical for the consolidation of both recent and remote episodic memories

Authors: ***L. P. BARBOZA**[1], B. BESSIÈRES[2], O. NAZARZODA[1], C. M. ALBERINI[1]; [1]NYU, New York, NY; [2]NYU, NYC, NY

Abstract: Oligodendrogenesis and *de novo* myelination have been implicated in motor learning and spatial memory consolidation. Whether these mechanisms play a role in episodic memory consolidation and in which specific brain regions remains unknown. Consolidation is a temporally graded process by which initially labile memories become stabilized across various brain regions including the anterior cingulate cortex (ACC). Studies indicate that the ACC is involved in the storage of remote but not recent memories; however, recent reports suggest that ACC is also critical for recent memory formation. Using RT-qPCR analyses of the ACC at various time points after training, we found significant upregulations of transcripts involved in oligodendrocyte differentiation and myelin biogenesis, including Olig2, Myrf, and Mbp, starting at one hour after training. Western blot and immunohistochemistry analyses confirmed

significant upregulation of corresponding proteins one day after training in the ACC. Furthermore, conditional global and ACC-targeted knockout, as well as ACC-targeted antisense-mediated knockdown of Myrf, a master regulator of myelin expression, impaired memory retention at both recent (1 day) and remote (28 days) time points after training. Thus, oligodendrocyte-specific mechanisms are regulated and required in the ACC for the consolidation of recent and remote episodic memories.

Disclosures: L.P. Barboza: None. **B. Bessières:** None. **O. Nazarzoda:** None. **C.M. Alberini:** None.

Digital Abstract Session

P085. Glia-Neuron Interactions in Injury and Disease

Program #/Poster #: P085.01

Topic: B.11. Glial Mechanisms

Support: PAPIIT UNAM Grant IN221820
PI Grant Facultad de Medicina UNAM
APEC Grant LOP-INV-001

Title: Palmitic acid modifies morphology and increases Vascular Endotelial Growth Factor A expression in Müller cells

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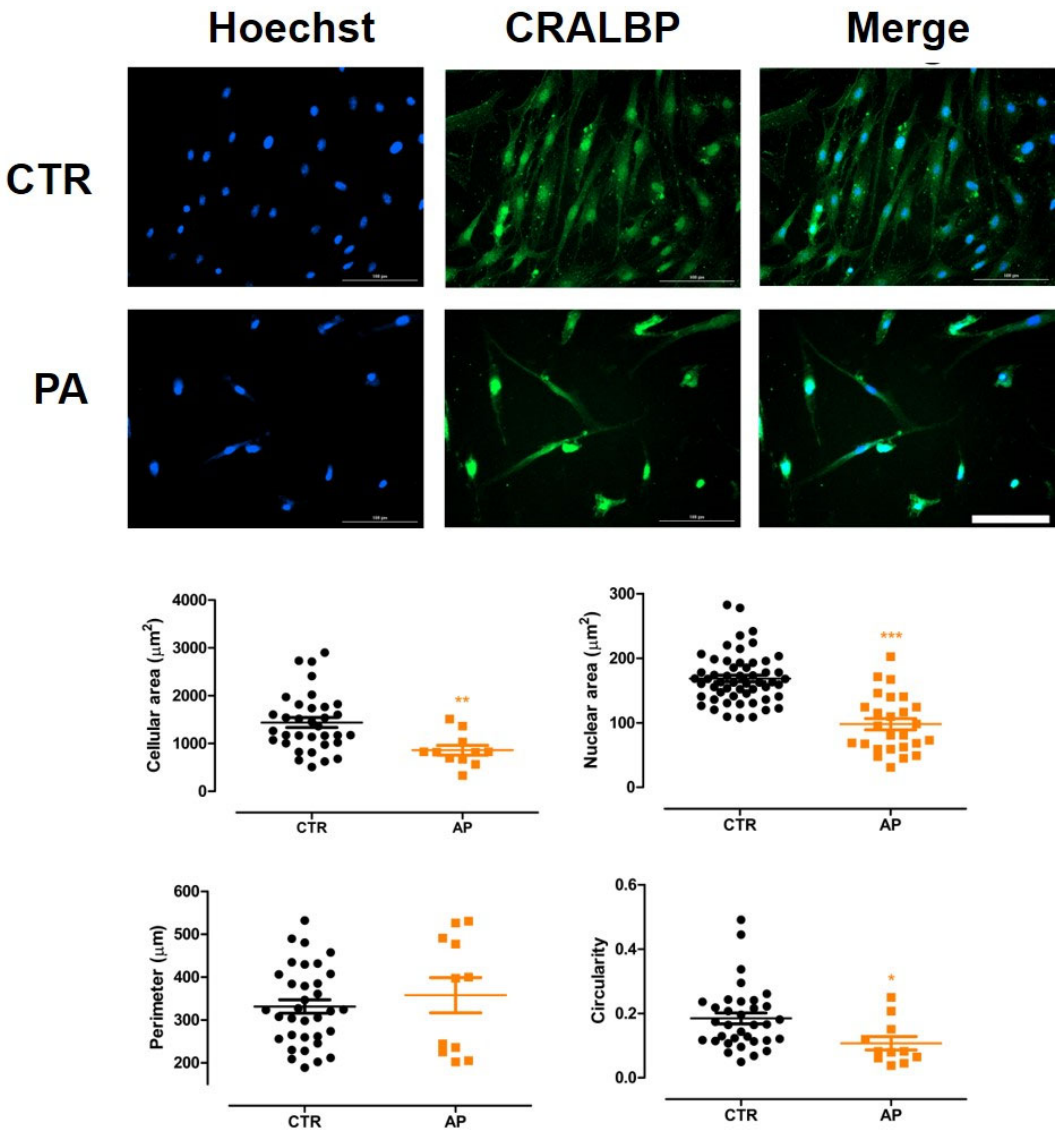
Abstract: Lipid study has traditionally been restricted to the formation of biological membranes, however studies in the last two decades have described new functions in these fatty acids; from energy supply via β-oxidation and protein post-traductional modifications, to intracellular lipid signaling pathways and modulation of ion channels properties. Besides that, recent studies have shown a key role of fatty acids in angiogenic processes, evidencing their importance in different diseases. Palmitic acid (PA), the most abundant saturated fatty acid in blood plasma, increases levels in patients with diabetic retinopathy (DR) or age-related macular degeneration (AMD), pathologies associated with neovascularization in the retina. In this neural tissue, Müller glial cells (MC) regulate a plethora of retinal functions, critical to the homeostasis of this neural tissue, such as potassium ion buffering, neurotransmitter recycling, and angiogenic factors regulation. We aimed to determine if the MC plays a critical role in processes related to PA effects. We use CD1 P5-7 mice MC primary cultures, exposed to 250 μM PA and vehicle (0.008% DMSO). Our results show a significant reduction in cell viability after 72h of incubation with PA and morphological changes associated with the presence of this lipid. We

also detect by immunofluorescence assays a significant increase of VEGF-A in MC cultures exposed to PA. Studies suggest that HIF-1α, a transcriptional factor related to VEGF, participates in the signaling pathway that links fatty acid and alterations in the retinal vasculature. Our preliminary data show that HIF-1α mRNA expression decreases in the MC during PA treatments. These results together suggest that MG plays a pivotal role in the VEGF synthesis, possibly through HIF1α, by an increment in the PA levels, an event associated with DR and AMD pathologies.



Disclosures: **A.E. Medina Arellano:** None. **E.J. Galvan:** None. **H. Sanchez-Castillo:** None. **L. Ochoa-de la Paz:** None.

Digital Abstract Session

P085. Glia-Neuron Interactions in Injury and Disease

Program #/Poster #: P085.02

Topic: B.11. Glial Mechanisms

Support: R01DE029493
R03DE027777
The Rita Allen Foundation Award in Pain
NYU Health and Hospital Corporation CTSI KL2 Scholar award

Title: Tnfα Promotes oral cancer growth, pain, and schwann cell activation

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Abstract: Oral cancer patients rate Pain as the worst symptom, which significantly impairs a patient's ability to eat, talk, and drink. Mediators such as TNFα, secreted from oral cancer and microenvironment, excite primary afferent neurons inducing pain. We aim to investigate whether TNFα can activate Schwann cells, peripheral glia, to further amplify pain signals and to increase cancer progression. We measured pain in patients using a validated University of California Oral Cancer Pain questionnaire. We used human primary cultures of Schwann cells, human oral cancer cell line HSC-3, and human dysplastic oral keratinocytes (DOK). Cell proliferation was measured using a real-time cell analyzer (RTCA) and the CellTiter 96® Aqueous One Solution Cell Proliferation Assay kit. Cell migration was examined using Transwell migration assays. We used two animal models of oral cancer: a tongue carcinogenesis model by feeding mice with 4-Nitroquinoline 1-oxide (4NQO), and a xenograft models by inoculating oral cancer cells (HSC-3) into the mouse right hind paw. Mechanical sensitivity was tested with von Frey filaments. Tumor size was measured using a plethysmometer. Schwann cell activation in the presence of TNFα or oral cancer cells was characterized using proliferation and migration assays and activation markers. To determine whether activated Schwann cells mediate oral cancer pain, we cultured Schwann cells in hypoxic conditions - a known cancer stimulus inducing robust Schwann cell activation. Schwann cell supernatant was then collected and injected into the mouse cheek; nociception in the face was measured using facial von Frey tests. C-87, a small molecule TNFα inhibitor was used to block the effect of TNFα on cancer proliferation, Schwann cell activation, and nociception in cell culture and animal models of oral cancer. We demonstrate that TNFα is overexpressed in human oral cancer tissues and correlates with increased self-reported pain in patients. C-87 reduced oral cancer proliferation, cytokine production, and nociception in animals with oral cancer. Oral cancer or TNFα increases Schwann cell activation, which can be inhibited by neutralizing TNFα. Cancer or TNFα activated Schwann cells release pro-nociceptive mediators such as TNFα and NGF. Activated Schwann cells induce nociceptive behaviors in mice, which is alleviated by blocking TNFα. Our study suggests that TNFα

promotes cancer proliferation, progression, and nociception at least partially by activating Schwann cells.

Disclosures: **Y. Ye:** None. **E. Salvo:** None. **N. Tu:** None. **Z. Dubeykovskaya:** None. **S. Shavan:** None. **N. Scheff:** None. **B. Aouizerat:** None.

Digital Abstract Session

P086. Oligodendrocyte Development and Demyelinating Diseases

Program #/Poster #: P086.01

Topic: B.12. Demyelinating Disorders

Title: Refinement of optic nerve function and myelination during postnatal development

Authors: *A. BALRAJ[1], R. H. MILLER[2];
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Abstract: Retinal ganglion cells generate a pattern of action potentials to communicate visual information. Myelin, an insulating sheath, facilitates signal propagation by wrapping around axons and, when deficient (as observed in patients with optic neuritis), can cause significant visual deficits. However, the relationship between optic nerve function and the extent of myelination is currently unknown. We tested if myelination patterns are correlated with changes in optic nerve function during postnatal development using extracellular nerve recordings, immunohistochemistry, western blot, and scanning electron microscopy. Comparing compound action potentials from C57Bl6 mice across ages 4-12 wks revealed an increase in the number of functional axons and shifts toward more fast-conducting axon populations at 5 and 8 wks ($p<0.05$, $n=8$). At these ages, nerve assessments suggest increases in myelin and neurofilament protein concentrations ($n=2$) and lower g-ratios ($n>2$). Increased expression of a mature sodium ion channel (Nav 1.6) at nodes of Ranvier was observed at 6 wks ($p<0.05$, $n=3$), while axon diameter, axon density, and nodal density remained unchanged across ages. Changes in the normal optic nerve to favor faster axonal conduction correlate with additional myelin proteins, thicker myelin around axons, and node maturity, suggesting that these properties are critical in the refinement of optic nerve signaling during postnatal maturation.

Disclosures: **A. Balraj:** None. **R.H. Miller:** None.

Digital Abstract Session

P086. Oligodendrocyte Development and Demyelinating Diseases

Program #/Poster #: P086.02

Topic: B.12. Demyelinating Disorders

Support: NIH Grant NS110627

Title: Yap and taz regulate schwann cell proliferation and differentiation during peripheral nerve regeneration.

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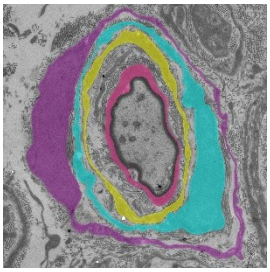
Abstract: YAP and TAZ are effectors of the Hippo pathway that controls multicellular development by integrating chemical and mechanical signals. Peripheral nervous system development depends on the Hippo pathway. We previously showed that loss of YAP and TAZ impairs the development of peripheral nerve as well as Schwann cell myelination. The role of the Hippo pathway in peripheral nerve regeneration has just started to be explored. After injury, Schwann cells adopt new identities to promote regeneration by converting to a repair-promoting phenotype. While the re-programming of Schwann cells to repair cells has been well characterized, the maintenance of such repair phenotype cannot be sustained for a very long period, which limits nerve repair in human. First, we show that short or long-term myelin maintenance is not affected by defect in YAP and TAZ expression. Using crush nerve injury and conditional mutagenesis in mice we also show that YAP and TAZ are regulators of repair Schwann cell proliferation and differentiation. We found that YAP and TAZ are required in repair Schwann cells for their re-differentiation into myelinating Schwann cell following crush injury. In this present study, we describe how the Hippo pathway and YAP and TAZ regulate remyelination over time during peripheral nerve regeneration.



Caption: *Yap*^{cHet} ; *Taz*^{cKO} crushed nerves present elevated formation of onion bulbs. Schwann cells in the onion bulb were pseudo-colored

Disclosures: H. Jeanette: None. **L. Marziali:** None. **L. Feltri:** None. **Y. Poitelon:** None. **S. Belin:** None.

Digital Abstract Session

P086. Oligodendrocyte Development and Demyelinating Diseases

Program #/Poster #: P086.03

Topic: B.12. Demyelinating Disorders

Support: NIH R56MH115201

NIA-AG039521
NIH T32GM007288
NICHD U54HD090260

Title: Haploinsufficiency in the ANKS1B gene encoding AIDA-1 leads to a neurodevelopmental syndrome

Authors: A. U. CARBONELL[1], C. CHO[1], H. ERDJUMENT-BROMAGE[2], J. VAZQUEZ[1], I. V. DEYNEKO[1], S. MOLHOLM[1], T. A. NEUBERT[2], **B. A. JORDAN**[1];
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Abstract: Neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD) and comorbid conditions, have complex polygenic etiologies. Single gene deletions and mutations identified in patients can help define causative molecular mechanisms. We recently identified patients around the world harboring monogenic deletions of the *ANKS1B* gene with confirmed haploinsufficiency. Affected individuals present with a spectrum of neurodevelopmental phenotypes, including autism and speech and motor deficits. Our findings corroborate previous genome-wide and genetic studies implicating *ANKS1B* in brain disorders and formalize a link between *ANKS1B* haploinsufficiency and a previously undefined syndrome. *ANKS1B* encodes for AIDA-1, a brain-specific protein that we have shown is enriched at neuronal synapses and regulates *N*-methyl-D-aspartate receptors (NMDARs) subunit composition and NMDAR-dependent synaptic plasticity. A transgenic mouse model of Anks1b haploinsufficiency recapitulates a range of phenotypes observed in patients, including social behavior deficits, hyperactivity, and sensorimotor dysfunction. Neurons generated from patient-derived induced pluripotent stem cells (iPSCs) show that the *ANKS1B* microdeletions lead to abnormal NMDAR subunit surface expression, confirming results in mice. Recent findings indicate that AIDA-1 may play a role in oligodendrocyte function and myelination. Loss of AIDA-1 leads to impaired oligodendrocyte maturation and migration throughout development, and following the application of demyelinating agents *in vivo*. Although we find that this novel NDD is rare, identification of the AIDA-1 interactome using isobaric tags and quantitative proteomics shows that AIDA-1 binds to diverse factors causative for NDDs, including Shank and SynGAP1. Moreover, single-nucleotide variants in *ANKS1B* have also been associated with NDDs, suggesting broader relevance for other monogenic or polygenic/complex forms of NDD.

Disclosures: A.U. Carbonell: None. **C. Cho:** None. **H. Erdjument-Bromage:** None. **J. Vazquez:** None. **I.V. Deyneko:** None. **S. Molholm:** None. **T.A. Neubert:** None. **B.A. Jordan:** None.

Digital Abstract Session

P086. Oligodendrocyte Development and Demyelinating Diseases

Program #/Poster #: P086.04

Topic: B.12. Demyelinating Disorders

Support: SCRMC Postdoctoral Fellowship
Wisconsin Alumni Research Foundation

Title: Demyelination derived TGFbeta1 signaling inhibits neural stem cells through the novel regulator Gpnmb

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Abstract: Demyelination in the CNS occurs across a range of neurodegenerative disorders, but the signaling pathways modulating subsequent repair and remyelination are incompletely understood. Specifically, the ability to recruit the multipotent periventricular adult neural stem cells (NSCs) to demyelinated lesions and promote oligodendrocyte generation and remyelination is of current interest. Using the cuprizone toxin model of demyelination, our group has identified glycoprotein non-metastatic melanoma b (*Gpnmb*), as a regulator of lesion derived TGFbeta1 signaling in Gli1 expressing ventral NSCs. GPNMB is a single pass, cleavable transmembrane protein whose function in NSCs is unknown. During cuprizone mediated demyelination, *Tgfbeta1* expression increases at the site of lesion and Gpnmb expression increases in *Gli1* vNSCs in parallel. Using a *Gpnmb-LacZ* reporter mouse, we show that GPNMB is predominantly expressed in periventricular neural stem cells, and oligodendrocyte precursor cells throughout white and gray matter. To determine if loss of *Gpnmb* alone can alter remyelination, cuprizone demyelinated *Gpnmb-Null* mice showed increased oligodendrocyte lineage number in the corpus collosum following remyelination. Reciprocally, *in vitro* overexpression of GPNMB resulted in a significant downregulation of oligodendrocyte genes, and interestingly an increase in TGFbeta receptor subunit 2 (*Tgfbeta-R2*) levels, indicating a potential feedforward mechanism with the TGFbeta1 pathway. First, in order to confirm TGFbeta1 directly activates *Gpnmb*, we treated mouse NSCs *in vitro* and found a significant increase in *Gpnmb* levels, with a corresponding decrease in oligodendrocyte lineage generation. Further evidence of direct regulation was the identification of a putative SMAD3/4 binding site, the downstream mediators of TGFbeta1, in the putative promoter region of *Gpnmb*, and a renilla-luciferase assay showed that TGFbeta1 activated the *Gpnmb* promoter. Finally, we show that knocking out *Tgfbeta-R2* in Gli1 vNSCs increases their recruitment to lesioned white matter following LPC mediated demyelination, indicating that part of the inhibition on endogenous vNSC recruitment is due directly to TGFbeta1 signaling. Our results indicate that lesion derived TGFbeta1 activates *Gpnmb* in Gli1 vNSCs via binding to TGFbeta-R2, and this activation inhibits the recruitment and oligodendrogenic potential of vNSCs to repair the CNS.

Disclosures: D.Z. Radecki: None. **J. Samanta:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Glixogen Therapeutics.

Digital Abstract Session

P086. Oligodendrocyte Development and Demyelinating Diseases

Program #/Poster #: P086.05

Topic: B.12. Demyelinating Disorders

Support: Research Grants Council of the Hong Kong Grant no. 21106716
Research Grants Council of the Hong Kong Grant no. 11101017
Research Grants Council of the Hong Kong Grant no. 11101019

Title: Demyelination regulates the circadian transcription factor BMAL1 to signal adult neural stem cells to initiate oligodendrogenesis

Authors: S. HUANG[1], M. CHOI[2], H. HUANG[1], X. WANG[2], Y. CHANG[1], ***J. KIM**[2];
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Abstract: Circadian clocks, endogenous oscillators generating cell-autonomous rhythms, are intrinsic inmost cells, including neural cells. Under physiological conditions, they are synchronized tolocal macro- and micro-environments by the action of external cues and regulate variouscellular processes. Demyelination is a common form of central nervous system pathology thathas variable degrees of recovery. Demyelination changes the surrounding microenvironmentvia damaged myelin and activation of glial cells. How these microenvironmental changesaffect circadian clocks in the lesions, and their consequences are mostly unknown. Here, weshow that circadian clocks are altered in demyelinating lesions. This initiates molecularsignals that induce adult neural stem cells (NSCs) in the subventricular zone (SVZ) toproduce oligodendrocyte lineage cells (OLCs) to enhance restoring myelin. Circadian clocksin demyelinating lesions shortened the period to transcribe more clock target genes, includingthe Wnt inhibitors SFRP1 and SFRP5. Unexpectedly, SFRP1 and SFRP5 signaled to the SVZto reduce the circadian transcription factor BMAL1, causing more SVZ NSCs to differentiateinto OLCs. We further found that inhibition of *Bmal1* expression in astrocytes, the signalproducing cells in the lesions, prevented the migration of OLCs from the SVZ todemyelinating lesions. Thus, our findings show that communication between demyelinatinglesions and the SVZ via local circadian clocks enhances the remyelination process bysupplying OLCs from the SVZ to the lesions.

Disclosures: S. Huang: None. **M. Choi:** None. **H. Huang:** None. **X. Wang:** None. **Y. Chang:** None. **J. Kim:** None.

Digital Abstract Session

P086. Oligodendrocyte Development and Demyelinating Diseases

Program #/Poster #: P086.06

Topic: B.12. Demyelinating Disorders

Support: National MS Society Pilot Research Award

Title: Circadian modulation of oligodendroglial lineage cells in development and demyelinating disease

Authors: *A. BADNER[1], J. J. GREENE[1], S. H. KIM[1], L. DAL CENGIO[1], C. ARELLANO-GARCIA[1], L. C. MEHL[1], E. EISINGER[1], E. GIBSON[1,2,3];
[1]Psychiatry and Behavioral Sci., [2]Wu Tsai Neurosciences Inst., [3]Maternal & Child Hlth. Res. Inst. (MCHRI), Stanford Univ., Palo Alto, CA

Abstract: Multiple sclerosis (MS) is a complex disease in which the underlying etiology includes loss of myelin, the multi-layered structure that surrounds and insulates neuronal axons, primarily through disordered glial cells. In MS, myelin-forming oligodendrocytes are the targets of an autoimmune inflammatory response that results in damage to myelin sheaths within the brain and spinal cord. While myelin-forming oligodendrocytes have limited innate repair capacity, largely stemming from a robustly cycling and proliferative population of oligodendrocyte precursor cells (OPCs), multiple demyelinating events hinder this response. Understanding the mechanisms underlying this lineage stability in health and MS is imperative to the development of regenerative strategies aimed at remyelination. Numerous lines of evidence suggest that dysregulation of the circadian or 24-hour clock is involved in the pathophysiology of MS. Nevertheless, how the circadian clock modulates myelin-forming cells and myelination remains unstudied, leaving a substantial gap in the field. This project explored the effect of circadian disruption on myelination in white matter development and remyelination in a lysolecithin-induced (L-a-Lysophosphatidylcholine, Sigma L4129 in 1% PBS; 1ul into the cingulum of the corpus callosum) model of demyelinating disease. Specifically, through knockdown of the molecular circadian clock regulator, *Bmal1*, in oligodendrocyte lineage cells (*NG2::Cre;Bmal1^{KO}*) at embryonic development, there was a substantial reduction in OPC and oligodendrocyte density, a ~40% decrease in myelin basic protein (MBP) in the corpus callosum and a significant dysregulation of motor function measured through CatWalk gait analysis. These results were further validated using the tamoxifen-inducible *PDGFRα::CreER^{T2};Bmal1^{KO}*, where *Bmal1* knockdown at postnatal days 18-20 also resulted in reduced OPC density and motor dysregulation. Assessment of remyelination potential between in *PDGFRα::CreER^{T2};Bmal1^{KO}* and *PDGFRα::CreER^{T2};Bmal1^{WT}* remains in progress. Together, this work highlights the novel role of the circadian clock in myelin maintenance and disease, opening new avenues of MS research and potential therapeutics.

Disclosures: A. Badner: None. **J.J. Greene:** None. **S.H. Kim:** None. **L. Dal Cengio:** None. **C. Arellano-Garcia:** None. **L.C. Mehl:** None. **E. Eisinger:** None. **E. Gibson:** None.

Digital Abstract Session

P086. Oligodendrocyte Development and Demyelinating Diseases

Program #/Poster #: P086.07

Topic: B.12. Demyelinating Disorders

Support: Mayo Clinic Center for Multiple Sclerosis and Autoimmune Neurology
Eugene and Marcia Applebaum Foundation
Mayo Clinic Center for Biomedical Discovery
NIH R01NS052741-10
National Multiple Sclerosis Society (G-1508-05951, RG-1901-33209 and FG-1908-34819)

Title: CD38 dependent NAD+ depletion contributes to oligodendrocyte loss following high fat diet consumption and impairs myelin regeneration

Authors: *M. R. LANGLEY[1], C. CHOI[1], T. R. PECLAT[2], W. SIMON[1], H. YOON[3], L. KLEPPE[1], C. C. S. CHINI[4], E. N. CHINI[4], I. A. SCARISBRICK[3];
[1]Dept. of Physical Med. & Rehabil., [2]Robert & Arlene Kogod Ctr. on Aging, [3]Dept. of Physical Med. & Rehabilitation, Physiol. & Biomed. Engin., [4]Robert & Arlene Kogod Ctr. on Aging, Anesthesiol., Mayo Clin., Rochester, MN

Abstract: Recent work from our laboratory and others demonstrate that Western-style diets cause disruptions in myelinating cells and astrocytes within the mouse central nervous system. CD38 is the main NAD+ depleting enzyme in CNS tissue, and altered NAD+ metabolism has been linked to both high fat consumption and Multiple Sclerosis (MS). We identified increased CD38 expression in the mouse spinal cord following chronic high fat consumption or focal demyelinating injury. We also show that male CD38-catalytically inactive mice are significantly protected from high fat-induced NAD^+ depletion, oligodendrocyte loss, oxidative damage, and astrogliosis. In primary murine glial cultures, 78c, a CD38 inhibitor, increased NAD^+ levels and attenuated neuroinflammatory changes in astrocytes induced by saturated fat. Conditioned media from saturated fat-treated astrocytes impaired oligodendrocyte differentiation, pointing to indirect mechanisms of oligodendrogliopathy. Combined saturated fat and lysolecithin demyelination in cerebellar slice cultures resulted in additional deficits in myelin proteins that were mitigated by concomitant 78c treatment. Importantly, oral 78c increased counts of oligodendrocytes and remyelinated axons after focal lysolecithin-induced demyelination to the adult male mouse spinal cord. Our findings suggest that high fat diet impairs oligodendrocyte survival and differentiation through astrocyte-linked mechanisms mediated by the NAD^+ase CD38, and highlight the use of CD38 inhibitors as potential therapeutic candidates to improve myelin regeneration.

Disclosures: **M.R. Langley:** None. **C. Choi:** None. **T.R. Peclat:** None. **W. Simon:** None. **H. Yoon:** None. **L. Kleppe:** None. **C.C.S. Chini:** None. **E.N. Chini:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); receipt of drug-containing chow from Calico. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds a patent on the use of CD38 inhibitors for metabolic diseases.. **I.A. Scarisbrick:** None.

Digital Abstract Session

P086. Oligodendrocyte Development and Demyelinating Diseases

Program #/Poster #: P086.08

Topic: B.12. Demyelinating Disorders

Support: The Shockey Family Foundation

Title: β-endorphin and opioid growth factor as biomarkers of physical ability in multiple sclerosis

Authors: C. PATEL, G. THOMAS, N. ZOMORODI, I. S. ZAGON, *P. J. MCLAUGHLIN; Penn State Univ. Coll Med., Hershey, PA

Abstract: Multiple sclerosis (MS) is an autoimmune mediated degenerative disease of the central nervous system with an estimated prevalence 2.5 million worldwide. Localized neuro-inflammation presents as demyelinating plaques within white matter monitored with magnetic resonance imaging. With the increasing MS-related healthcare costs, the need to validate minimally invasive biomarkers is imperative. In this study, conducted between 2019-2020 at the Penn State Hershey Neurology Clinic, 60 male and female relapsing-remitting MS patients on disease modifying therapies were consented to provide blood samples for analysis of serum cytokines (TNFα, IL6, and IL-17A) and endogenous opioid peptides (OGF and β-endorphin), as well as to complete the MSQOL-54 survey. Vitamin D levels were obtained from patient records. Patient age ranged from 23-78 years, with length of disease ~ 14 years. Mean serum OGF levels in all MS patients were elevated ($p<0.01$) and ranged from 193 -393 pg/ml in comparison to age matched controls (~98 pg/ml). The increased interaction between OGF and its receptor was hypothesized to facilitate a modulation of cytokines TNFα and IL-17A. Elevated OGF levels corresponded to increased levels of TNFα ($r = 0.78$) and IL-17A ($r = 0.81$). Analyses of MS-QOL data showed no significant differences in physical or mental composite scores between treatment groups. However, β-endorphin levels correlated with physical health composite score ($r = 0.70$), physical function score ($r = 0.68$), and social function ($r = 0.68$). Social function scores were indirectly correlated with increases in TNFα ($r = 0.63$) and IL6 ($r = 0.63$). Although the correlations do not demonstrate causality of disease, the data provide strong associations suggesting that serum levels of OGF, β-endorphin, and Vitamin D are potential biomarkers of patient perception of their disease.

Disclosures: C. Patel: None. **G. Thomas:** None. **N. Zomorodi:** None. **I.S. Zagon:** None. **P.J. McLaughlin:** None.

Digital Abstract Session

P087. Neuro-Oncology

Program #/Poster #: P087.01

Topic: B.13. Neuro-Oncology

Title: Analysis of ELAVL1-dependent transcripts involved in cell fusion and tunneling membrane nanotube formations in gliomas.

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Abstract: Homotypic and heterotypic cell fusions via permanent membrane fusions and temporal tunneling nanotube formations (TNT) in the glioma microenvironment were recently documented in vitro and in vivo and mediate glioma survival, plasticity, and recurrence. Cell fusion is a multistep process, which consists of activation of the cellular stress response, autophagy formation, rearrangement of cytoskeletal architecture in the areas of cell-to-cell contacts, and the expression of proinflammatory cytokines and fusogenic proteins. The mRNA-binding protein of ELAV-family HuR is a critical node, which orchestrates the stress response, autophagy formation, cytoskeletal architecture, and the expression of proinflammatory cytokines and fusogenic proteins. Our work provides analyses of HuR role in the expression of fusogen and TNT-related transcripts in gliomas. Meta-analysis and the data from R2: Genomics Analysis and Visualization Platform confirmed the significant expression of the following known fusogens in the glioma microenvironment: i) the fusogen transcripts from genomes of pathogenic enveloped viruses; ii) the fusogen transcripts encoding endogenous retroviral envelope proteins; iii) the fusogen transcripts encoding proteins essential for sexual reproduction and gamete fusions; iv) the muscle-specific fusogen transcripts at low levels; v) the transcripts of fusogens involved in the intercellular and extracellular vesicle-specific transfers. The type-1 TNT-related transcripts were significantly overexpressed in gliomas compared to normal brain samples. The type 2 TNT-transcripts (GJA1, GAP43), which overlap with gap junction formations, exhibited diverse expression in gliomas (GJA1 transcript was significantly overexpressed; however, GAP43 expression was significantly decreased compared to normal brain samples.We developed transcriptomic data (RNA-seq) of five patient-derived glioma xenograft (PDGx) cell lines of classic, proneural, and mesenchymal subtypes to analyze the expression of the fusogens and the TNT-related transcripts in gliomas of different subtypes alone and in the presence of the newly developed inhibitor of HuR dimerization. The analyses of the transcriptome signatures of five PDGx neurosphere cell lines confirmed the expression of a variety of fusogen and TNT-related transcripts in different glioma subtypes and the modulation of the expression of the key transcripts by inhibitors of HuR dimerization. The clinical outcome of patients with gliomas harboring low or high expression of key HuR-dependent fusogens and the TNT-related transcripts is provided.

Disclosures: **N. Filippova:** None. **X. Yang:** None. **J.A. Calano:** None. **E.N. Hayward:** None. **H.D. Namkoong:** None. **L.B. Nabors:** None.

Digital Abstract Session

P087. Neuro-Oncology

Program #/Poster #: P087.02

Topic: B.13. Neuro-Oncology

Support: ISF Grant 1431/16

Title: Blood glutamate scavengers increased pro-apoptotic signaling and reduced metastatic melanoma growing in-vivo

Authors: Y. GOLDSHMIT[1], E. BANYAS[2], A. YAKOVCHUK[2], ***A. RUBAN**[2];
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Abstract: Blood glutamate scavengers increased pro-apoptotic signaling and reduced metastatic melanoma growing in-vivo In the past few years, an ever-increasing body of data has suggested that glutamate (Glu) plays a crucial role in the growth of glioma cells, their invasiveness and ability to destroy neighboring brain tissue. The ability of glioma to secrete Glu into the tumor microenvironment has prompted the question of whether this feature is common to other types of cancer like melanoma. It has been shown that transgenic mice, which conditionally express mGluR1 in melanocytes, secret Glu and produce melanomas at a frequency of 100% 52 weeks after transgene activation. Furthermore, it is possible that more advanced tumors in stage III and IV metastases have a higher frequency of mGluR1 expression and that ectopic mGluR1 is more frequent in more advanced stages of the disease. In such case, the inhibition or reduction of extracellular brain glutamate could be very effective in the management of brain metastatic melanoma.The overall objective of this study was to evaluate the therapeutic efficacy of an entirely novel approach in which excess Glu is removed from brain into blood as a result of blood Glu scavenging. Materials and methods: mCherry RET melanoma cells were implanted intracranially in C57BL mice and treated with glutamate scavenger (BGS) vs. vehicle-control starting one hour after the implantation and for 7 consecutive days. CSF and blood Glu levels were measured at days 3 and 7 post implantation to confirm a continuous increase in Glu levels following the tumor progression, and to prove the scavenging activity of the treatment. The tumors' growth was examined 2- and 7-days post implantation by bioluminescence imaging. At day 7 mice were sacrificed and the tumors were analyzed for pro-apoptotic and DNA markers by immunohistochemistry. Results: Our results demonstrate that in control mice, Glu levels in the CSF have increased more than 5 folds at 7 days post cell implantation compared to the 3-fold in the BGS treated group. Moreover, BGS 7 days treatment has significantly increased expression of DNA suppressor marker 53BP1, active caspase-3 expression and has mediated CD8+ cells infiltration into the tumors. Importantly, BGS 7 days treatment has significantly decreased the tumor's growth in-vivo.Discussion: We have shown that the administration of blood Glu scavengers is highly effective in reducing the proliferation of RET melanoma cells in brain metastatic mice model. Our results suggest that BGS treatment is mediating DNA damage and apoptotic cell death in brain metastatic melanoma model. This is the first time that this approach is used in metastatic melanoma.

Disclosures: **Y. Goldshmit:** None. **A. Ruban:** None. **E. Banyas:** None. **A. Yakovchuk:** None.

Digital Abstract Session

P087. Neuro-Oncology

Program #/Poster #: P087.03

Topic: B.13. Neuro-Oncology

Support: NMSU Foundation

Title: Aminoquinolines impede proliferation of brain cancer cell lines

Authors: ***A. SANGAMI**, E. E. SERRANO;
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Abstract: FDA approved drugs Chloroquine and Hydroxychloroquine are alkylated 4-aminoquinolines that can be repurposed to treat brain cancer. Our previous reports have suggested 96-hour chloroquine exposure had significant effects in reducing the proliferation of a glioblastoma cell line (Sangami et al.; 2019; SFN Abstract 557.08.). To further evaluate drug activity, a double-blind experiment was conducted with drug exposure times of 24 hours and 48 hours. Glioblastoma cells (CCF-STTG1; ATCC CRL-1718; RRID: CVCL_1118) were maintained according to vendor guidelines and treated with 50 μM or 400 μM of chloroquine diphosphate (CQ; Sigma12084-10MG-F) or hydroxychloroquine sulphate (HCQ; Sigma H0915-15MG). We evaluated cell viability with Calcein-AM (Invitrogen C3099) and counted cell numbers by labelling nuclei with Hoechst 33342 (Invitrogen H3570). Images captured with the Nikon Eclipse TE 2000-S microscope were analyzed using the open access image analysis tool, ImageJ, by counting nuclei in 2% area of the chamber to determine cell proliferation. Analysis revealed that longer exposure times and higher concentrations of CQ and HCQ were correlated with reduced cell counts as compared with controls. Cells exposed to 50 μM CQ and HCQ showed negligible treatment reduction and low effect sizes (MD, SMD) at 24 and 48 hours. Visible changes and greater effect sizes were observed in cell lines treated with 400 μM chloroquine diphosphate ($p = .048$) and hydroxychloroquine sulphate ($p = .078$) at 24 hours, and this effect was more pronounced at 48 hours. Analysis of controls indicated the process of plating cells introduced technical variation that we aim to minimize. Future experiments will implement a newly acquired Incucyte® S3 Live-Cell Analysis System to capture image data from the entire well in real time. Cells will be treated with multiple concentrations of CQ and HCQ and to evaluate the proliferation and viability of cell lines, reduce technical variation due to plating, and further confirm the validity of results obtained.

Disclosures: **A. Sangami:** None. **E.E. Serrano:** None.

Digital Abstract Session

P087. Neuro-Oncology

Program #/Poster #: P087.04

Topic: B.13. Neuro-Oncology

Support: K08 110919-01

Title: Adult language process is influenced by thrombospondin-1 paracrine signaling from glioma-neuron interactions

Authors: *S. KRISHNA[1], A. CHOUDHURY[1], K. SEO[1], L. NI[2], A. LEE[1], A. AABEDI[1], D. BRANG[3], S. HERVEY-JUMPER[1];
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Abstract: The cellular biology of human language processing remains largely unknown given the limited scope of animal models. We now know that a subpopulation of gliomas interacts with neurons via electrochemical synapses. Little is known about the consequences of glioma-neuron interactions on cognitions in humans. We therefore studied the cellular mechanisms underlying network integration of gliomas as a model to study human language processing. We used electrocorticography to measure event related potentials during language task performance in IDH-wild-type glioblastoma (GBM). Site-directed tumor biopsies from high (HFC) and low (LFC)-functional connectivity intratumoral regions identified by magnetoencephalography were analyzed by RNA sequencing, immunohistochemistry (IHC), and patient-derived xenografts. Secreted proteins from patient serum was measured using ELISA. Language evaluation of patients were performed to correlate with functional connectivity measures. We found that speech initiation recruit glioma-language network activity with both tumor-infiltrated and non-tumor regions generating similar 'normal' neural responses. HFC regions showed increased expression of the neurogenic factor, thrombospondin 1 (TSP-1) originating from glioma cells in HFC regions and non-tumor astrocytes in LFC regions. This was further confirmed at protein level by IHC. The number of intratumoral HFC sites positively correlated with the serum TSP-1 of glioma patients. Compared to LFC, primary patient samples from HFC regions showed increased expression of pre- and post-synaptic markers in tissues and cells co-cultured with hippocampal neurons and neuron organoids. Furthermore, the integration of HFC cells with neurons was significantly higher than LFC cells and addition of TSP-1 to the LFC-neuron co-culture rescued the integrative phenotype. Mice bearing HFC xenografts showed increased total number of synapse structures. Importantly, compared to patients without intratumoral high connectivity sites, GBM patients with intratumoral HFC showed worse language task performance. To conclude, we found that glioma infiltrated regions generate task-relevant neural responses, with speech production evoking neuronal activity throughout tumor-involved cortex in the dominant hemisphere. A population of synaptogenic glioma cells within high connectivity intratumoral regions maintains their affinity for neurons through TSP-1 mediated paracrine signaling. Together, these findings indicate that malignant gliomas can functionally remodel

neural circuitry, thereby impairing neurological function and conferring negative cognitive outcomes.

Disclosures: **S. Krishna:** None. **A. Choudhury:** None. **K. Seo:** None. **L. Ni:** None. **A. Lee:** None. **A. Aabedi:** None. **D. Brang:** None. **S. Hervey-Jumper:** None.

Digital Abstract Session

P087. Neuro-Oncology

Program #/Poster #: P087.05

Topic: B.13. Neuro-Oncology

Title: Outcomes of Awake versus Sleep Craniotomy for Brain Tumors in a Developing Country

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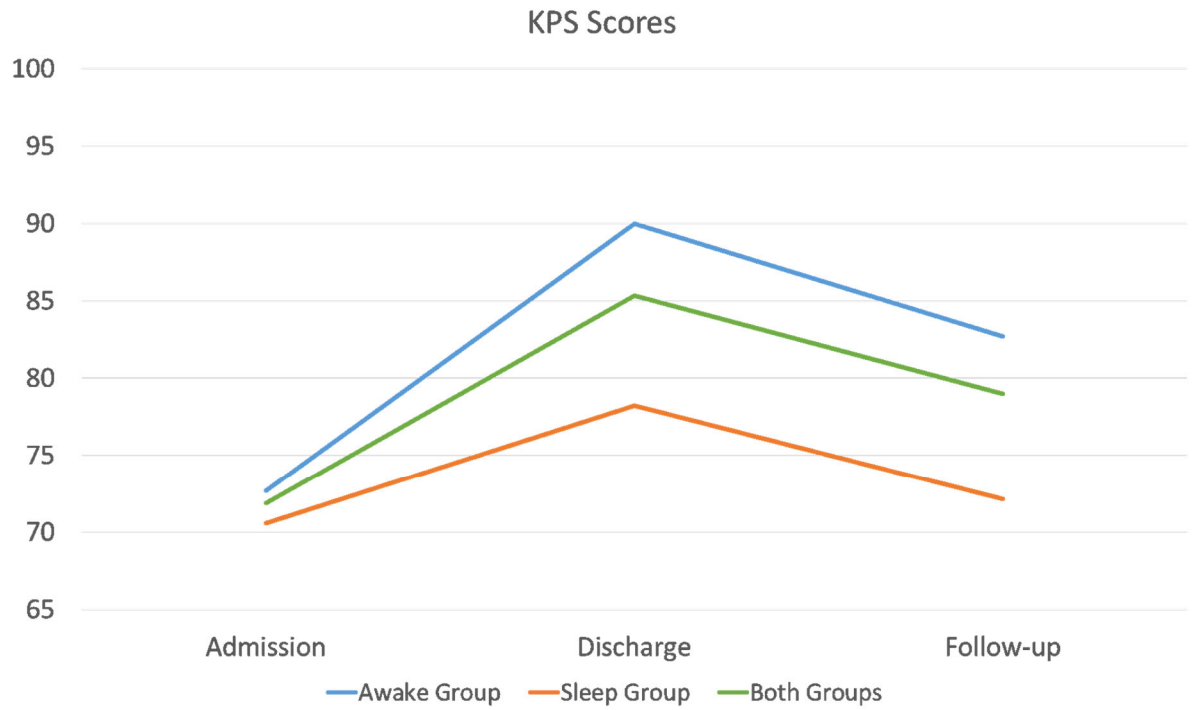
Abstract: Objectives: Awake Craniotomy for brain tumors is a neurosurgical procedure in which the patient is intraoperatively assessed for neurological deficits to avoid damage to an eloquent cranial region. Despite being popular in the developed world, it is still a rare technique in the rest of the globe. This study aimed to assess the outcomes of awake craniotomy, which was recently introduced at our facility, and compare it to craniotomy under general anesthesia for intracranial brain tumors in a developing country. **Methods:** A retrospective cohort study was conducted with 43 patients who underwent craniotomy for intracranial tumor resection by a single neurosurgeon between November 2015 and October 2016 at The Aga Khan University Hospital, Pakistan. The patients were assigned to an awake or sleep group based on the type of craniotomy they underwent. Medical records were reviewed for basic demographics, preoperative characteristics and postoperative outcomes, including KPS scores. **Results:** The mean age of the enrolled patients was 43.37 ± 13.24 years. For resection of the tumor, 26 (60.5%) patients underwent awake craniotomy, whereas 17 (39.5%) patients underwent sleep craniotomy. Awake craniotomy was associated with significantly higher discharge KPS score, shorter duration of surgery and lower length of hospital stay. Both groups had similar KPS scores at last follow up, with similar follow up periods. **Conclusion:** Awake craniotomy has proved to be an advantageous neurosurgical technique within the first twelve months of its introduction. It offers better postoperative functional status along with lower treatment cost, which can be valuable in a developing country.

Outcomes of patients undergoing Awake or Sleep Craniotomy

Outcomes	**Awake Craniotomy**		**Sleep Craniotomy**		**p-value**
	n	**mean ± s.d.**	**n**	**mean ± s.d.**	
Operating Time (hours)	25	3.10 ± 1.02	16	4.64 ± 1.10	**0.002**
Stay at Hospital (days)	26	4.65 ± 1.38	17	8.35 ± 6.67	**0.014**

Last Follow-up (months)	26	13.91 ± 12.81	14	12.97 ± 9.28	0.804
Gross Total Resection Achieved[1]	24	17 (70.8)	13	6 (46.2)	0.121
Discharge KPS of 100[1]	26	15 (57.7)	17	1 (5.9)	**0.001**
Discharge KPS score	26	90.00 ± 16.00	17	78.24 ± 14.25	**0.030**
Follow-up KPS score	26	82.69 ± 25.23	14	72.14 ± 29.92	0.247
1n (%)					



Disclosures: **J.A. Gilani:** None. **K. Nathani:** None. **M. Barakzai:** None. **H. Iftikhar:** None. **S. Khan:** None. **S. Enam:** None. **F. Shafiq:** None.

Digital Abstract Session

P088. Molecular and Cellular Changes in Neurodegeneration

Program #/Poster #: P088.01

Topic: C.01. Brain Wellness and Aging

Support: USUHS

Title: Histone H2A ubiquitination resulting from Brap loss of function connects multiple aging hallmarks and accelerates neurodegeneration

Authors: Y. GUO[1], A. CHOMIAK[1], Y. HONG[2], C. C. LOWE[3], W.-C. CHAN[4], J. ANDRADE[4], H. PAN[3], X. ZHOU[3], E. S. MONUKI[5], ***Y. FENG**[3,1];

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Abstract: Aging is an intricate process that is characterized by multiple hallmarks including stem cell exhaustion, genome instability, epigenome alteration, impaired proteostasis, and cellular senescence. While each of these is detrimental at the cellular level, it remains unclear how they are interconnected to cause systemic organ deterioration. Here we show that abrogating Brap, a BRCA1 associated protein, results in cellular senescence with persistent DNA double-strand breaks and elevation of histone H2A mono- and poly-ubiquitination (H2Aub). The high H2Aub initiates proteasome-dependent histone proteolysis, leading to global epigenetic alteration, ubiquitinated protein accumulation, and senescence reinforcement. When these defects occur in mice carrying Brap deletions in cerebral cortical neural progenitors or postnatal neurons, they accelerate brain aging, induce neurodegeneration, and shorten lifespan. As we show H2Aub is also increased in human brain tissues of Alzheimer's disease, these data together suggest that chromatin aberrations mediated by H2Aub act as a nexus of multiple aging hallmarks.

Disclosures: Y. Feng: None. **C.C. Lowe:** None. **H. Pan:** None. **X. Zhou:** None. **Y. Guo:** None. **A. Chomiak:** None. **E.S. Monuki:** None. **W. Chan:** None. **J. Andrade:** None. **Y. Hong:** None.

Digital Abstract Session

P088. Molecular and Cellular Changes in Neurodegeneration

Program #/Poster #: P088.02

Topic: C.01. Brain Wellness and Aging

Support: NIA AG057558
NIA AG051807
NIA AG054349
OD CA250382

Title: Examining age-dependent DNA methylation patterns and gene expression in the male and female hippocampus

Authors: *C. CHINN[1], H. REN[2], Q. NIE[2], M. A. WOOD[1], T. L. DOWNING[3];
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Abstract: Epigenetic signatures of biological age have recently been generated based on measurements of cytosine methylation within CpG dinucleotide contexts throughout the genome. Patterns of methylation are highly correlated with chronological age and form the molecular basis for the establishment of epigenetic clocks. However, CpG sites identified from different tissues in mice, such as liver or brain, show that these epigenetic clocks are largely tissue specific and do not share the same DNA methylation patterns across the genome. Current epigenetic

clocks from brain tissue include cortex and cerebellum combined, but a multi-tissue approach creates a signature not comparable to clocks made from other, single tissues. Given their tissue specificity, clocks generated from a brain region that is crucial to learning and memory may reveal key sites in the genome where methylation changes correlate with age. Here, we performed reduced representation bisulfite sequencing (RRBS) from mouse dorsal hippocampus in young (2 months) and old (20 months) mice of both male (n=10 young, 10 old) and female (n=10 young, 10 old) sex to investigate whether baseline tissue specific age-related methylation differences can be observed in a brain region involved in memory formation. After statistical analysis to define differentially methylated regions (DMRs), genomic 3'-UTR regions were found to have the highest bimodal degree of change in methylation levels in aging, displaying hypermethylation in females but hypomethylation in males. Furthermore, motif enrichment analysis revealed the AP-1 complex family of transcription factors, a major regulator of gene expression, as the most commonly associated transcription factor binding sites within hypomethylated regions in aged mice when compared to young. Using RNA sequencing data from the same brain region in young and aging mice, we identified differentially expressed genes with DMRs in their enhancer and/or promoter regions, suggesting that these genes may be important to understanding how methylation markers of epigenetic age correlate with age-related impairments in gene expression and long-term memory formation. Overall, our findings demonstrate that methylation levels within the dorsal hippocampus are divergent between sexes during aging, particularly in regions correlating to mRNA functionality, transcription factor binding sites, and gene regulatory elements. These results provide a foundational analysis for creating an epigenetic clock from a brain region involved in memory and defining age-related changes in the methylome across the lifespan.

Disclosures: C. Chinn: None. **H. Ren:** None. **Q. Nie:** None. **M.A. Wood:** None. **T.L. Downing:** None.

Digital Abstract Session

P088. Molecular and Cellular Changes in Neurodegeneration

Program #/Poster #: P088.03

Topic: C.01. Brain Wellness and Aging

Title: Epigenomic and transcriptomic profiling revealed molecular pathways participating in mouse neurological aging

Authors: Y. XU, C. NGUYEN, Z. LIU, J. OLLAR, E. BILLINGS, *J. A. VALADEZ, Z. ZHANG, K. BOOHER, Y. CHEW, X. YANG, X. JIA;
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Abstract: Aging is one of the most impactful risk factors for the onset and progression of cognitive and neurodegenerative diseases such as Alzheimer disease (AD) and Parkinson disease (PD). Previous researches have showed that the lifespan of the AKR/J mice is significantly shorter than that of the mice from the more common laboratory strain, C57BL/6J (median

lifespan for AKR/J male: 288 days; median lifespan for C57BL6/J male: 901 days). Moreover, age acceleration (AA) was observed in both blood and brain tissues of AKR/J mice compared to C57BL6/J mice measured by DNA methylation-based epigenetic clocks. This proved that AKR/J strain of mice is a valid model organism for AA study using genome-wide NGS methods that was not adequately exploited previously.To elucidate the underlying mechanisms of neurological aging, we used reduced representation bisulfite sequencing (RRBS) and total RNA sequencing (RNA-seq) to profile DNA methylation and whole transcriptome in the cortex and the hippocampus extracted from these two strains of mice at age of 24 weeks, respectively (6 biological replicates per brain region for the AKR/J strain, 4 biological replicates per brain region for the C57BL/6J strain). Distinctive DNA methylation patterns and transcriptome patterns were identified among these two brain regions of the two strains. Specifically, we identified more than 14,000 differentially methylated cytosines (DMCs, Benjamini-Hochberg FDR <0.05; methylation difference ≥0.15), more than 200 differentially methylation regions (DMRs, p-value <0.05; methylation difference ≥0.15), and more than 500 differentially expressed genes (DEGs, adjusted p-value <0.05) from each of the cortex and hippocampus regions, respectively. Interestingly, multiple genes with DMRs were among the DEGs, presenting possible interplays between the levels of DNA methylation and gene expression. These genes were further identified to participate in several biological pathways for metabolism, immune response, development and beyond. In conclusion, this study leveraged NGS profiling of DNA methylation and transcriptome of the cortex and hippocampus regions between the AKR/J and the C57BL/6J mice to reveal the genes and pathways playing roles in the neurological aging process. This may in turn help uncover the mechanisms connecting lifespans and neurological aging, and shed light on the understanding of the pathogenesis of related neurological diseases. Future work should continue probing for the interactions between genetic and epigenetic factors contributing to neurological aging, as well as capturing the transcriptomic consequences of such interplay.

Disclosures: **Y. Xu:** None. **C. Nguyen:** None. **Z. Liu:** None. **J. Ollar:** None. **E. Billings:** None. **J.A. Valadez:** None. **Z. Zhang:** None. **K. Booher:** None. **Y. Chew:** None. **X. Yang:** None. **X. Jia:** None.

Digital Abstract Session

P088. Molecular and Cellular Changes in Neurodegeneration

Program #/Poster #: P088.04

Topic: C.01. Brain Wellness and Aging

Support: TUBİTAK Grant-116S408

Title: Ultrastructural alterations in the upper motor neurons of rats after systemic delivery of genes encoding TDP-43 via viral vectors

Authors: *E. POLAT CORUMLU[1], H. A. KAPKAC[3], M. ARSLANYOLU[3], E. ULUPINAR[2]; [1]Interdisciplinary Neuroscience Department, [2]Interdisciplinary Neuroscience And Anatomy

Departments, Eskisehir Osmangazi University, Eskisehir, Turkey; [3]Department Of Molecular Biology, Eskisehir Technical University, Eskisehir, Turkey

Abstract: TDP-proteinopathies include cytoplasmic mislocalization, formation of toxic TDP-43(TarDNA-binding protein-43) fragments and aggregates, deposition of ubiquitinated and hyper-phosphorylated TDP-43 into inclusion bodies in the cortical and spinal motor neurons. In an effort to generate a novel rat model with TDP-43 pathology, we used AAV-mediated gene transfer method and aimed to investigate ultrastructural changes in the upper motor neurons. AAV9 was used in the design of construction cassette for expression of gene encoding TDP-43 with eGFP under the control of a cell-type specific promoter (UCHL1). The tail vein of 30-day old Sprague-Dawley rats was used for systemic injection of vectors at the dose of $1.77X10^{12}$vg/kg. Animals were sacrificed by intracardiac perfusion under ketamine and xylazine anesthesia on postnatal day 80. Ultra-thin sections obtained from the layer V of motor cortex were treated with 2% osmium tetroxide and then counterstained with 1% uranyl acetate and 0.2% lead citrate. Electron microscopic examination of motor neurons revealed disorganization in the cristae and the mitochondrial matrix. Various shapes and sizes of vacuoles were observed in the soma as an early stage symptom in the process of neurodegeneration. The most prominent alterations were observed in the nuclear envelope of Betz cells which displayed ondulations, large defects, notches and indentations. Membrane integrity of the endoplasmic reticulum was disrupted and broken pieces of membranes were detectable in autophagosomes and/or were engulfed by lysosomes. Taken together, these observations suggest that TDP-43 proteinopathy might cause defects in the nucleocytoplasmic transport by disrupting nuclear membrane and nuclear por complexes of the upper motor neurons. Therefore, better understanding of the role of TDP-43 in nucleostoplasmic transport is necessary in developing novel strategies for both neurodegeneration and physiological aging.

Disclosures: E. Polat Corumlu: None. **H.A. Kapkac:** None. **M. Arslanyolu:** None. **E. Ulupinar:** None.

Digital Abstract Session

P088. Molecular and Cellular Changes in Neurodegeneration

Program #/Poster #: P088.05

Topic: C.01. Brain Wellness and Aging

Support: NINDS Grant R21-NS111174
NINDS Grant U01 NS076474
NINDS Grant RF1 AG043640

Title: Metabolic labeling of newly synthesized myelin in the rhesus monkey brain

Authors: *K. R. BOTTENFIELD, T. L. MOORE, F. MORTAZAVI, D. L. ROSENE; Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: Pathology in primate brains often involves damage to myelin and impacts normal function as myelin constitutes 40% or more of the forebrain. Myelin can be assessed by electron microscopy, histological stains (e.g. Luxol Fast Blue), immunohistochemistry (e.g. for myelin basic protein), and a label free confocal method (SCoRe). Each has advantages but none offer a reliable, validated method to assess microscopic synthesis or repair of myelin across the entire primate forebrain. Interestingly, newly synthesized myelin can be labeled by in vivo administration of a choline analog, propargylcholine (P-Cho). It is readily incorporated into phosphatidylcholine and sphingomyelin which are major components of the myelin sheath. Importantly, P-Cho contains an alkyne moiety that can be labelled by conjugation with a fluoro-labeled azide using click chemistry and visualized with confocal microscopy. Since previous research had only demonstrated the use of P-Cho to assess remyelination in mice, we decided to explore the efficacy and safety in non-human primates by administering P-Cho by intraperitoneal injection (IP) daily for 6 days (3.5 - 4.0 mg/kg) to 6 young adult and 2 older adult monkeys at different time points before euthanasia (1, 2, 4 and 6 weeks). After fixation and cryoprotection, frozen brain sections were cut, labeled with a fluorescent azide using Click-iT technology, and the sections evaluated for the presence and specificity of P-Cho in white and gray matter regions throughout the brain by co-labeling the tissue with antibodies for axons, oligodendrocytes, and myelin basic protein using immunohistochemistry. In addition, we co-localized P-Cho labeling with spectral confocal reflectance microscopy (SCoRe), a label free method to visualize myelinated axons. Analyses show effective incorporation of P-Cho into newly synthesized myelin within 1 week of dosing and sustained presence beyond 6 weeks. These results validate P-Cho as a promising new method for investigating myelin plasticity and white matter pathologies related to aging, neurodegeneration, brain injury, and neurological disease in an animal model that is highly translatable to humans. This method can also be instrumental for assessing pharmacotherapies for inducing remyelination and myelin synthesis (Supported by NINDS grants R21-NS111174, U01 NS076474 and RF1 AG043640).

Disclosures: K.R. Bottenfield: None. **T.L. Moore:** None. **F. Mortazavi:** None. **D.L. Rosene:** None.

Digital Abstract Session

P088. Molecular and Cellular Changes in Neurodegeneration

Program #/Poster #: P088.06

Topic: C.01. Brain Wellness and Aging

Title: Imaging the age-related decline of neuronal function in C. elegans

Authors: *G. S. WIRAK, C. W. CONNOR, C. V. GABEL;
Physiol. and Biophysics, Boston Univ. Sch. of Med., Boston, MA

Abstract: Title: Imaging the age-related decline of neuronal function in *C. elegans*
Neuronal function declines with age resulting in behavioral deficits. How this age-related decline is related to changes in neuronal connectivity and circuit function on the cellular level is largely

unknown. Here we employ pan-neuronal fluorescence imaging in the nematode worm *C. elegans* to measure changes in neuronal activity and connectivity with age across the animal's nervous system. Prior research into the aging *C. elegans* nervous system has largely focused on the cell biology and accompanying morphological and ultrastructural changes. Employing transgenic expression of the fluorescent calcium indicator GCaMP6s in specific neurons, we identify changes in activity of individual neurons and circuits linked to the locomotory behaviors that are modulated by age. We further perform pan-neuronal imaging, employing light sheet microscopy to capture neuronal activity at single-cell resolution across the organism's entire head region. We identify substantial age-related alterations in the activity dynamics of neurons as well as changes in connectivity and functional organization across the entire nervous system. These age-related effects are recapitulated in young animals bearing a gain-of-function mutation within the *unc-2* gene, encoding a pre-synaptic voltage-gated calcium channel. Conversely, a loss-of-function mutation diminishes such effects. As the technologies enabling functional neuronal imaging rapidly advance, the simple and highly tractable nematode *C. elegans* might be employed to further our understanding of the breakdown of nervous system function with age.

Disclosures: G.S. Wirak: None. **C.W. Connor:** None. **C.V. Gabel:** None.

Digital Abstract Session

P089. Oxidative Stress, Mitochondria, and Metabolism in Brain Disorders

Program #/Poster #: P089.01

Topic: C.01. Brain Wellness and Aging

Support: Ontario Graduate Scholarship to AS
Canadian Institutes of Health Research grant (PJT-159493) to MF

Title: The effect of oxidative stress on proNGF transport in basal forebrain cholinergic neurons

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Abstract: Profound and early basal forebrain cholinergic neuron (BFCN) degeneration is a hallmark of Alzheimer's disease. BFCNs depend for survival and function on neurotrophins like nerve growth factor (NGF) which are retrogradely transported from BFCN target tissue. We have recently shown that the retrograde axonal transport of proNGF, the only detectable form of NGF in the brain, is reduced with age in cultured BFCNs and coincides with the loss of proNGF receptor tropomyosin-related kinase A (TrkA). We sought to determine whether mechanisms related to oxidative stress, a longstanding hypothesized contributor to Alzheimer's disease, account for these reductions. We found that elevation of intracellular oxidative stress via antioxidant deprivation significantly reduced both TrkA immunoreactivity and proNGF retrograde transport in rat BFCNs cultured in microfluidic chambers. TrkA levels are partially regulated by protein tyrosine phosphatase 1B (PTP1B), an enzyme whose activity is reduced by

the oxidation of a cysteine residue within its active site. siRNA knockdown of PTP1B and pharmacological PTP1B antagonism via selective inhibitor TCS401 significantly reduced TrkA levels in BFCNs. Furthermore, TCS401 treatment reduced proNGF retrograde axonal transport in BFCNs and reduced axonal uptake of proNGF-KKE, a mutant form of proNGF that only binds TrkA. Finally, treatment of BFCNs with thioredoxin-1, an antioxidant responsible for the reactivation of oxidized PTP1B, significantly increased TrkA levels in BFCNs following antioxidant deprivation. Our results suggest that, during aging and in Alzheimer's disease, increased oxidative stress reduces TrkA levels and proNGF transport through a PTP1B-dependent mechanism. Impaired proNGF retrograde transport due to TrkA loss may contribute to BFCN degeneration in Alzheimer's disease.

Disclosures: **A. Shekari:** None. **C. Wu:** None. **M. Fahnestock:** None.

Digital Abstract Session

P089. Oxidative Stress, Mitochondria, and Metabolism in Brain Disorders

Program #/Poster #: P089.02

Topic: C.01. Brain Wellness and Aging

Support: NIH ZIA NS003029
NIH ZIA NS002946

Title: The cross-talk of energy sensing and mitochondrial anchoring sustains synaptic efficacy by maintaining presynaptic metabolism.

Authors: ***S. LI**, G.-J. XIONG, N. HUANG, Z.-H. SHENG;
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Abstract: Mitochondria supply ATP essential for synaptic transmission. Neurons face exceptional challenges in maintaining energy homoeostasis at synapses. Regulation of mitochondrial trafficking and anchoring is critical for neurons to meet increased energy consumption during sustained synaptic activity. However, mechanisms recruiting and retaining presynaptic mitochondria in sensing synaptic ATP levels remain elusive. Here we reveal an energy signalling axis that controls presynaptic mitochondrial maintenance. Activity-induced presynaptic energy deficits can be rescued by recruiting mitochondria through the AMP-activated protein kinase (AMPK)-p21-activated kinase (PAK) energy signalling pathway. Synaptic activity induces AMPK activation within axonal compartments and AMPK-PAK signalling triggers phosphorylation of myosin VI, which drives mitochondrial recruitment and syntaphilin-mediated anchoring on presynaptic filamentous actin. This pathway maintains presynaptic energy supply and calcium clearance during intensive synaptic activity. Disrupting this signalling cross-talk triggers local energy deficits and intracellular calcium build-up, leading to impaired synaptic efficacy during trains of stimulation and reduced recovery from synaptic depression after prolonged synaptic activity. Our study reveals a mechanistic cross-talk between

energy sensing and mitochondria anchoring to maintain presynaptic metabolism, thus fine-tuning short-term synaptic plasticity and prolonged synaptic efficacy.

Disclosures: **S. Li:** None. **G. Xiong:** None. **N. Huang:** None. **Z. Sheng:** None.

Digital Abstract Session

P089. Oxidative Stress, Mitochondria, and Metabolism in Brain Disorders

Program #/Poster #: P089.03

Topic: C.01. Brain Wellness and Aging

Support: Basque Government - Eusko Jaurlaritza; BIKAINTEK 2019 DI

Title: Differential effects of ketamine and fluoxetine on the mitochondrial respiratory chain of brain regions involved in depression

Authors: A. ELEXPE[1], J. AURREKOETXEA[1], N. AZKARGORTA[1], E. ASTIGARRAGA[1], C. BRUZOS-CIDÓN[2], M. TORRECILLA[2], ***G. BARREDA-GÓMEZ**[1];
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Abstract: Depression is the most disabling illness worldwide that highly strikes life quality of the patients. Although antidepressant drugs relieve clinical depression symptoms after 2-4 weeks of treatment an important group of patients presents incomplete or lack of response after two or more trials of monoaminergic antidepressants and they are considered to have treatment-resistant depression. In current clinical practice, a new line of research suggests that mitochondrial dysfunction may be involved in the late antidepressant response. Recently, ketamine has been reported as a rapidly acting antidepressant that is effective in patients with treatment-resistant depression. Its mechanism of action involves activation of the mTOR signaling which affects mitochondrial energy metabolism. However, its action on mitochondrial respiratory chain needs further investigation.
To study the action of fluoxetine and ketamine on the activity of mitochondrial respiratory chain in multiple brain areas simultaneously, we developed microarrays using cell membranes isolated from mouse cerebral tissues. Subsequently, the superoxide formation capacity of each cerebral region was determined on the cell membrane microarrays using complexes I and II substrates and the specific inhibitors rotenone and azide as standards. Rotenone induced a similar reduction of the superoxide production rate in all the brain areas studied, according to the blockage of the mitochondrial complex I. However, the inhibition of the cytochrome c oxidase evoked by azide enhanced the superoxide production depending on the brain area, showing specific differences in the potency and in the maximal effect. While ketamine did not alter the activity of the respiratory chain at physiological concentrations, fluoxetine inhibited the complex I activity in the same range as rotenone in all brain areas studied. This inhibition was attenuated by decylubiquinone, suggesting that fluoxetine exerts a competitive action on the binding site of the electron transporter.
In conclusion, these data indicate that unlike ketamine, fluoxetine reduces mitochondrial

respiration of central neurons, compromising their energetic state. This restriction may contribute to delay the fluoxetine antidepressant response.

Disclosures: **A. Elexpe:** None. **J. Aurrekoetxea:** None. **N. Azkargorta:** None. **E. Astigarraga:** None. **C. Bruzos-Cidón:** None. **M. Torrecilla:** None. **G. Barreda-Gómez:** None.

Digital Abstract Session

P089. Oxidative Stress, Mitochondria, and Metabolism in Brain Disorders

Program #/Poster #: P089.04

Topic: C.01. Brain Wellness and Aging

Support: NIH/NIA R21 AG06703-A01A1

Title: The role of microglia in blood-brain barrier cholesterol synthesis and uptake

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Abstract: The blood-brain barrier (BBB) is a set of properties unique to central nervous system (CNS) vasculature that enables tight control of the brain microenvironment. Approximately 10-30% of microglia closely associate with CNS blood vessels and can reach their processes in between astrocyte endfeet to touch the endothelial basement membrane. Despite this intimate interaction, it is unknown whether microglia play a functional role at the healthy BBB. To answer this question, we used the colony-stimulating factor 1 inhibitor PLX5622 to deplete microglia and assessed the structural, functional, and transcriptomic effects on brain endothelial cells. Microglial depletion did not alter the ultrastructure of the BBB, its permeability to hydrophilic or hydrophobic molecules, or its expression of genes associated with classic BBB properties. However, to our surprise, PLX5622 treatment significantly increased the expression of cholesterol synthesis enzymes and uptake receptors in brain endothelial cells. This effect was extremely stable throughout time; the increased expression was still evident in mice on the PLX5622 diet for over a year. As cellular cholesterol levels can influence processing of amyloid precursor protein (APP), this result has particularly important implications for the role of microglial dysfunction in cerebral amyloid angiopathy (CAA) - the accumulation of vascular Aβ plaques that can cause stroke, dementia, inflammation, cortical microbleeds, and hemorrhage. It has long been thought that blood cholesterol and brain cholesterol are two separate pools, with glial cells making brain cholesterol *de novo*. Our results instead suggest that cholesterol synthesis and uptake at the BBB can be dynamically regulated by the brain microenvironment. We further investigated the effect of brain microenvironment on endothelial cholesterol metabolism and found that endothelial expression of cholesterol synthesis enzymes and uptake inhibitors is directly correlated to levels of neural activity. We are now performing further experiments to understand how microglia and neural activity interact in the context of endothelial cholesterol metabolism. We are also investigating the functional consequences of increased cholesterol uptake by injecting microglia-deficient and control mice with labeled low density lipoprotein.

Together, these experiments will shed light on the role of microglia in regulating brain and BBB cholesterol metabolism and uptake with potential implications for vascular diseases such as CAA and atherosclerosis.

Disclosures: C.P. Profaci: None. **T.Z. Zhang:** None. **R. Daneman:** None.

Digital Abstract Session

P089. Oxidative Stress, Mitochondria, and Metabolism in Brain Disorders

Program #/Poster #: P089.05

Topic: C.01. Brain Wellness and Aging

Support: FWO Grant 1S16617N

Title: Imperfect regeneration in the perfect aging model: functional recovery impaired by cellular senescence?

Authors: *S. VANHUNSEL[1], S. BERGMANS[1], A. BECKERS[1], J. VAN HOUCKE[2], L. MOONS[1];
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Abstract: Purpose: As the number of elderly in our society is growing, more and more age-associated neuropathies affect our world population. Research efforts therefore focus on stimulating repair in the (degenerating) central nervous system (CNS). Inducing neuroregeneration, however, remains challenging, especially in an aging environment. As the African turquoise killifish has a remarkable regenerative potential in its adult CNS and displays aging features similar to humans, this fast-aging teleost is very well-suited to investigate the underlying mechanisms that underlie successful restoration in an aging environment.
Methods and results: An in-depth evaluation of the aging hallmarks in the visual system of young, middle-aged, old and very old killifish, using techniques such as qPCR, (immuno)histochemistry ((I)HC) and the optokinetic response (OKR) test, revealed several age-associated manifestations in old fish, including decreased visual acuity, declined neurogenesis, cellular senescence, inflammaging and gliosis. To unravel the effect of these aging processes on CNS repair, and more specifically on axonal regrowth, we compared optic nerve regeneration in the 4 age groups subjected to optic nerve crush (ONC) injury. Strikingly, biocytin tracing experiments show that both the number of regenerating retinal ganglion cells and the level of tectal reinnervation are reduced in old fish. As a consequence, old fish do not recover their vision, as evaluated by OKR and dorsal light reflex tests. To investigate why there is no functional recovery and where in the regenerative process old fish are failing, we are currently performing experiments in which neurodegeneration, synaptic repair and the role of the innate immune system upon ONC are being examined.
Conclusion: Our current results point towards the presence of several aging hallmarks in the killifish visual system, which seem to impact the regenerative capacities in these old fish. These

findings urge further investigations into the underlying cellular aging processes affecting the regenerative potential, thereby contributing to the search for effective neuroregenerative therapies.

Disclosures: S. Vanhunsel: None. **S. Bergmans:** None. **A. Beckers:** None. **J. Van houcke:** None. **L. Moons:** None.

Digital Abstract Session

P090. Biomarkers and Brain Wellness in Aging

Program #/Poster #: P090.01

Topic: C.01. Brain Wellness and Aging

Support: McKnight Brain Research Foundation
NIH/NIA 1R01AG049722

Title: Expression of the Immediate-early Genes Arc and Narp during Cognitive Multitasking is Attenuated in Aged Rats

Authors: *C. N. LOGAN, K. LUBKE, S. N. BURKE;
Univ. of Florida, Gainesville, FL

Abstract: Aged humans (Clapp et al., 2011), monkeys (Gray et al., 2017) and rats are impaired at cognitive multitasking (Hernandez et al., 2015; Hernandez et al., 2019). The working memory/biconditional association task (WM/BAT), which requires the perirhinal cortex (Hernandez et al., 2017), has been used in rats to examine the neurobiological basis of these decificits. For this task, an animal must simultaneously perform and spatial alternation task that while also performing an object discrimination task in which the correct choice updates based on the animal's position in the maze. A previous study has shown that the expression of the immediate-early gene Arc is altered in the perirhinal cortex (PER) of aged rats. The expression patterns of other immediate-early genes that may be critical for updating PER cortical circuits in response to behavior, however, have not been examined. The PER is hypothesized to function as a wall of inhibition between the entorhinal cortex and the hippocampus since PER cortical interneurons project to layer II entorhinal cells that synapse in the hippocampus (de Curtis & Pare, 2004; Pinto et al., 2006). Aged rats have reduced monosynaptic coupling between excitatory principal cells and interneurons in the PER (Maurer, Burke, et al., 2017). Reduced afferent drive onto PER interneurons may manifest as increased activation in the lateral entorhinal cortical neurons that project to the hippocampus and hyperactivity in CA3 of the hippocampus (Maurer, Johnson, et al., 2017). Together these data suggest that a disruption in the balance between inhibition and excitation within the PER may aggregate across the medial temporal lobe circuit. A candidate gene that could be critical for regulating PER excitatory balance is neuronal activity-regulated pentraxin (Narp). Narp is up-regulated following behavior and encodes an effector protein that is released from the axons of excitatory neurons to cluster AMPA receptors on parvalbumin-positive interneurons (Chang et al., 2010; O'Brien et al., 1999).

The current work quantified the co-expression of Arc and Narp in young and aged rats that performed the WM/BAT and a control alternation task. The proportion of cells that were positive for Narp mRNA and co-expression of Arc and Narp were decreased in aged compared to young rats. These data provide additional evidence that a dysregulation of the balance between inhibition and excitation within the PER contributes to the interruption in feedforward inhibition necessary for cognitive multitasking. Future studies will continue to examine the relationship between the hyperactivation and hypoactivation in cognitive deficits in aging.

Disclosures: C.N. Logan: None. **K. Lubke:** None. **S.N. Burke:** None.

Digital Abstract Session

P090. Biomarkers and Brain Wellness in Aging

Program #/Poster #: P090.02

Topic: C.01. Brain Wellness and Aging

Support: NIA/NIH Grant 3R01AG053961-03S1
NIA/NIH 1R01AG053961
NIA/NIH 2R56AG045571
NJDOH MH-STT-15-001
NJDOH OMMH21HDP002

Title: Higher body mass is associated with smaller select hippocampal subfields in older African Americans at increased genetic risk for Alzheimer's disease

Authors: *B. A. FAUSTO, Z. OSIECKA, N. SINHA, M. A. GLUCK;
Ctr. for Mol. & Behavioral Neurosci., Rutgers University–Newark, Newark, NJ

Abstract: Obesity disproportionately affects African Americans and has been inconsistently associated with Alzheimer's disease (AD) risk in later-life. Examining the influence of the strongest genetic risk factor for AD, the APOE ε4 allele, may provide additional insight into the complex relationship between late-life obesity and AD. The purpose of this study was to: (1) compare hippocampal-dependent cognitive performance among older African Americans in the normal, overweight, and obese ranges of body mass index (BMI); and (2) explore the independent and interactive effects of BMI and genetic risk for AD on medial temporal lobe (MTL) cortical region and hippocampal subfield structure. Analyses included 70 cognitively normal older African Americans (ages 60-90, 86% female, mean education level = 14 years, 47% BMI >= 30 kg/m^2, 39% APOE ε4 allele carriers) who completed cognitive testing, saliva sampling for genotyping, and structural neuroimaging. Separate ANCOVAs were used to compare the three BMI groups on the following cognitive outcome measures of generalization, feedback-based learning, and verbal episodic memory, respectively: Concurrent Discrimination and Transfer Task, Acquired Equivalence Task, Rey Auditory Verbal Learning Test. Moderated hierarchical linear regressions were performed to determine the independent association between BMI, APOE ε4 status, and MTL cortical region/hippocampal subfield volume and surface area

and whether APOE ε4 status moderates the BMI-structure relationship while controlling for age, sex, education, literacy, and depressive symptomology. There was a statistically significant difference between BMI groups on Concurrent Discrimination and Transfer Task errors, $F(2, 60) = 3.60$, $p = .03$, partial eta-squared = .11. The normal BMI group committed significantly greater errors (17.22 +/- 2.88) than the overweight group (7.87 +/- 2.08), $p_{corrected} = .03$. BMI and APOE ε4 status were independently but inconsistently associated with MTL cortical regions and hippocampal subfield structure. APOE ε4 status moderated the relationship between BMI and hippocampal subfield structures. With increasing BMI levels, the following hippocampal subfield volumes and surface areas decreased but *only* in the APOE ε4 allele carriers: right and left dentate gyrus/CA3, left CA1, $ps < .05$. These results suggest being overweight may confer some neuroprotection in older African Americans. However, higher BMI is associated with smaller hippocampal subfields in APOE ε4 allele carriers. Future studies should investigate the potential overlap between the metabolic sequelae of late-life obesity and genetic mechanisms underlying AD.

Disclosures: B.A. Fausto: None. **Z. Osiecka:** None. **N. Sinha:** None. **M.A. Gluck:** None.

Digital Abstract Session

P090. Biomarkers and Brain Wellness in Aging

Program #/Poster #: P090.03

Topic: C.01. Brain Wellness and Aging

Support: NIH RF1AG057264
NIH R03AG063215

Title: Skeletal muscle circulating factors as novel regulators of CNS aging

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Abstract: Abstract
Dalton Patterson, Constanza J. Cortes
Department of Cell, Development and Integrative BiologyUniversity of Alabama at Birmingham
Exercise is a powerful behavioral intervention against central nervous system aging and Alzheimer's Disease, and plays an essential role in maintaining healthy neurocognitive function and immune metabolism in the aging brain. Physical activity is a positive regulator of hippocampal plasticity, stimulating adult neurogenesis, increasing levels of BDNF and ultimately preserving neurocognitive function in the aging CNS. Moreover, physical activity decreases the risk of developing Alzheimer's Disease, is associated with better Alzheimer's Disease prognosis, and positively affects cognitive function in Alzheimer's patients. Mechanistic studies in murine

Alzheimer's Disease models show that exercise rescues impaired neurogenesis, enhances synaptic plasticity, and attenuates the accumulation of tau tangles and Aβ-plaques, the two histopathological hallmarks of Alzheimer's Disease. Multiple epidemiological studies have suggested that skeletal muscle aging is a risk factor for the development of age-associated disease, including those of the CNS. By observing the effects of exercise, we saw that the effects of this opposes the local deleterious effects of aging on skeletal muscle, reversing age-associated transcriptional and metabolic dysregulation. We hypothesize that the differential expression and secretion of exerkines may underlie responsiveness to the CNS-targeting effects of exercise. We have identified differential expression of cytokines in circulation of exercised and exercise-mimetic transgenic mice with CNS targeting effects. We are currently investigating the role of these cytokines in neuronal survival using in vivo and in vitro approaches, measuring neuroinflammation, neurogenesis and AD-associated phenotypes, with the ultimate goal of developing novel CNS-targeting therapeutics that mimic the neuroprotective effects of exercise.

Disclosures: D.C. Patterson: None. **C.J. Cortes:** None.

Digital Abstract Session

P090. Biomarkers and Brain Wellness in Aging

Program #/Poster #: P090.04

Topic: C.01. Brain Wellness and Aging

Support: NSERC RGPIN-2020-06403
NSERC USRA-552599-2020
NSERC CGS-M
MITACS IT21365

Title: Characterizing lifespan changes in the expression of SARS-CoV-2 receptors and co-receptors in the human brain

Authors: N. HALABIAN, B. KUMAGAI, K. NAEEM, D. AHUJA, E. JEYANESAN, *K. M. MURPHY;
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Abstract: Many individuals infected with the SARS-CoV-2 virus will experience neurological symptoms ranging from headaches to more serious events such as delirium and ischemic stroke. The symptoms tend to differ across age groups. For example, younger adults with COVID-19 are vulnerable to stroke, while older adults also experience delirium. Even children may be affected, with distinct neurological symptoms in children with severe systemic inflammatory response to SARS-CoV-2 infection. Some of these neurological complications could be secondary to the infection. However, others may result from the direct effects of the SARS-CoV-2 virus on the brain. At present, there is little information about lifespan changes in expression of the virus receptor, ACE2, in the human brain that might help to understand the age-related impacts of COVID-19. We used transcriptomic data from postmortem tissue samples to determine the

lifespan trajectories (n=83 samples, age range: 2nd trimester - 80 years) for a network of 37 SARS-CoV-2-related genes in 16 areas of the human brain. The genes included coronavirus receptors, ACE2, DPP2, ANPEP, ENPEP, CD209, CLEC4G, CLEC4M, CECAM1, CECAM2, and the proteases (e.g.TMPRSS2) and integrins (e.g. ITGB3) that act as co-receptors for SARS-CoV-2. First, we characterized the lifespan changes for ACE2 and TMPRSS2. We found that most subcortical and 3 cortical (VFC, IPC, V1) areas followed undulating trajectories where those genes increased into young childhood, declined into teens then increase through younger and older adults. In 4 cortical areas (OFC, M1, S1, ITC), ACE2 and TMPRSS2 expression did not undulate but instead increased to a peak in young childhood and then declined across the rest of the lifespan. Thus, there is more than one lifespan trajectory for the expression of ACE2 and TMPRSS2 in the human brain. Next, we used high dimensional analyses to identify 4 clusters of brain areas with common developmental trajectories. The clusters represent anatomical regions: frontal cortex, parietal cortex, occipital/temporal cortex, and subcortical areas. Finally, we used a new analysis and visualization technique (PHATE) to compare the 16 brain areas' development using all 37 SARS-CoV-2-related genes. The high dimensional analyses identified regional and age-related differences in the expression of SARS-CoV-2-related genes in the human brain. The long-term neurological consequences of COVID-19 have the potential to be debilitating. So this new information about lifespan changes in the expression of SARS-CoV-2 receptors and co-receptors may help understand the neurological symptoms suffered by COVID-19 patients.

Disclosures: **N. Halabian:** None. **B. Kumagai:** None. **K. Naeem:** None. **D. Ahuja:** None. **E. Jeyanesan:** None. **K.M. Murphy:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U24AG061340
NIH Grant RF1AG057443
NIH Grant U54AG065187

Title: Agora, an open platform for the exploration of alzheimer's disease evidence

Authors: *A. K. GREENWOOD, J. GOCKLEY, J. WILEY, K. LEAL, D. ALUTHGAMAGE, K. WOO, S. SIEBERTS, S. SIMON, Z. LEANZA, A. GENDEL, E. MILLS, T. THYER, K. DO, M. PETERS, L. OMBERG, L. MANGRAVITE;
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Abstract: There is an urgent need for new therapies for the treatment of Alzheimer’s Disease. Discovery-based research studies have identified a suite of promising new AD targets across a spectrum of therapeutic hypotheses. However, validation of targets and development of new experimental reagents and therapeutics requires efforts broader than the focus of any single

research group. We developed the Agora site as a public resource to openly share information about new AD targets in order to support evaluation by the broader AD research community. Agora is freely available at https://agora.adknowledgeportal.org. Agora aggregates information and experimental resources in support of emerging AD targets and hypotheses. Agora presents a list of nominated targets stemming from the Accelerating Medicines Partnership in AD (AMP-AD) consortium and the broader AD research community. Agora also hosts interactive visualizations designed to support non-bioinformaticians in the evaluation of data from RNAseq, proteomic, and metabolomic studies. We are expanding Agora to catalog evidence and experimental reagents being generated by the TaRget Enablement to Accelerate Therapy Development for AD Centers (TREAT-AD). Updates to Agora in support of TREAT-AD will include individual scorecards for nominated targets based on multiple lines of evidence. In summary, Agora is a platform aimed at enabling the AD research community to evaluate promising target hypotheses.

Disclosures: **A.K. Greenwood:** None. **J. Gockley:** None. **J. Wiley:** None. **K. Leal:** None. **D. Aluthgamage:** None. **K. Woo:** None. **S. Sieberts:** None. **S. Simon:** None. **Z. Leanza:** None. **A. Gendel:** None. **E. Mills:** None. **T. Thyer:** None. **K. Do:** None. **M. Peters:** None. **L. Omberg:** None. **L. Mangravite:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: L.I.F.E. FOUNDATION Research Grant

Title: In silico gene expression analysis of Dek in the human brain across the lifespan

Authors: ***A. GREENE**[1], L. PRIVETTE VINNEDGE[2], M. SOLOMON[1];
[1]Univ. of Cincinnati, Cincinnati, OH; [2]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: DEK, a chromatin-associated phosphoprotein, mainly studied in the periphery, is known for its role in DNA repair, cellular proliferation and apoptosis inhibition. Our group is the first to associate DEK loss with central nervous system diseases, including Alzheimer’s disease. Consistent with this, we determined that DEK is prominently expressed in the murine brain in learning and memory-relevant brain regions, such as the hippocampus, prefrontal cortex, and amygdala. However, Dek expression has not yet been characterized in the human brain. Therefore, we leveraged data from the BrainCloud and Human Brain Transcriptome databases to assess Dek mRNA expression in human brain across the lifespan. Specifically, we examined the impact of age and biological sex on Dek expression in the dorsolateral prefrontal cortex (dlPFC; BrainCloud and Human Brain Transcriptome) and hippocampus (Human Brain Transcriptome). Given its role in cellular proliferation, DNA repair, and chromatin remodeling, we hypothesized that DEK expression would be highest in fetal development relative to other developmental

stages (e.g., adulthood) because this period is associated with cellular proliferation and synaptic growth. We also hypothesized that women would have higher Dek expression than men because it is an ER-alpha target gene. Consistent with our hypothesis, Dek expression in the hippocampus and dlPFC is highest during fetal development and decreases with aging in both sexes, which is consistent across databases. Contrary to our hypothesis, we observed elevated Dek expression in males over females across the lifespan in the dlPFC (BrainCloud). Given the prominent expression of Dek in the fetal brain and its subsequent decline in aging, the data suggest a role of DEK in brain development. This assertion is consistent with our previous findings whereby DEK loss *in vitro* and in mouse models induces impaired neurite length, DNA damage, and apoptosis. Further analyses will include correlations of Dek expression with expression of genes associated with proliferation and brain development. Assessing Dek mRNA expression patterns in human brain across lifespan not only informs us of the possible roles of DEK in normal brain development, but may also hint at its potential role in age-related brain diseases.

Disclosures: **A. Greene:** None. **L. Privette Vinnedge:** None. **M. Solomon:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant U54 AG054345

Title: Validation of late-onset Alzheimer's disease risk variants in mouse models

Authors: ***M. SASNER**[1], A. OBLAK[2], D. GARCEAU[1], K. KOTREDES[1], P. LIN[2], D. SONI[2], R. PANDEY[1], A. UYAR[1], C. PREUSS[1], A. GREENWOOD[3], G. CARTER[1], G. HOWELL[1], B. T. LAMB[2];
[1]The Jackson Lab., Bar Harbor, ME; [2]STARK Neurosci. Res. Inst., Indiana Univ. Sch. of Med., Indianapolis, IN; [3]Sage Bionetworks, Seattle, WA

Abstract: Alzheimer’s disease (AD) is the most common form of dementia, and has a significant genetic component. While rare familial causative mutations have been known for decades, the genetic etiology of the common (>95%) late-onset AD (LOAD) is still unknown. Numerous AD risk loci and variants have been identified by large-scale genetic studies, but few have been functionally verified and models to study their mechanism of action are lacking. The Model Organism Development and Evaluation for Late-onset AD (MODEL-AD) Center was created to develop, characterize, and distribute more precise preclinical models for late-onset AD by engineering disease-associated variants into mouse models, and characterizing the phenotypes using clinically relevant assays. Criteria to prioritize risk variants include replication in multiple studies, predicted pathogenicity of variant, sequence conservation between human and mouse, and expression in relevant cell types. We have created coding variants in the *Abca7, Clasp2, Mthfr, Mtmr4, Picalm, Plcg2, Shc2, Slc6a17, Snx1, Sorl1* and other loci. Knockouts of *Abca7*,

Ceacam1, *Il1rap*, and *Plcg2* have been created to model human loss-of-function variants. Because a large percentage of AD risk variants occur in non-coding regions, we have most recently created 6 alleles in promoter or enhancer regions in the murine *Adamts4, Bin1, Cd2ap, Epha1, Ptk2b, and Scimp* loci.These variants have been engineered into a mouse model that expresses the AD risk variants APOE4 and Trem2*R47H as well as a humanized Abeta allele. Primary screening was completed by transcriptomic analysis using the nanoString Mouse AD Panel at 4 and 12 months, which demonstrated that the *Abca7*A1527G*, *Mthfr*C677T*, and *Plcg2*M28L* models exhibited age-dependent similarities to transcriptomic changes observed in post-mortem human samples from the AMP-AD cohort. These are now being aged for comprehensive phenotyping at 12, 18 and 24 months of age to include biomarker analysis, transcriptomics, proteomics, metabolomics, *in vivo* neuroimaging of glucose utilization and amyloid deposition, neuropathology and cognitive assays. Additional models will be added as primary screening is completed. All models are made available for both academic and for-profit use from The Jackson Laboratory, and all validation protocols and data will be shared via the AD knowledge portal (https://adknowledgeportal.synapse.org/). For more information see www.model-ad.org.

Disclosures: **M. Sasner:** None. **A. Oblak:** None. **D. Garceau:** None. **K. Kotredes:** None. **P. Lin:** None. **D. Soni:** None. **R. Pandey:** None. **A. Uyar:** None. **C. Preuss:** None. **A. Greenwood:** None. **G. Carter:** None. **G. Howell:** None. **B.T. Lamb:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HRC Programme Grant 16-697

Title: Novel whole-transcriptome RNA-Seq protocol reveals major perturbations in microglial function in a transgenic mouse model of Alzheimer's Disease.

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Abstract: RNA sequencing (RNA-Seq) can offer unique insights into the pathology of disease. In particular, simultaneous identification and quantification of both coding and non-coding RNA bypasses many shortcomings of more traditional RNA-Seq protocols. In Alzheimer's Disease, non-coding RNA species such as microRNA, long-non-coding RNA, small nuclear RNA, and small nucleolar RNA, are increasingly being implicated in the pathogenesis of the disease. Here we implement a novel method of ribosomal RNA depleted RNA-Seq that encompasses coding- and non-coding RNA, to investigate changes in the transcriptome of APPswe/PSEN1dE9 transgenic mice and wild-type controls at 15 months (n=4). We identified >30,000 transcripts

corresponding to messenger RNA and numerous species of non-coding RNA. Differential expression analysis using edgeR found in total 615 differentially-expressed genes including 336 protein-coding genes, 26 miRNA and 15 pseudogenes (Exact Test; $p<0.05$). Bioinformatics and functional enrichment analysis of these differentially-expressed transcripts (coding *and* non-coding) reveals significant changes in microglial function. One major enriched pathway is the Complement Cascade activation pathway. This is an immune pathway responsible for microglial phagocytosis of damaged neurons and cellular debris, which has been implicated in the later stages of Alzheimer's Disease pathogenesis involving clearance of Amyloid-ß. Our results show that this protocol allows deep simultaneous sequencing of coding and non-coding RNA, and reveals some of the extensive changes to biological processes that underpin Alzheimer's Disease-like pathology in this mouse model.

Disclosures: **N. Potemkin:** None. **S.M. Cawood:** None. **B. Mockett:** None. **J.L. Stanton:** None. **J.M. Williams:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant DGAPA/PAPIIT number IA210620

Title: In Silico analysis of differential expression genes in databases of Alzheimer´s disease patient's vs controls, to identify potential biomarkers of the disease

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Abstract: Introduction. Alzheimer's disease (AD) is the most common cause of dementia. Symptoms begin with mild memory difficulties and evolve towards cognitive impairment. The discovery of biomarkers for AD diagnosis is a valuable tool to study the etiology of the disease, to find risk factors, and to discover new treatments. Bioinformatics has become a relevant research tool in which computational models of biological processes and expression analysis have lead to the identification of a number of pathways whose relationship with the diseases was still unknown. Methods. RNA seq databases or RNA microarrays of tissue from the medial temporal lobe and peripheral blood of patients with AD, which were processed by TrueSeq RNA-seq. Values were normalized with the Limma program of the statistical R programming language. We performed a computational analysis of the genetic differential expression using t-statistic-Limma. Data were corrected with the Benjamini and Hochberg approach and the genes with *p* values equal to or less than 0.05 were considered significant. The direction of the change

in gene expression was determined by its variation in the "log2-fold change" between controls and patients. We performed a functional enrichment analysis of GO was performed using goana and topGO-Limma (Smyth et al., 2016). Results were compared with clusterProfilerR. Results. With brain tissue database of medial temporal lobe (EMEXP 2280) we obtained 359 down regulated genes (DR) and 324 up regulated genes (UR), the UR pathways were: transcription, mitochondrial function and hydrolase activity, while DR were: gene expression and regulation of cell proliferation, nuclear alterations, transmembrane receptor signaling. Analysis with KEGGp showed that metabolic pathways were UR and cytokine pathways and their receptors were DR. The blood samples analysis (PBMC, E-GEOD-18309) showed 383 genes DR and 257 genes UR. UR: negative regulation of Wnt signaling, hormonal metabolism processes, and DR: in subcellular components and homeostatic processes, cytosol and extracellular space alterations. KEGGp analysis showed UR: alteration in the Retinol and Cytochrome P450 and DR of the actin cytoskeleton. Finally, the intersection of the differentially expressed genes in the two databases showed 34 genes shared between the brain and blood arrangements: UR: SCG2, CA14, AHSP, HAPLN2, RALY-AS1, PLEKHG4, PRSS35, LINC01116; DR: DBH, NME8, CXCL6, SERPINA7, OR10H3, NYNRIN, MOS, C3, IL10RB-DT, CDKN2A-DT, CCDC60, CPB2, RBPMS6 CRST, T4ENT5D, DGKG. TRIM58 matched in both databases. Conclusion. *In silico* search for altered gene expression in AD will allow us to identify new biomarkers for AD.

Disclosures: M. Silva-Lucero: None. **J. Rivera-Osorio:** None. **Y. Muley VijayKumar:** None. **G. Lopez-Toledo:** None. **C. Sanchez-Hernandez:** None. **M. Cardenas-Aguayo:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA grant # AG061831

Title: Circadian clock is a central regulator of genes disrupted in early Alzheimer's disease pathology

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Abstract: Background: Circadian disruptions are experienced by more than 80% of Alzheimer's disease (AD) patients, and emerging data strongly supports a causal role for circadian abnormalities in AD pathophysiology including exacerbation of inflammation and Amyloid-β accumulation, cognitive impairment and oxidative stress. Circadian rhythms are generated by oscillation of clock genes and regulate up to 30% of genome. Studying the nature of circadian alterations in the brain early in disease progression is therefore fundamental to our understanding of AD pathology development.
Methods: APP23 transgenic mice, overexpressing human APP_{Swe} (n = 26), and non-transgenic

littermates (NTG; n = 28) were housed in individual cages under 12:12 LD cycle. Cage activity was monitored and analyzed with ClockLab. Mice were sacrificed at 10-months old, at six-hour intervals and brains were collected for neuropathology. RNA-Seq was used to analyze gene expression in hippocampus (HIPP) and frontal cortex (FC). MetaCycle package and Ingenuity Pathway Analysis (IPA) software were used to detect rhythmic signals in the data and for transcriptomics analysis, respectively.
Results: APP23 mice were at early stages of pathology at the time of sacrifice, with mild behavioral abnormalities and low levels of amyloid plaque accumulation. Global transcriptional changes between TG and NTG mice were modest: 22 and 157 genes with higher than 30% expression level changes in FC and HIPP respectively. We identified a large number of genes with circadian rhythmicity in HIPP and FC of NTG mice: 868 and 1190 respectively. According to IPA analysis, many of them are associated with neurological disease pathways, including AD, as well as senescence pathway. Crucially, we observed significant changes in the expression profile of oscillating genes between NTG and APP23 mice. We identified 257 genes in the HIPP samples that lost their 24-hour periodicity, exhibited more than two-fold change in amplitude, or more than 3-hour phase shift. Importantly, more than 15% of these genes are involved in the immune system development and response. In FC, 294 genes exhibited similar loss/changes of circadian rhythmicty in APP23 TG mice, with more than 70% of these associated with neurological disease.
Conclusions: Transcriptomic analysis in this AD mouse model suggests that early gene expression changes are highly associated with abnormalities in circadian rhythmicity. In NTG mouse brains, a large number of AD-related genes exhibit circadian oscillations, which are lost or severely disrupted early in disease progression in APP23 mice, potentially driving later neuropathological changes.

Disclosures: **L. Akhmetova:** None. **J. Mott:** None. **D. Whittaker:** None. **P. Desplats:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Institutes of Health T32HD007065
National Institute on Aging (AG054345)
National Institute on Aging (AG038070)

Title: Determining the mechanisms by which APOE$^{\varepsilon 3/\varepsilon 4}$ modifies risk for AD and related dementias

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Abstract: Background: Human and murine studies have parsed out mechanisms by which *APOE*$^{\varepsilon4}$ differs from *APOE*$^{\varepsilon3}$ to increase risk for Alzheimer's disease (AD). However, little is known about the ways *APOE*$^{\varepsilon3}$ and *APOE*$^{\varepsilon4}$ interact to affect risk for AD. This is due in part to legal restrictions of existing humanized *APOE* strains – including breeding of *APOE*$^{\varepsilon4/\varepsilon4}$ to *APOE*$^{\varepsilon3/\varepsilon3}$ mice to create the commonly understudied risk genotype in human AD cases - *APOE*$^{\varepsilon3/\varepsilon4}$. To overcome these limitations, here we describe the creation of a new set of humanized *APOE* alleles and test our hypothesis that *APOE*$^{\varepsilon3/\varepsilon4}$ modifies risk for AD and related dementias in ways distinct from *APOE*$^{\varepsilon4/\varepsilon4}$.
Methods: A new allelic series of humanized *APOE* mice that includes *APOE*$^{\varepsilon4/\varepsilon4}$, *APOE*$^{\varepsilon3/\varepsilon3}$, and *APOE*$^{\varepsilon2/\varepsilon2}$ mice were generated by MODEL-AD using homologous directed repair followed by CRISPR, and validated using PCR, sanger sequencing, RNA sequencing and western blotting. Female and male *APOE*$^{\varepsilon3/\varepsilon3}$, *APOE*$^{\varepsilon3/\varepsilon4}$ and *APOE*$^{\varepsilon4/\varepsilon4}$ mice were then established and characterized at 2 mos, 4 mos and 12 mos of age. Two voluntary running cohorts (1-4 mo and 1-12 mos) were included to determine whether the effects of *APOE*$^{\varepsilon4}$ genotype are modified by exercise. Our extensive assays included blood lipid/cholesterol profiles, novel spatial memory, advanced metabolic assessment, immunofluorescence and cortical transcriptional profiling. Linear modeling and weighted gene co-expression network analysis (WGCNA) were used to identify effects of sex, genotype, and running in gene expression data.
Results: At both 2 and 4 mos, linear modeling revealed more significant genes unique to either *APOE*$^{\varepsilon3/\varepsilon4}$ or *APOE*$^{\varepsilon4/\varepsilon4}$ when compared back to *APOE*$^{\varepsilon3/\varepsilon3}$, than in the intersection of these two comparisons. We also show that genes unique to *APOE*$^{\varepsilon3/\varepsilon4}$ at 4 mo enriched for extracullar matrix (ECM) and coagulation related terms. Further, WGCNA identified gliogenesis and myelination terms affected by exercise regardless of *APOE* genotype. Additionally, we show an increased in collagen deposition in *APOE*$^{\varepsilon4/\varepsilon4}$ mice compared to *APOE*$^{\varepsilon3/\varepsilon3}$ mice at 12 mos.
Conclusions: Our study predicts important differences between *APOE*$^{\varepsilon3/\varepsilon4}$ and *APOE*$^{\varepsilon4/\varepsilon4}$ genotypes on AD-relevant cerebrovascular transcriptional profiles.This work challenges the well hypothesized notion that *APOE*$^{\varepsilon3/\varepsilon4}$ shows an intermediate dose response between *APOE*$^{\varepsilon3/\varepsilon3}$ and *APOE*$^{\varepsilon4/\varepsilon4}$. Specifically, our results suggest that cerebrovascular health through ECM homeostasis is altered at a young age by *APOE*$^{\varepsilon4}$ allele dosage, and remains affected at one year. This work suggests that therapies aimed at modifying APOE biology to treat dementias may need to be targeted to specific *APOE* genotypes.

Disclosures: K. Foley: None. **D. Garceau:** None. **K. Kotredes:** None. **G. Carter:** None. **M. Sasner:** None. **G. Howell:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: U54AG054349

Title: Long-read RNA-seq and microRNA-seq analysis of the 5xFAD transcriptome

Authors: *G. BALDERRAMA-GUTIERREZ[1], E. REBBOAH[1], C. MCGILL[2], D. WYMAN[1], F. REESE[1], A. MORTAZAVI[1], C. MODEL-AD[1];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative pathology characterized by loss of memory and cognitive impairment due to cell loss in the cortex and hippocampus, which are the first affected regions. The cells in our brain, like in any other tissue, are under constant transcriptional regulation to respond to environmental stimuli. Alternative splicing is a process where a gene can have multiple protein products by choosing different combinations of exons. Some genes involved in AD have shown changes at isoform level as a contributor for the pathology, Mapt (Tau) being a known example. The 5xFAD mouse model has 5 familial mutations associated with AD and presents aggressive pathology since early age. As one of the most popular AD mouse models, it has been widely characterized using short-read sequencing, but as long-read technologies improve their yield and accuracy full-length transcriptomes promise a different insight at an isoform level. To complement already available short-read data deposited in Synapse, MODEL-AD has matching cortex and hippocampus samples characterized using PacBio (Sequel II platform) and microRNA sequencing from 8-month-old 5xFAD mice. In this poster, we will explore the full-length transcriptome using TALON for long-read annotation and quantification and Swan for differential transcript analysis and transcript visualization. Additionally, characterization and differential analysis will be done for the microRNA data.

Disclosures: G. Balderrama-Gutierrez: None. **E. Rebboah:** None. **C. McGill:** None. **D. Wyman:** None. **F. Reese:** None. **A. Mortazavi:** None. **C. Model-ad:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: U54AG054349

Title: Bulk and single-nucleus analysis of the 3xTG cortex and hippocampus transcriptome

Authors: *N. REZAIE, G. BALDERRAMA-GUTIERREZ, H. LIANG, C. MCGILL, A. MORTAZAVI, C. MODEL-AD;
Univ. of California, Irvine, Irvine, CA

Abstract: Clinical trials for potential Alzheimer disease (AD) drugs have a high failure rate due to the fact that most drugs are successful in mouse models but are not translatable to humans. Mouse models fail to fully recapitulate the pathology, specially presenting plaques and tangles at the same time.
The 3xTg mouse model contains three mutations associated with familial AD (APP Swedish, MAPT P301L, and PSEN1 M146V), and it presents both plaques and tangles accumulation at later stages of development. Cognitive impairments happen by 4 months, but there is no data that

indicates neuronal loss.
Responses to the accumulation of plaques and tangles will involve all cell diversity in the brain. In particular, activation of glial cells such as microglia will start and promote neurodegeneration by eliminating debris from cells of protein aggregates around them. Model-AD has collected cortex and hippocampus of 3xTg mice at different time points (4,12,18 and 24 months) to be characterized with RNA-seq. We generated matching single-cell and single-nucleus from 12, 18 and 24-month 3xTg mice using the ddSeq platform. The scRNA-seq datasets were produced exclusively from isolated microglia. As an alternative to performing separate experiments for nuclei and cells, we have generated microglia + nuclei datasets from a 24-month female 3xTg mouse using the SplitBio barcoding kit.
In this poster, we will explore brain cell diversity as well as microglial subpopulations in the 3xTg mouse models to generate additional insights about the cell response to AD.

Disclosures: **N. Rezaie:** None. **G. Balderrama-Gutierrez:** None. **H. Liang:** None. **C. McGill:** None. **A. Mortazavi:** None. **C. Model-ad:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01NS095799

Title: Tip60 HAT/HDAC2 subcellular localization is critical for neuroprotection

Authors: *E. ARMOUR, J. PERHACS, F. KHOA, H. ZHANG, F. ELEFANT;
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Abstract: Neurodegenerative diseases such as Alzheimer’s disease (AD), Parkinson’s disease (PD), and Amyotrophic Lateral Sclerosis (ALS) that involve cognitive defects are becoming increasingly common in our aging population. Epigenetic mechanisms, such as histone acetylation, have proven to reinstate cognition and are critical for proper neuronal function, although the exact mechanisms are yet to be elucidated. Previous work in our lab has shown the imbalance of Tip60 histone acetyltransferase (HAT) and histone deacetylase 2 (HDAC2) in a well characterized *Drosophila* AD model results in epigenetic repression of synaptic plasticity genes and functional cognitive defects. Remarkably, Tip60 overexpression restores AD-induced neuropathology, emphasizing Tip60’s neuroprotective role. Recent work in our lab has identified Tip60 as having shuttling capabilities between the nucleus and the cytoplasm. Moreover, we have shown that in the human AD hippocampus, Tip60 is largely excluded from the nucleus, suggesting that disruption of Tip60 subcellular localization may contribute to AD. To further elucidate the importance of localization and the HAT/HDAC balance, we examined the subcellular localization of Tip60 and HDAC2 in the *Drosophila* brain. Under normal conditions, Tip60 is localized mainly in the cytoplasm, while HDAC2 is localized mainly in the nucleus. By

elucidating Tip60 HAT/HDAC2 shuttling mechanisms, we can better understand the interaction between these two epigenetic modulators in an attempt to develop more specific therapeutic targets for neurodegenerative diseases.

Disclosures: E. Armour: None. **J. Perhacs:** None. **F. Khoa:** None. **H. Zhang:** None. **F. Elefant:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U19AG060909

Title: Transcriptomic cell type analysis of human middle temporal gyrus in the context of Alzheimer's disease

Authors: *A. BUCHIN[1], R. D. HODGE[1], K. A. SMITH[1], J. GOLDY[1], A. TORKELSON[1], E. S. KAPLAN[1], E. J. MELIEF[2], M. J. HAWRYLYCZ[1], D. C. KEEN[2], E. S. LEIN[1], J. A. MILLER[1]; [1]Allen Inst. For Brain Sci., Seattle, WA; [2]Univ. of Washington, Seattle, WA

Abstract: Dementia affects nearly ~11% of the U.S. population over the age of 65, with the incidence doubling every 5 years up to 40-60% after the age of 90. Around 2/3 of these cases are correlated with Alzheimer's disease. Even though Alzheimer's disease has been studied intensively for decades there is still no clear understanding of its underlying cellular mechanisms. Recent advances in single cell or single nucleus transcriptomics have provided a new strategy to understand cellular diversity in normal brain and cell type specific changes in disease. Indeed, recent work has begun to identify selective cell type vulnerability in AD using these techniques [Mathys et al. 2019]. One key strategy to identify AD-related changes in cell type proportions and properties is to generate a well-annotated reference cell type classification of the neurotypical brain, and then map AD data to that reference to discern pathological changes in specific cell types during disease progression. Here we present a workflow for constructing a neurotypical brain reference classification and strategies for mapping disease datasets for middle temporal gyrus (MTG) based on 10x Genomics single nucleus RNA sequencing data. Non-linear dimensionality reduction and clustering of nearly 90 thousand nuclei from 3 patients identified 29 glutamatergic neuron, 41 GABAergic neuron and 10 non-neuronal types, very similar to recently published results using SMART-seq methodologies [Hodge et al. 2019]. To identify known cell types, we applied neural network and random forest classifiers to the tissue from aged and Alzheimer's patients. We found that that the new reference dataset based on 10X Genomics MTG data could be successfully used to identify known cell types in AD tissues based on patterns of gene expression. This novel reference dataset could enable high resolution tracking of cell type-specific changes associated with Alzheimer's disease and normal aging.

Disclosures: **A. Buchin:** None. **R.D. Hodge:** None. **K.A. Smith:** None. **J. Goldy:** None. **A. Torkelson:** None. **E.S. Kaplan:** None. **E.J. Melief:** None. **M.J. Hawrylycz:** None. **D.C. Keen:** None. **E.S. Lein:** None. **J.A. Miller:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R01AG057914
NIA R01AG057914-02S1
NIA R01AG057914-03S1
NIA R01AG054180
Bright Focus Foundation A2016397S
Human Brain Project - ID66

Title: Using brain-wide spatial analysis to identify region-specific changes in cell composition and define the nature of cognitive resilience to Alzheimer's Disease in a model mouse population

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Abstract: Age is currently the greatest risk factor for developing Alzheimer's disease (AD); however, it is increasingly evident that there is also a strong genetic component to AD. Identifying neuroanatomical correlates of cognitive resilience and their underlying genetic modifiers can potentially elucidate why there is significant variation in the progression of AD. Recent advances in large-scale immunohistochemistry (IHC), now make it feasible to assess brain-wide regional and cell-type-specific hallmarks of AD progression in terms of pathology and clinically relevant traits in well-characterized cohorts of AD model mice that include extensive behavior and omics data. To identify differences in such traits, hemibrains of 34 male and female, adult(6m) and middle-aged(14m), C57BL/6J(B6) and B6/D2 F1 (D2) mice harboring five causal human familial AD mutations (5XFAD) and their non-transgenic (Ntg) littermates were stained to evaluate neurodegeneration (NeuN), gliosis (Iba1&GFAP), and amyloid pathology (AB1-42) using IHC. The QUINT workflow was implemented to measure individual differences in cellular composition. This workflow allowed for experimental slices to be systematically segmented and registered to the Allen Brain Atlas to gain a global perspective of % coverage of each stain across brain regions. Compositional differences between the B6 and F1-D2 strains became apparent when evaluating potential neurodegeneration, as measured by reduced NeuN coverage across regions. Despite comparable levels of amyloid, the B6-5XFAD strain exhibited a greater decrease in % neuron coverage than the D2-5XFADs by 14m in the hippocampus and cortex. Surprisingly this smaller cell coverage did not relate to deficits in short or long-term memory. Thus, although the B6-5XFAD mice were not resilient to AD-induced

neurodegeneration, on average the B6-5XFAD mice were more cognitively resilient and performed better than their D2-5XFAD counterparts on memory tasks at both 6m and 14m. A significant interaction between background strain and 5XFAD mutation was also revealed by the analysis of Iba1 coverage specifically in the cortex and striatum. Interestingly, our results suggest that B6-5XFAD Iba1-positive microglia were reduced relative to Ntg counterparts, and the opposite was observed in the D2-5XFAD mice. In summary, performing brain-wide spatial mapping fostered an unbiased approach to identifying cell types and regions vulnerable to AD among two commonly used mouse strains. Future studies will investigate how genetics affect regional and cell-type composition and employ genetic mapping to identify loci associated with these endophenotypes of resilience.

Disclosures: B. Gurdon: None. **N. Hadad:** None. **M. Telpoukhovskaia:** None. **J.G. Bjaalie:** None. **C. Kaczorowski:** None. **S. Yates:** None. **M. Amedjkouh-Puchades:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PICT/2016-2513-ANPCyT-Argentina
UBACyT-20020170100745BA-Argentina

Title: Learning and memory is differentially affected in middle aged wild type and McGill-R-Thy1-APP rats according to sex

Authors: M. HABIF[1], N. COLETTIS[1], F. FILIPPIN[1], S. DO CARMO[2], C. SISTER[1], V. BERKOWICZ[1], M. CERCATO[1], A. CUELLO[2], *D. A. JERUSALINSKY[1];
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Abstract: Recent studies have shown a sex difference in neuropathology and cognition in mouse models of Alzheimer’s disease (AD). In this study, we investigated the influence of sex on short-term (STM) and long-term memory (LTM) in middle-aged McGill-R-Thy1-APP transgenic (Tg) rats, a model of AD-like brain amyloidosis. In heterozygous (+/-) McGill Tg rats the amyloid pathology is mainly intraneuronal with extracellular plaques developing at advanced age, offering an opportunity for testing cognitive deficits at preplaque stages. 12-13 month old McGill +/- male and female rats and their wild-type (wt) littermates were left to explore an open field for 5min and tested 24hr later. Horizontal (traveled distance) and vertical (rearings) exploration as well as grooming and time spent in the center of the arena vs periphery, were quantified. Most of the exploratory parameters were significantly lower along a session, as well as in the 2nd vs. the 1st exposure, denoting habituation in the 4 groups, without significant differences between them. However, the number of rearings appeared lower in males than in females. Rats were then

trained in 2-novel object recognition (NOR) and location (NOL) tasks. Both wt and Tg rats discriminated well a new vs. a familiar object 1hr later (short-term memory, STM). However, 24h later (long-term memory, LTM), Tg rats either female or male, did not reach the discrimination criteria. In the NOL task, only wt females performed well to discriminate a new location for a familiar object. For inhibitory avoidance (IA), rats were left in an enlighten compartment, receiving a mild foot shock when entering a dark compartment, and latency to enter was recorded. 24h later test latencies were significantly higher for both wt and Tg females, and for wt males, though not for Tg males. Furthermore, 14 days later only wt females reached the criteria. In conclusion, both 12-13 month-old wt and Tg+/- rats showed sex-dependent impairments in some cognitive functions: wt males and Tg (both female and male) rats exhibited selective LTM and persistence deficits in associative memories involving spatial reference and/or aversive stimulus compared to wt females. This highlights the importance of including sex as a variable when interpreting cognitive behavior data.

Disclosures: **M. Habif:** None. **N. Colettis:** None. **F. Filippin:** None. **S. Do Carmo:** None. **C. Sister:** None. **V. Berkowicz:** None. **M. Cercato:** None. **A. Cuello:** None. **D.A. Jerusalinsky:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R01NS093362-01
NIH Grant 3R01NS093362-04S1

Title: N-terminal phosphorylation of fused in sarcoma (FUS) linked to DNA damage leads to distinct changes in the FUS proteome

Authors: *M. A. JOHNSON[1], T. A. NUCKOLS[1], S. NANDY[1], G. TAYLOR[1], J. ROOT[1], T. KUKAR[1,2,3];
[1]Dept. of Pharmacol. and Chem. Biol., [2]Ctr. for Neurodegenerative Dis., [3]Dept. of Neurol., Emory Univ., Atlanta, GA

Abstract: Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are two neurodegenerative disorders that exhibit overlapping clinical symptoms and pathological markers. One such neuropathological marker is the abnormal cytoplasmic aggregation of fused in sarcoma (FUS). FUS is an RNA/DNA binding protein involved in gene transcription, mRNA splicing, DNA-repair pathways, and mRNA transport. While FUS is primarily a nuclear protein, FUS must shuttle between various cellular compartments to function properly. When shuttling is disrupted, FUS accumulates into cytoplasmic aggregates leading to the loss of FUS nuclear functions and a toxic gain of cytoplasmic functions. FUS shuttling can be disrupted through genetic mutations, but most of these cases have been linked to either ALS or mixed ALS/FTD.

Therefore, non-genetic factors such as post-translational modifications may drive pure FTLD-FUS pathology. Our lab discovered that double-strand DNA damage induces phosphorylation of FUS (p-FUS) at multiple N-terminal sites leading to an accumulation of p-FUS in the cytoplasm, yet the consequence of this cytoplasmic p-FUS remain unknown. To investigate this, we used an unbiased proximity labeling approach termed APEX2 to interrogate how phosphorylation may change the FUS proteome. We and others have mapped the major N-terminal phosphorylation sites, enabling the creation of a phosphomimic variant of FUS (PM FUS) that mimics the phosphorylation of FUS caused by double-stranded DNA damage. We then linked WT FUS, PM FUS, or the ALS-linked mutant P525L FUS to the engineered peroxidase, APEX2, and used proximity-dependent biotin tagging to enrich for all possible interacting partners. Biotin-tagged proteins were identified and quantified using label-free quantitative proteomics. We identified a total of 3,313 differentially expressed proteins within our three groups. From this, we identified 228 proteins significantly enriched in PM FUS over WT FUS and 1,332 proteins significantly enriched in PM FUS over P525L FUS. Overall, we found PM FUS enriched for cytoplasmic proteins involved in mRNA catabolic process, translation initiation, and stress granule assembly over WT FUS. In contrast, PM FUS enriched for nuclear proteins involved in functions such as spliceosome, ribonucleoprotein complex biogenesis, and covalent chromatin modification over P525L FUS. Taken together, these data suggest that phosphorylation results in a novel FUS interaction state that exists between the toxic P525L and the homeostatic WT functions. Findings from these studies will help to inform whether FUS phosphorylation status is important to FTLD-FUS pathology.

Disclosures: **M.A. Johnson:** None. **T.A. Nuckols:** None. **S. Nandy:** None. **G. Taylor:** None. **J. Root:** None. **T. Kukar:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant Number:UL1TR001449

Title: Interactions Between Autophagy, Herpesvirus and Neurodegeneration in Alzheimer's Disease

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Abstract: Introduction: Various microbes and viruses have been associated with the pathogenesis of Alzheimer’s Diseases (AD), with many studies dating back to the 1950s. There are challenges in establishing direct links between the microbes and AD pathology. Since microbes and viruses have been observed to be dormant and reactivate in aging brains, it is not

clear if the microbes are a byproduct of AD pathology or a direct cause of it. Recently, various publications considered the link between several herpes simplex viruses and neurodegeneration in AD. For example, Readhead et al. utilized multiomics data from post-mortem brain samples meeting the neuropathological criteria for AD to construct biological networks and look for associations between viral genome, transcription and AD. This study established associations between human herpesviruses HHV-6A and HHV-7 and various aspects of AD. The herpes simplex viruses (HSV1 & 2) interact with intracellular membranes during egress. After viral replication and exit from the cell's nucleus, a secondary envelopment process, by which HSV acquires its envelope, structurally resembles the process of autophagy. Thus, autophagy might have significant interactions with HSV. In this study, we are interested in investigating whether autophagy-related genes are regulated by herpesviruses to determine molecular relationships between autophagy, herpesviruses, and AD.**Methods:** We have downloaded the R codes and datasets published by Readhead et al. available on synapse.org. We were able to run the code and reproduce the figures in their paper. We've also compiled a list of 180 autophagy-related genes. We plan to mine viral quantitative trait loci (vQTL) to correlate host genomic and RNA-seq to viral load in preclinical and clinical AD; and Quantitative trait of host genes (eQTL) to identify genes associated those host sites that also correlate with cognition and Braak stage. This produces 91,000 sites which we will mine for autophagy genes to construct a network of autophagy, viral activity, and AD traits. **Results:** We hypothesize that there exists a correlation between autophagy related genes and the reactivation of the dormant viruses and the progression of AD pathology.**Conclusions:** The integration of autophagy related genes in this project will potentially clarify the role microbes and viruses play in the progression and onset of AD pathology and how they interact with viral products in the brain.

Disclosures: **A. Nafchi:** None. **M. Anani:** None. **E. Bearer:** None. **T. Oprea:** None. **B. Readhead:** None.

Digital Abstract Session

P091. Alzheimer's Disease: Genetics

Program #/Poster #: P091.16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: U01DA047713
NIA supplement to U01DA047713

Title: Ptprd phosphatase positive allosteric modulation and flavanol benefits for reducing alzheimers disease incidence

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Abstract: Alzheimers disease (AD) and the key elements of AD neuropathology receive contributions from genetics and environmental influences that include diet. Variants in intron 10 of the gene encoding the receptor type protein tyrosine phosphatase PTPRD are associated with individual differences in densities of neurofibrillary tangles, the AD pathological element that is rich in hyperphosphrylated tau. Serine threonine kinases most associated with tau phosphorylation include the glycogen synthase kinases GSK3 alpha and beta and the cyclin dependent kinase CDK5. Activity of each of these kinases is enhanced by phosphorylation of their own tyrosines. We now report that a phosphopeptide that has a phosphotyrosine in sequences that are identical between GSK3 alpha and GSK3 beta and a CDK5 phosphopeptide (pYGSK3 and pYCDK5) are avidly dephosphorylated by recombinant human PTPRD phosphatase with several lines of evidence for specificity, including effects of varying sequence and comparisons with activities of the related phosphatases PTPRS and PTPRF. We report that PTPRRD activities on the pYGSK3 phosphopeptide are almost doubled by addition of quercetin and related flavanols, but not by flavones or flavanones. This structure-activity relationship is similar to that for dietary flavanol intake in preventing AD incidence in studies of aging. PTPRD is well positioned to contribute to both genetic and environmental influences on Alzheimer's pathophysiology. Improved PTPRD positive allosteric modulators should come from testing our in silico models for PTPRD phosphatase interactions with phosphopeptides and flavanols.

Disclosures: **G.R. Uhl:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent submitted by VA. **I.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent submitted by VA. **M. Martinez:** None. **K. Dokladny:** None. **D. Johnson:** None.

Digital Abstract Session

P092. Alzheimer's Disease: Neuroinflammation in Vitro Models

Program #/Poster #: P092.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 NS100714
NIH R01 AG067258

Title: APOE genotype-dependent secretion and expression of apoE in astrocytes and microglia after inflammation

Authors: ***M. LANFRANCO**[1], J. SEPULVEDA[2], G. KOPETSKY[1], G. W. REBECK[1]; [1]Neurosci., [2]Pharmacol., Georgetown Univ., Washington, DC

Abstract: Neuroinflammation is a common feature in neurodegenerative diseases, characterized by activated glial cells and increased levels of cytokines and chemokines. Apolipoprotein E (apoE) is a protein with immunomodulatory properties, but the effect of inflammation on apoE production and processing is poorly understood. In the brain, apoE is synthesized by astrocytes

and microglia. We isolated both astrocytes and microglia from human *APOE (E2, E3* and *E4)* targeted-replacement mice, collected cells and conditioned media, and analyzed levels and forms of apoE. We found that astrocytes secrete two species of apoE, and that cellular apoE consists only of one of those species. In contrast to cellular apoE, both forms of secreted astrocytic apoE were glycosylated evidenced by glycoprotein isolation, and by a convergence to a single form after enzymatic removal of the glycans. Microglia release only a single species of apoE, and cellular apoE consists of two forms. Secreted apoE and only one of the two forms of cellular apoE were glycosylated. We tested whether the levels of forms of apoE were affected by inflammation, using either endogenous (TNFα) or exogenous (LPS) pro-inflammatory stimuli. While LPS had no effect on astrocytic apoE, *APOE2* and *APOE3* microglia increased the release of apoE; *APOE4* microglia showed no effect. *APOE4* microglia showed higher baseline secretion of TNFα compared to *APOE2* and *APOE3* microglia. Treatment with TNFα reduced the secretion and cellular expression of apoE only in *APOE4* astrocytes. The patterns of apoE species produced by astrocytes and microglia were not affected by inflammation. No detectable changes in *APOE* mRNA was observed in astrocytes after both treatments. Together, our data support a model by which astrocytic and microglial apoE secretion and expression are affected by inflammation in an *APOE* genotype-dependent manner, such that *APOE4* astrocytes and microglia are deficient in immunomodulation compared to *APOE2* and *APOE3*, leading to excessive inflammation in response to stimuli.

Disclosures: M. Lanfranco: None. **J. Sepulveda:** None. **G. Kopetsky:** None. **G.W. Rebeck:** None.

Digital Abstract Session

P092. Alzheimer's Disease: Neuroinflammation in Vitro Models

Program #/Poster #: P092.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Generation of iPSC-derived TREM2 R47H and isogenic TREM2 Loss of Function (LOF) microglia to investigate Alzheimer’s disease risk

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Abstract: Recent Genome-wide association studies (GWAS) in Alzheimer’s disease (AD) have uncovered a number of single nucleotide polymorphisms (SNPs) or genetic variants within genes predominantly expressed in microglia in the CNS (TREM2, CD33, TYROBP, PLCG2). Microglia are the resident immune cell of the brain, and numerous studies have linked genetic variants of triggering receptor expressed on myeloid cells 2 (TREM2), including the R47H variant, with impairment of microglia function affecting ligand binding/sensing, phagocytosis, metabolism, and inflammatory responses. Since primary human microglia from living donors are not accessible for research, we generated microglia from episomally reprogrammed human

induced pluripotent stem cells (iPSCs) from donors harboring either WT TREM2 or the R47H mutation. In addition, end-stage microglia were generated from both heterozygous (TREM2 HT) and homozygous (TREM2 HO) TREM2 KO gene edited isogenic iPSCs derived from an apparently healthy normal (AHN) donor. To generate a large batch of cryopreserved end-stage microglia, iPSCs were first differentiated to hematopoietic progenitor cells (HPCs) and purified by MACS of $CD34^+$ cells (>90%) along with CD43, CD45, and CD235/CD31 coexpression. Next, HPCs were differentiated to pure end-stage microglia and cryopreserved. Upon thaw, the microglia (i) retained a high purity based on CD45, CD11b, CD33, P2RY12, TREM2, CX3CR1, and IBA-1 expression, (ii) retained the ability to be polarized towards an inflammatory or anti-inflammatory subtype by specific stimuli, and (iii) are indistinguishable from freshly differentiated microglia. This panel of iPSC-derived microglia expressing WT TREM2, TREM2 R47H, TREM2 HT, TREM2 HO was used to understand the role of TREM2 R47H or loss of function (LOF) of TREM2 in isogenic microglia on phagocytic function, cytokine response and whole transcriptome gene expression for predictive *in vitro* disease modeling applications. This unique tool set of microglia is useful for interrogating the role of TREM2 function and for identification of compounds that restore TREM2 function in high throughput screening assays. Thus, both patient-derived TREM2 R47H and isogenic variants of TREM2 LOF microglia are valuable platforms for investigating TREM2's role in AD pathogenesis.

Disclosures: **W.W. Poon:** A. Employment/Salary (full or part-time):; FUJIFILM CDI. **C. Munn:** A. Employment/Salary (full or part-time):; FUJIFILM CDI. **S. Burton:** A. Employment/Salary (full or part-time):; FUJIFILM CDI. **I. Gurevich:** A. Employment/Salary (full or part-time):; FUJIFILM CDI. **M. Goedland:** A. Employment/Salary (full or part-time):; FUJIFILM CDI. **M. McLachlan:** A. Employment/Salary (full or part-time):; FUJIFILM CDI. **M. Xong:** A. Employment/Salary (full or part-time):; FUJIFILM CDI. **D. Rajesh:** A. Employment/Salary (full or part-time):; FUJIFILM CDI. **S. Hilcove:** A. Employment/Salary (full or part-time):; FUJIFILM CDI.

Digital Abstract Session

P092. Alzheimer's Disease: Neuroinflammation in Vitro Models

Program #/Poster #: P092.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant - AG067781
NIA Grant - AG045571
NINDS Grant - NS104160
NINDS Grant - AG13854

Title: Cultured Microglia from Cognitive SuperAgers Show High Rates of Proliferation

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Abstract: Cognitive SuperAgers are individuals 80 years of age or older, with episodic memory performance that is equal or better than those of individuals 20-30 years their junior. The Northwestern University SuperAging project was established to identify cognitive, behavioral, anatomic, pathologic, molecular, cellular and neurochemical factors that contribute to the SuperAging phenotype and distinguish these individuals from their cognitively normal peers. Accordingly, studies of SuperAgers have identified a number of signatures that distinguish them from cognitively normal elderly, including thicker cerebral cortices, lower Alzheimer's disease pathology, increased density of von Economo neurons in the anterior cingulate cortex, and greater preservation of cortical cholinergic innervation. Given the recent surge of evidence indicating a central role for microglia function in the pathobiology of cognitive decline, we sought to determine if microglia cultured from brains of SuperAgers display different characteristics when compared with those from cognitively normal elderly. Fresh autopsied prefrontal cortical tissue from 9 SuperAgers and 10 cognitively normal elderly were used in these experiments. Microglia cultures were prepared from whole cell suspensions in the presence of microglia medium (SienCell, Inc), supplemented with 5% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 ml/500ml primocin, 1%microglia growth supplement (ScienCell), and 10 ng/ml GM-CSF (Sigma-Aldrich). Cultures were passaged after they were 70% confluent and until growth had significantly slowed or stopped. Cultured microglia from all participants were viable and continued proliferation at least up to passage 7-10. The average time, in days, to reach confluence was considerably shorter in microglia cultures from SuperAgers when compared with cognitively normal controls (7.1 ± 1.7 days in SuperAgers vs 12.3 ± 0.76 days in cognitively normal controls; $p<0.03$). Moreover, the highest passage reached among all participants was passage 21, which was achieved in the SuperAger group. These observations point to significant enhancement of the proliferative capacity of microglia in SuperAgers, which may relate to the protective functions of these cells in the brain. The precise mechanisms that contribute to differences in microglia proliferation in SuperAgers remain to be elucidated.

Disclosures: E. Taefi: None. **I. Ayala:** None. **T. Gefen:** None. **M.E. Flanagan:** None. **E. Rogalski:** None. **M.M. Mesulam:** None. **C. Geula:** None.

Digital Abstract Session

P092. Alzheimer's Disease: Neuroinflammation in Vitro Models

Program #/Poster #: P092.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS104160
NIH Grant AG13854

Title: Characterized Adult Primary Human Microglia Cells for Research

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Abstract: Microglia have diverse functions in the healthy and diseased brain. Much of what has been learned regarding microglia biology is based on *in vitro* studies the overwhelming majority of which used cells isolated from the rodent brain. However, higher anatomical and functional complexity of the human brain and species differences in microglia response and function make imperative the use of human microglia to ascertain that the results are applicable to man. Investigation of microglia function in the adult brain, in which many inflammatory and protective microglia responses occur, requires use of human microglia from adult brains. Microglia cultured from embryonic human brain show substantial proliferative capacity. However, while methods for isolation of microglia from adult postmortem human brains had been described, they allowed only use of a limited quantity of microglia isolated and cultured from each case due to low levels of proliferation. We have developed a method that allows culturing microglia from adult postmortem human brains to high passage and have found that such cells maintain their phenotype in high passage cultures. Briefly, microglia cultures were prepared from whole cell suspensions in the presence of microglia medium (SienCell, Inc), supplemented with 5% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 ml/500ml primocin, 1%microglia growth supplement (ScienCell), and 10 ng/ml GM-CSF (Sigma-Aldrich). Microglia maintained their phenotype in culture as evidenced by uptake of acetylated low density lipoprotein, a marker of scavenger receptors, presence of CD68 immunoreactivity, and production of reactive oxygen species in response to fibrillary amyloid-β peptide. We have built a library of primary adult human microglia cells of various passages from young and aged postmortem brains and from brains of individuals with various neurodegenerative disorders. The goal of the current project is to continue to culture and characterize adult primary human microglia cells of various passages from normal and diseased human brains and to share them with researchers nationally and internationally. High yield and well characterized adult human microglia cultures from our library will allow mechanistic *in vitro* studies with cells from the same case used in different experimental conditions. Availability of large numbers of cells would also allow initial testing of drugs that can regulate microglial functions and permit isolation of large amounts of RNA and/or protein for transcriptomic or proteomic studies. To obtain primary adult human microglia for your research, please contact Changiz Geula at c-geula@northwestern.edu.

Disclosures: C. Geula: None. **E. Sabeti:** None. **A. Bahrami:** None. **I. Ayala:** None.

Digital Abstract Session

P092. Alzheimer's Disease: Neuroinflammation in Vitro Models

Program #/Poster #: P092.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NEI grant EY005121 (NGB)

Eye, Ear, Nose, Throat Foundation (NGB)

Title: Novel neuroprotective Elovanoids (ELVs) modulate Tau hyperphosphorylation (Tau-HP), microtubule-associated protein tau (MAPT) missorting, and neuronal integrity in a cellular model of Alzheimer's disease.

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Abstract: *Rationale:* AD involves accumulation of amyloid-β, Tau hyperphosphorylation (Tau-HP), neuroinflammation and homeostasis perturbations. In this project, we tested a new conceptual framework to understand early events in the pathophysiology of AD. It focuses on the novel pro-homeostatic, neuroprotective lipid mediators (LMs) - elovanoids (ELVs) and its targeting of events that counteract neuroinflammation, in human neuronal cells in primary culture as well as a rodent cellular model of AD. Our lab in 2017 reported the finding that ELVs are novel LMs endogenously produced in the CNS and derived from 32:6n3 and 34:6n3. In the 5XFAD model, we recently demonstrated altered pro-homeostatic lipid signalling, including downregulation of Neuroprotectin D1 (NPD1) and ELVs early in the retina, preceding photoreceptor cell loss. This project brings novel LMs as key in the initiation and early progression of AD, senescence gene programming (SASP) and microtubule associated protein Tau (MAPT). Hyperphosphorylated forms of MAPT are constituent of neurofibrillary tangles (NFT), a histopathological hallmark of AD and appear in dendrites, “somatodendritic missorting.” *Methods:* Primary hippocampal cultures were exposed to Oaβ (10μM) and treated with ELVs or other LMs. Tau-HP, missorting was assessed by confocal microscopy. Images were acquired using Olympus Fluoview 3000 confocal microscope at a size 512x512 pixels. Z section images were taken, with focal plane +/- 20 micrometers captured. Images were captured from random sites in a well. Using Olympus Multi-Area Time Lapse (MATL) function, wells were duplicated, resulting in identical locations imaged in each well of a standard 12 well cell culture plate (Corning). Images were quantified in an unbiassed manner using Bitplane Imaris software 9.5.1. The Intensity Mean function of Imaris software was used to assess the signal intensity of captured images using surfaces parameter. Signal intensity data was statistically analyzed using GraphPad Prism software 8.4.3. Protein abundance of phosphorylated tau residues p-Tau-Thr181, p-Tau-Thr217, p-Tau-Thr231 and (pSer202/pThr205) (AT8) was checked by Western Blotting (WB). *Results/Conclusions:* Confocal imaging demonstrates a significant difference ($p<0.0001$) in signal intensity of AT8, Thr231 from wells exposed to Oaβ in comparison to Oaβ+ELVs/NPD1 at 250 nM - 1μM. WB results show significant upregulation of Tau-HP at AT8, p-Tau-Thr181, p-Tau-Thr217, p-Tau-Thr231 which is abrogated by LMs. ELVs arrest senescence gene programming expression, including SASP secretome. ELVs and NPD1 reduce Oaβ-induced MAPT hyperphosphorylation.

Disclosures: S. Bhattacharjee: None. **P.K. Mukherjee:** None. **A.J. Ledet:** None. **C. Polk:** None. **N. Desai:** None. **N.A. Petasis:** None. **N.G. Bazan:** None.

Digital Abstract Session

P093. Altered Energy Homeostasis in Alzheimer's Disease

Program #/Poster #: P093.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Instituto Nacional de Pediatría

Title: Metabolic syndrome effect on olfactory function, memory and neurodegeneration

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder distinguished by an advancing cognitive decline. Although memory loss is evident, olfactory dysfunction can arise years before any cognitive symptoms. Metabolic syndrome (MS) is a significant risk factor for AD development. Insulin resistance and oxidative stress, two core MS dysfunctions, promote the development of neuritic plaques that characterize AD. However, whether these alterations participate in early olfactory dysfunction remains unknown. We investigated cerebral insulin resistance and oxidative stress induced by a model of metabolic syndrome without alterations in glucose metabolism. For this we used Wistar rats, with 250-300 g body weight on arrival. They were randomly divided in two groups: 1. a control group (n=3-4) that received standard chow plus water and 2. the MS group (n=4-5) that received standard chow plus 30 % sucrose in their drinking water. Both groups were kept on their respective diet for 23 weeks and 3 independent replications were done (30 rats in total). Olfactory and memory function were evaluated using behavioral tests. Insulin pathway activation and amyloid precursor protein (APP) expression were assessed with western blots. Lipid peroxidation was evaluated by the determination of malondialdehyde levels. Neurodegeneration was assessed by measuring the fluorescence intensity of Fluro-Jade-C. All these techniques were performed in the olfactory bulb and hippocampus. Rats receiving the high-carbohydrate diet had olfactory dysfunction, but interestingly memory appeared to be better. Systemic hyperinsulinemia hyper-activated the insulin pathway and APP expression was reduced in the hippocampus. These effects were not present in the olfactory bulb, insulin activation and APP expression were similar in both groups. Malondialdehyde, a lipid peroxidation marker, was reduced in the hippocampus but remained unchanged in the olfactory bulb. We did not find signs of neurodegeneration in these tissues. Insulin cascade stimulation, reduced APP expression and malondialdehyde levels suggest that compensatory mechanisms likely occurr in the hippocampus. We propose that the olfactory bulb has early insulin resistance, accounting for the olfactory impairment. We provide novel information about olfactory dysfunction in a model of MS induced by high carbohydrate. Insulin pathway impairment and accumulation of amyloid beta in the olfactory bulb merit further research.

Disclosures: **B. Phillips-Farfan:** None. **P.I. Clavel Pérez:** None. **K.G. Carvajal Aguilera:** None.

Digital Abstract Session

P093. Altered Energy Homeostasis in Alzheimer's Disease

Program #/Poster #: P093.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH PPG P01AG014930

Title: An in vitro model of neurogenesis using iPSC derived neural stem cells and neurons to study the interaction of metabolism and differentiation

Authors: ***A. C. CROSS**[1], E. IVANOVA[1,2], H. CHEN[1], B. T. SAGDULLAEV[1,3], G. E. GIBSON[1,2];
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Abstract: Neurogenesis, the conversion of neural stem cells to neurons, declines in Alzheimer's disease (AD), and the mechanisms are unknown. In cancer cells and in other cell types, mitochondria and metabolism change dramatically with differentiation. Indeed, differentiation can be modulated by manipulating mitochondria. Since reduced metabolism always accompanies AD, we hypothesize that the decline in neurogenesis with AD is caused by diminished metabolism and altered mitochondrial function. Differentiation from neural stem cells to neurons is accompanied by a "glycolytic switch," and the cell's energy production shifts the cells from depending primarily on glycolysis to utilizing oxidative phosphorylation. This shift has downstream effects on the differentiation of stem cells to neurons. The link between metabolism and neurogenesis is unknown. We have focused on the key mitochondrial enzyme complex, the alpha-ketoglutarate dehydrogenase complex (KGDHC), which is often rate limiting in the regulation of the tricarboxylic acid cycle. The reduction of KGDHC has a surprisingly large number of consequences, and we postulate that many of its actions are related to KGDHC mediated post-translational modifications. The second enzyme of the KGDHC is a succinyl-transferase (DLST). We have shown that altered metabolism changes the subcellular distribution of DLST, and DLST regulates the lysine succinylation of hundreds of proteins in the mitochondria and cytosol. We have not yet studied nuclear succinylation. To determine the role of KGDHC and succinylation in neurogenesis, we established a model of *in vitro* neurogenesis using cells derived from induced pluripotent stem cells (iPSC), which were derived from fibroblasts. To verify this model, we quantified the temporal pattern of protein and RNA markers that change during normal neurogenesis (SOX1, Nestin, ASCL1, Doublecortin, and Calbindin). Exploratory experiments examined changes in the levels and subcellular localization of succinylation and DLST during early neurogenesis. When compared to neural stem cells, immature neurons show a decrease in nuclear succinylation and an increase in mitochondrial succinylation. There is also a slight, not statistically significant increase in DLST staining in the

mitochondria and nuclei of immature neurons, as compared to neural stem cells, which agrees with the well-established increase in KGDHC in brain with maturation. The experiments demonstrate that iPSC derived neural stem cells and neurons are a valid model to explore the interaction of succinylation, mitochondria and differentiation.

Disclosures: A.C. Cross: None. **E. Ivanova:** None. **H. Chen:** None. **B.T. Sagdullaev:** None. **G.E. Gibson:** None.

Digital Abstract Session

P093. Altered Energy Homeostasis in Alzheimer's Disease

Program #/Poster #: P093.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JSPS KAKENHI (JP26282026)
JSPS KAKENHI (JP17K19951)
JSPS KAKENHI (JP17H02188)

Title: Type2 diabetes-induced cognitive deterioration in AD is associated with IRS modifications but not with amyloid beta accumulation

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Abstract: Type2 diabetes (T2DM) is associated with an increased risk for Alzheimer's disease (AD), suggesting that insulin signaling that regulates glucose metabolism may be involved in the development of AD. Our recent studies show that alterations in the hippocampal insulin receptor substrates (IRSs) signaling are associated with cognitive decline in T2DM model mice and middle-aged APP knockin (APP KI) mice for AD model. However, the effect of T2DM on APP KI mice and the changes of hippocampal IRSs signaling in T2DM-induced APPKI mice remain unknown. We found that T2DM deteriorates memory impairment, decline in neurogenesis, and modification in IRSs in middle-aged APP KI mice, while T2DM has no effect on amyloid β(Aβ) accumulation and microglial activation. On the other hand, T2DM-induced glucose intolerance is suppressed in middle-aged APP KI mice. These results suggest that specific modifications in IRSs maybe related to the development and progression of memory impairment caused by AD with/without T2DM and T2DM-induced cognitive dysfunction may occur independent of Aβ accumulation and impaired systemic glucose metabolism.

Disclosures: D. Tanokashira: None. **W. Wang:** None. **M. Maruyama:** None. **C. Kuroiwa:** None. **T. Saito:** None. **T. Saido:** None. **A. Taguchi:** None.

Digital Abstract Session

P093. Altered Energy Homeostasis in Alzheimer's Disease

Program #/Poster #: P093.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG059762
NIGMS 5T32GM008541-22
Harold and Margaret Southerland Alzheimer's Research Fund

Title: Splicing landscape of ApoER2 in Alzheimer's disease, mapped by single molecule, long read RNA sequencing

Authors: *C. M. GALLO[1,3], A. T. LABADORF[2,4], U. BEFFERT[3], A. HO[3,1];
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Abstract: Age and the ε4 allele of apolipoprotein E (apoE) are the two greatest risk factors for developing Late Onset Alzheimer's Disease (LOAD). As there is no known cure for or cause of LOAD, understanding the mechanism underlying known risk factors is paramount for moving therapeutic research forward. ApoE mediates its physiological effects in the brain by binding to cognate receptors like apoER2. Using long read single molecule RNA sequencing, we have identified over 40 isoforms of apoER2 in the human cerebral cortex indicating vast diversity at the level of apoE receptors. This diversity is reflected at the protein level, as multiple apoER2 isoforms have already been shown to have distinct functions in the brain and majority of splicing events remove or add unique functional domains. Recent studies have discovered a novel link between aging, neurodegeneration and alternative splicing. Interestingly, alternative splicing of the apoE receptor apoER2 is altered in Alzheimer's disease (AD). Therefore, abnormal alternative splicing in aging and neurodegeneration may alter the interactome of the key AD risk factor APOE through apoER2, influencing APOE physiological function and highlighting a new scientific paradigm through which to investigate the role of APOE in LOAD. Based on this rationale, we hypothesize that apoER2 isoform balance is progressively perturbed in AD contributing to synaptic dysfunction. To characterize apoER2 isoform distribution in the AD hippocampus, we have utilized long read single molecule RNA sequencing to generate novel apoER2 transcript maps from three female control patients and three female Braak Stage IV LOAD patients. Our data indicates common isoforms amongst control and AD patients and highlights several isoforms of interest for quantitative follow-up including a potentially novel 3' splicing event. To ensure experimental rigor and analyze apoER2 splicing trends quantitatively, we plan to interrogate apoER2 splicing patterns using qPCR across over 200 control and AD tissue samples in the hippocampus, as well as the prefrontal cortex, parietal cortex and control region cerebellum. Our findings suggest a novel scientific model through which to view conferred APOE risk in AD: altered splicing of the cognate receptor apoER2.

Disclosures: **C.M. Gallo:** None. **A.T. Labadorf:** None. **U. Beffert:** None. **A. Ho:** None.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DST Inspire
CSIR, Govt of India

Title: Tissue inhibitor of matrix metalloproteinase-1 modulates Akt phosphorylation in promoting neuronal survival and in ameliorating cognitive functions in 5xFAD mouse

Authors: S. SARKAR, *R. PAIDI, ***S. C. BISWAS**;
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Abstract: Alzheimer's disease (AD), a progressive neurological disorder develops over several decades with reactive astrocytes displaying major functional dichotomy across disease stages. Reactive astrocytes are characterized by dramatic transcriptional, morphological and biochemical changes and secrete several cytokines dictating their role in regulating neuronal health in neurological diseases including AD. Recently, we have shown that tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) is rapidly secreted by reactive astrocytes as early as 6 hours in response to synthetic oligomeric $A\beta_{1-42}$ (Aβ) treatment in primary culture and promotes neuronal survival in primary rat cortical neurons. Moreover, intracerebroventricular TIMP-1 infusion in Aβ-injected rat arrests apoptotic cell death, reduces Aβ load and ameliorates cognitive functions. However, the underlying signaling pathway/s responsible for TIMP-1's neuroprotective functions or how TIMP-1 binds to neuron was unclear, especially in a more biologically relevant AD model. In our present work we show that TIMP-1 can activate the serine-threonine kinase, Akt by phosphorylating at serine 473, a major regulator of both autophagic and apoptotic pathways in 5xFAD mice model of AD and downregulates the apoptotic pathway while upregulating autophagy flux, in cortex and hippocampus as seen by western blot and immunofluorescence analyses. Intracerebroventricular TIMP-1 infusion ameliorated cognitive behaviours assessed by fear conditioning, passive avoidance, elevated plus maze tests etc. in 6-month old 5xFAD mice. Improvement in cognition may be attributed to the underlying recovery in their synaptic deficits. Indeed, recombinant TIMP-1 treatment improved synaptic health by inducing expression of pre- and post-synaptic proteins SNAP-25 and PSD-95 in hippocampus and cortex of 5xFAD mice assessed by western blotting and immunohistochemistry. However, we noted that TIMP-1 mediated phosphorylation of Akt at threonine 308, different from the earlier site, which further inactivates the apoptogenic protein glycogen synthase kinase-3β playing a pivotal role in regulating synaptic plasticity. Importantly, we emphasize that TIMP-1 mediates its neuroprotective role in a matrix metalloproteinase-9 independent manner by binding through CD63 receptor on neuronal surface. Thus, TIMP-1 binds to CD63 on neurons activating Akt by phosphorylating it at distinct sites and driving TIMP-1's neuroprotective role in models of AD. TIMP-1's ability to induce recovery in major cognitive deficits in an advanced AD transgenic model projects it as a major candidate in cytokine-mediated therapy of AD.

Disclosures: **S. Sarkar:** None. **R. Paidi:** None. **S.C. Biswas:** None.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1 AG060057

Title: A Novel Inducible Complement C3 Conditional Global Knockout Mouse Model to Investigate Neurodegeneration in Alzheimer's disease

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Abstract: The complement pathway, part of the innate immune system, is involved in the elimination of dead cells, and pathogens, and as well as brain wiring during development. Complement protein C3, a central component of the complement pathway, participates in eliminating synapses and is elevated with aging and neurodegenerative diseases, including Alzheimer's Disease. Although we previously showed that lifelong, germline C3-deficiency protected aged male wild-type and APPswe/PS1dE9 mice against hippocampal neuronal dysfunction and cognitive decline, despite increasing the Aβ plaque load, it remains unknown whether suppressing complement C3 signaling during early stages of AD pathogenesis, when relevant therapeutic interventions might be considered, can confer neuroprotection. Therefore, we generated a C3 floxed (C3fl/fl) mouse line, and crossed it to an inducible, global Cre line (Rosa26-Cre-ERT2+/-) for 2 generations to generate novel inducible C3 conditional knockout C3fl/fl; Rosa26-Cre-ERT2+/- (C3iKO) mice on a C57BL/6J background. Mice aged 2-3 months received intraperitoneal injections of either corn oil or tamoxifen (75 mg/kg) once a day for 5 consecutive days. We analyzed the C3 serum levels at 7, 14, 30, 60, 90, 120, 150, 180 and 210 days post-tamoxifen treatment. We also analyzed the mRNA expression of complement proteins and receptors in the brain and liver 60 days and 150 days following tamoxifen treatment. C3iKO mice had a significant 85-90% reduction in serum C3 levels compared to controls, which was consistent at all timepoints analyzed. C3 mRNA expression in the liver, the main source of complement proteins, as well as in the brain was significantly reduced, demonstrating effective recombinase activity in these organs. We confirmed in the brain that C3 protein levels remain suppressed at 60 and 150 days after tamoxifen treatment. Surprisingly, C1q mRNA expression was increased in the brain and liver at 60 days but not at 150 days following tamoxifen treatment. None of the other complement component/receptor mRNAs were altered. Some brain homeostatic genes which include TMEM-119, P2ry12; Cx3cr1 and TGFβR1 were not altered.

Interestingly, we observed a significant reduction in TGF-β mRNA expression only on day 150. Further studies are underway. In conclusion, we present a novel mouse model, C3iKO, in which tamoxifen treatment resulted in sustained lowering of C3 in the serum, liver, and brain. We will next cross this model with AD-like mouse models of amyloidosis and tau pathology to evaluate whether global C3 lowering in early stages AD pathogenesis is protective and if so, our data would support targeting complement as a therapy for AD.

Disclosures: **A.F. Batista:** None. **M. Schroeder:** None. **K. Khan:** None. **J. Presumey:** None. **E. Yalcin:** None. **M. Carroll:** None. **C.A. Lemere:** None.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DFG German Research Foundation
BrightFocus Foundation
National Institutes of Health AG049952
National Institutes of Health. NS108472
German National Academic Scholarship Foundation

Title: Inhibition of peripheral VEGF signaling rapidly reduces leucocyte obstructions in brain capillaries and improves cortical blood flow in an Alzheimers disease mouse model

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Abstract: Inhibition of peripheral VEGF-A signaling rapidly reduces leucocyte obstructions in brain capillaries and improves cortical blood flow in an Alzheimer's disease mouse model
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We recently found that increased obstructions in capillaries caused by adhesion of leucocytes in the cortical microvascular leads to a ~20% reduction of cerebral blood flow (CBF) in mouse models of Alzheimer's disease. Here, we explore the contribution of peripheral Vascular Endothelial Growth Factor (VEGF-A) signaling at the luminal side of the brain microvasculature as a driver of capillary stalling and blood-brain barrier (BBB) integrity in the APP/PS1 mouse model of AD. The number of stalled cortical capillaries and blood flow speeds were measured in cortical capillaries using high resolution in vivo two-photon imaging, before and after one hour

and two weeks of i.p. anti-VEGF-A164 antibody injection. Capillary stalls were measured using the citizen science platform Stall Catchers. The blood-brain barrier integrity was analyzed using immunohistochemistry. We found that inhibition of VEGF-A signaling in APP/PS1 mice reduces capillary stalling by ~75% and improves average capillary speed by ~50%. In contrast, no such effects were seen in wild-type saline-injected controls. The anti-VEGF164 injection also reduced overall eNOS protein concentrations and increased the tight junction protein levels of occludin. Intriguingly, the few APP/PS1 capillaries that were still prone to obstructions after anti-VEGF-A treatment had lower occludin concentrations between their endothelial cells than flowing capillaries. We further demonstrate that capillary stalling reductions and capillary speed improvements occur within an hour of intraperitoneal anti-mouseVEGF164 injection. At the same time scale, anti-VEGF-A treatment reduced Evan's blue diffusion across the BBB.This data demonstrates that peripheral inhibition of VEGF-A signaling in APP/PS1 mice restores aberrant eNOS/occludin-associated BBB permeability, and is associated with decreased capillary stalls and increased CBF.

Disclosures: **O. Bracko:** None. **M. Ali:** None. **C.B. Schaffer:** None. **N. Nozomi:** None. **K. Falkenhain:** None. **M. Haft-Javaherian:** None. **M. Murtaza-Ali:** None. **B.N. N. Njiru:** None.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG049952
NIH NS108472

Title: Oxidative stress contributes to capillary stalling and cerebral blood flow reductions in the APP/PS1 mouse model of Alzheimer's disease

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Abstract: Cerebral blood flow (CBF) is decreased by ~30% in both patients and animal models of Alzheimer's disease (AD). Previously, we identified that about 2% of cortical capillaries in the APP/PS1 mouse model of AD had stalled blood flow due to white blood cell arrest in the capillary segment. In comparison, only 0.4% of capillaries were stalled in wild type controls. Antibodies against the neutrophil surface protein Ly6G reduced the incidence of capillary stalls, leading to increased CBF and improved short-term memory. Here, we report on the upstream molecular mechanisms contributing to increased neutrophil arrest, focusing on oxidative stress pathways that have previously been implicated in endothelial dysfunction in mouse models of

AD, including loss of neurovascular regulation. We inhibited NOX2-containing NADPH-oxidase, a reactive oxygen species producing enzyme shown to be activated in AD, in 10-11 month old APP/PS1 mice for two weeks. We found that, in APP/PS1 mice, the fraction of capillaries with stalled blood flow was decreased by 67%, CBF was increased by 29%, and performance on short-term memory tasks was improved. Enzyme-linked immunosorbent assays showed no significant changes in amyloid-beta monomeric species, and the overall plaque load was not affected. NOX2 inhibition was also associated with a decrease in blood brain barrier dysfunction and neurovascular inflammation. This study implicates the NOX2 pathway as a molecular mechanism contributing to capillary stalling and CBF reductions in a mouse model of AD and could represent a molecular pathway with therapeutic potential for AD.

Disclosures: **N.E. Ruiz-Uribe:** None. **O. Bracko:** None. **M. Swallow:** None. **M. Ali:** None. **B. Njiru:** None. **K. Falkenhain:** None. **H. Chang:** None. **L. Park:** None. **N. Nishimura:** None. **C.B. Schaffer:** None. **C. Iadecola:** None.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Field Neurosciences Institute, Saginaw, MI
John G. Kulhavi Professorship in Neuroscience, Central Michigan University

Title: Hm15211 can reduce spatial memory deficits, reverse neuroinflammation and restore insulin signaling pathway in aged triple transgenic mouse model of alzheimer's disease.

Authors: *L. PALADUGU, P. MAITI, Z. BOWERS, J. ROSSIGNOL, G. DUNBAR; Neurosci., Central Michigan Univ., Mount Pleasant, MI

Abstract: Despite extensive research on potential treatments for Alzheimer’s disease (AD), no effective therapy has emerged. Previous studies have shown a strong link between AD and type 2 diabetes mellitus (T2DM) to an extent that AD has been called type 3 diabetes mellitus. In this context, a promising approach is to repurpose anti-diabetic drugs as a potential way to treat the pathological hallmarks of the disease and restore cognitive function. Apart from the traditional anti-diabetic drugs, like metformin and insulin, incretin analogues, like liraglutide, a glucagon-like peptide-1 (GLP-1) and peptides that have anti-diabetic effects, like gastric inhibitory peptide (GIP), have shown potential for reversing the cognitive dysfunction and ameliorating the effects of AD. In the present study we tested the efficacy of a triple receptor agonist (TA), in aged triple transgenic (3xTg) mice, that carry transgenes for AD. TA activates GLP-1, GIP and glucagon (Gcg) receptors at the same time in the brain. Fourteen-month old 3xTg mice and their WT counterparts were treated with 10- and 25- nM of TA or saline vehicle with daily intra-peritoneal injections (i.p) for thirty days. Following a battery of behavioral tests including open field, novel object recognition, and Morris water maze, several neuropathological parameters, including

markers for neurodegeneration, neuroinflammation, insulin signaling, tau, and amyloid beta (Aβ) were studied in both cortical and hippocampal regions of the brain via immunohistochemical and Western Blot studies. Our results indicated that treatment with TA significantly reversed memory deficits in the Morris water maze task. Western Blot analysis indicated that the drug reduced the levels of type 1 and type 2 macrophages in the brain and normalized the expression of proteins associated with insulin signaling pathway in the brain. Moreover, TA also reduced the expression of phosphorylated tau in both cortical and hippocampal regions of the brain. Collectively, our results suggest that TA can be a promising drug to restore the disrupted insulin signaling pathway and reduce memory deficits and reduce neuroinflammation in AD.

Disclosures: L. Paladugu: None. **Z. Bowers:** None. **J. Rossignol:** None. **G. Dunbar:** None. **P. Maiti:** None.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ERC 741744
ISF 991/16
ISF 1354/15
Thompson and Adelis foundations
Ministry of Science and Technology of Israel

Title: Scientific Abstract--Membership being added to reg

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Abstract: Immune gateway to repair the brain – A role for Interferon-γ-Receptor at the choroid plexus in a mouse model of Alzheimer's disease

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A transient blockade of the PD-1/PD-L1 immune checkpoint pathway was shown to improve cognitive performance, and to reduce pathology in Alzheimer's disease (AD) in the mouse. Based on our previous studies, we hypothesized that such a treatment leads to systemic elevation of IFN-γ producing T cells, and thereby to an increase of availability of IFN-γ signaling at the choroid plexus (CP) that needed for supporting trafficking of repairing immune cells to the brain. Here we challenged this hypothesis using a mouse model of 5xFAD bred with IFN-γ-Receptor loxP/loxP mice. At the age of 5.5 months old these mice received a bilaterally

intracerebroventricularly (i.c.v.) TAT-CRE recombinase injection to locally downregulate IFN-γ-Receptor within the CP. Following a 2-week recovery period, mice were given a single injection (i.p.) of either anti-PD-L1 antibody or vehicle. Multi-parameter immune profiling using flow cytometry was performed on spleen, blood and brains 1, 3 or 7 days following anti-PD-L1 injection, while cognitive performance and AD-related neuropathologies were assessed 4-5 weeks following the anti-PD-L1 injection. Our results show that 4 weeks following anti-PD-L1 injection, INF-γ-Receptor flox/flox mice that received vehicle i.c.v. injection showed a robust improvement in cognitive performance as well as a reduction in hippocampal soluble and insoluble β-amyloid levels relative to a matched IgG2b-treated group. TAT-CRE i.c.v. injected mice showed a significant reduction in the response to anti-PD-L1 treatment with respect to cognitive improvement and a complete loss of the beneficial effect on hippocampal β-amyloid levels. Importantly, i.c.v. TAT-CRE injection had no effect on the systemic response to anti-PD-L1 measured by the levels of splenocyte T cells and FOXP3+ regulatory CD4+ T cells in the blood. Downregulation of IFN-γ-Receptor at the CP following TAT-CRE injection was verified using Real-Time PCR. We conclude that these results strengthen our suggested mechanism that the therapeutic effect of immune checkpoint pathway blockade on AD-related pathology involves IFN-γ-dependent signaling at the brain's CP epithelium.

Disclosures: **M. Arad:** None. **J.M. Peralta Ramos:** None. **H. Ben-Yehuda:** None. **G. Castellani:** None. **S. Suzzi:** None. **A. Tsitsou-Kampeli:** None. **M. Schwartz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); M.S. is an inventor of the intellectual property that forms the basis for development of PD-L1 immunotherapy for AD.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: F31 NS115290-01A1
NINDS/NIA R01 NS110749

Title: Sex differences in the impact of prediabetes on multi-etiology dementia

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Abstract: It is estimated that 60-80% of Alzheimer's disease (AD) cases also have underlying vascular contributions to cognitive impairment and dementia (VCID). This overlap of vascular and Alzheimer's pathology is termed multi-etiology dementia (MED). AD and VCID have many mutual risk factors; however, men are more likely to develop VCID and women are more likely

to develop AD. When diabetes is present, this sex difference reverses, with women having a higher risk of VCID (19% greater risk compared to men). The effects of prediabetes, which is three times more common than diabetes, on this sex difference and on MED are unknown. We hypothesize that prediabetes will exacerbate cognitive deficits and pathology in MED, with females having greater deficits. We used a triple transgenic (3xTg-AD) model of AD in which mice develop amyloid and tau pathology and wild type mice (B6129SF1/J; WT) as controls. To model MED, we performed a unilateral common carotid artery occlusion surgery on 3xTg-AD mice, which produces chronic cerebral hypoperfusion. Prediabetes was modeled using a high fat (HF) diet. We have previously shown that 3xTg-AD females suffer greater metabolic consequences of a HF diet than WT females. This did not occur in 3xTg-AD males. To assess cognitive function, we performed Morris water maze (spatial learning and memory), novel object recognition (episodic memory), and nest building (activities of daily living) tests. Episodic memory was impaired by the HF diet in control females but not males, and all dementia groups were impaired by a HF diet regardless of sex. Spatial memory was impaired by HF diet in male and female AD and MED mice. HF diet caused deficits in spatial learning in female dementia groups only. AD and MED females, regardless of diet, had deficits in activities of daily living while the males were unaffected. Using immunohistochemistry and Western blot, we are assessing sex differences in the effects of dementia and HF diet on AD pathology, including amyloid pathology, cerebral microbleeds, and tau/p-tau levels. In conclusion, HF diet resulted in a wider array of adverse cognitive effects in AD and MED females compared to males. These results support the role of prediabetes as a potentially greater dementia risk factor for women.

Disclosures: **O.J. Gannon:** None. **L. Robison:** None. **A. Salinero:** None. **F. Mansour:** None. **D. Riccio:** None. **A. Tyagi:** None. **M. Thomas:** None. **K. Zuloaga:** None.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U01 AG052460
NIH Grant R21 NS10167

Title: Neuroinflammatory signals correlate with age related cognitive decline in 5XFAD (B6SJL) mouse model of Alzheimer’s disease

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Abstract: Alzheimer’s disease (AD) is the third leading cause of death, and the leading cause of dementia in the USA. AD is also associated with substantial medical and societal burden with currently no approved therapy that can clearly alter the disease progression. Decades of research on amyloid and tau pathological markers helped us to elucidate the pathophysiological features

of AD, but they have not yet culminated into disease modifying therapies. Recent findings implicate that neuroinflammatory pathways play a key role in exacerbating AD pathology and ensuing cognitive and behavioral deficits in patients. However, it is not clearly investigated the relative induction of neuroinflammatory markers during the ageing of AD transgenic animal models to design an effective anti-inflammatory therapy. To answer this question, we investigated 5XFAD mouse model, which carries five AD-linked mutations for human APP and PSEN1 genes. 5XFAD line has been maintained on both genetic backgrounds (hybrid B6SJL and congenic C57BL6). Our previous studies using 5XFAD mouse on C57BL6 background indicated that they do not display a robust neuroinflammatory signaling until 5 months, and no behavioral deficits until 9 months of age. Therefore, we investigated the neuroinflammatory and cognitive profiles of 5XFAD mice in B6SJL background longitudinally from 5-9 months. 5XFAD transgenic and non-transgenic littermates in both sexes were tested in Y-maze and Morris water maze (MWM) and then perfused at 5, 7 and 9 months of their age to harvest the brains. A series of neuroinflammatory cytokines, chemokines and astrocytic and microglial markers were analyzed in the brain regions. The Y-maze data showed little difference in working memory in 9 months old mice compared to their younger counterparts while their performance in MWM showed an age dependent decline in spatial memory with maximum impairment at 9 months of their age. mRNA levels of several neuroinflammatory markers indicated that significant changes in the level of proinflammatory cytokines, IL1β, IL6, CCL3, CCL4, immunomodulatory markers including chemokines such as EP2, TREM2, NOX2 and CXCL10, and astrocytic and microglial markers such as GFAP, CD68, IBA1 and CD11b. These data suggest the role of neuroinflammation in associated cognitive decline in AD brains and further downstream molecular pathway analysis will help us to find prudent anti-inflammatory targets for future therapies.

Disclosures: **A. Banik:** None. **T. Ganesh:** None.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Areas of Excellence Scheme of the University Grants Committee (AoE/M-604/16)
the National Key R&D Program of China (2017YFE0190000 and 2018YFE0203600)
the Research Grants Council of Hong Kong (the Collaborative Research Fund [C6003-14G], the Theme-Based Research Scheme [T13-605/18W], and the General Research Fund [HKUST16100418 and HKUST16103017])
the Innovation and Technology Commission (ITCPD/17-9)
the Guangdong Provincial Key S&T Program (2018B030336001)

the Shenzhen Knowledge Innovation Program (JCYJ20180507183642005 and JCYJ20170413173717055)
S.-F.L. and W.W. are recipients of the Hong Kong PhD Fellowship Award.

Title: Il-33 promotes a stepwise functional state transition of microglia to alleviate alzheimer's disease pathology

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Abstract: Impaired clearance activity of microglia results in the accumulation of beta-amyloid (Aβ) deposition and contributes to Alzheimer disease (AD) pathogenesis. While previous studies have demonstrated that modulating cytokine milieus enhances microglial clearance activity in AD, it remains unclear how microglia response to cytokine and regulate their clearance capacity. Here, we demonstrate that interleukin (IL)-33 enhances microglial clearance activity through stimulating a stepwise state transition in AD. Upon IL-33 treatment, microglia undergo stepwise transcriptome programming and lead to the induction of IL-33-responsive microglia (IL-33RM). These IL-33RM exhibit enhanced Aβ phagocytic and clearance activity. Furthermore, our epigenetic profiling reveal that IL-33RM induction is controlled by the remodeling of chromatin accessibility and PU.1 transcription factor binding. Inhibiting PU.1-DNA interaction abolishes the IL-33-induced microglial state transition and Aβ clearance. Thus, we define a IL-33-induced functional state transition in microglia that drives their beneficial functions in AD.

Disclosures: **S. Lau:** None. **W. Wu:** None. **A. Fu:** None. **N.Y. Ip:** None.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: UND VPRED Postdoctoral support
DaCCoTA CTR NIH grant (U54GM128729)
NIH/NIGMS award (P20GM103442)
INBRE (P20GM103442)
NIH/NIGMS award (P20GM113123)
RO1AG048993
RF1AG069378

Title: Splenectomy reduces Aβ plaque load ameliorating the Alzheimer's pathology potentially by increasing beneficial neuro-inflammatory cycle

Authors: *B. SAHU, M. SOHRABI, A. M. FLODEN, G. D. MANOCHA, S. NOOKALA, C. K. COMBS;
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of amyloid-β (Aβ) plaques, neuroinflammation, and neuronal death affecting nearly 5 million individuals in the United States. Unfortunately, current therapeutic options are limited in efficacy requiring additional understanding for disease intervention. Numerous studies support the idea that peripheral immune dysfunction likely influences immune cell phenotype in the brain. As the secondary lymphoid organ for the circulatory system, the spleen is constantly monitoring antigens to modulate both innate and adaptive immune responses. Since splenic control of immune cell phenotype is associated with both pro and anti-inflammatory effects in the brain in paradigms such as stroke and traumatic brain injury, we hypothesized that a similar spleen to brain communication was relevant in AD. To test this idea, splenocytes from 12-14 months old male and female C57BL/6 wild type, APP/PS1, and App^{NL-G-F} mice were analyzed by flow cytometry. Male but not female APP/PS1 and App^{NL-G-F} mice had altered spleen immune cell numbers and phenotype compared to C57BL/6 mice indicating sex selective peripheral immune dysfunction as a consequence of disease. To better understand the contribution of these splenic changes to the brain we removed the spleen in male C57BL/6 wild type, APP/PS1 and App^{NL-G-F} mice. The mice were divided into 3 groups: control, sham surgery, and splenectomy. Mice were sacrificed 2 weeks post-surgery for analysis of brains. Splenectomy led to elevated brain CD68 but not GFAP immunoreactivity and increased concentrations of proinflammatory cytokines, TNF-α, IL-1β, and IL-6. Interestingly, splenectomy also significantly reduced Aβ levels and plaque load in comparison to controls. Overall, our data suggest that splenocyte phenotype directly influences immune status and Aβ levels in the brain.

Disclosures: B. Sahu: None. **M. Sohrabi:** None. **A.M. Floden:** None. **G.D. Manocha:** None. **S. Nookala:** None. **C.K. Combs:** None.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R21AG060430-02S1

Title: A western diet combining high fat, sugar, and salt induces muscular and sensory dysfunctions and neurodegeneration in the aging mice: ameliorative action of Metformin

Authors: *S. HONG[1], A. NAGAYACH[2], Y. LU[2], Q.-V. A. DUONG[2], H. PENG[3], N. B. PHAM[2], C. A. VUONG[2], N. G. BAZAN[2];
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Abstract: Research objective and rationale. The Western diet, which combines high saturated fat, sugar, and salt (HFSS), is negatively linked to human health disorders, including brain dysfunctions that are amplified during aging and likely involved in the onset of age-related neurodegeneration. Our objective is to explore the specific behavioral (muscular and sensory), cellular, and molecular consequences of the HFSS diet on the aged brain using mice in aging. **Methods and results.** Aged male C57BL/6 mice were fed with HFSS, and tested for their muscular and sensory functions. Our study then followed glial activation and associated inflammatory reactions, neurodegeneration, and accumulation of amyloid β (Aβ) and phosphorylated tau (pTau) in brain, using immunohistochemistry and cellular quantification. Significant reductions were observed in the motor-muscle grip strength and sensory behaviors of mice fed a HFSS diet, concomitant with an increase in inflammatory $iNOs^+$ microglia and a decrease in inflammation-resolving reparative $Arg\text{-}1^+$ microglia in the motor and sensory cortex, with simultaneous signs of neurodegeneration. The HFSS diet promotes the deposition of pTau and Aβ around the neurons and blood vessels, respectively, in both the motor and sensory cortexes, indicating the early onset of AD. Metformin (1,1-dimethylbuguanide hydrochloride) administration resulted in neuroprotection in both aging and HFSS-fed mice. **Conclusions.** A HFSS diet damages the brain and behavior of aging mice by promoting the progression of neurodegeneration, whereas metformin counteracts the majority of these effects. The appearance of Alzheimer's disease (AD) pathology following HFSS consumption and the related motor and sensory behavioral alterations disclosed here provide an effective window into the mechanisms involved, while targeting potential new therapeutic avenues. The new animal model described here is valuable for these endeavors. The ameliorating neuroprotective effects of Metformin in aging and HFSS-diet related dysregulation at different ages also require additional approaches and further exploration to better understand the neuroprotective therapeutic effects of Metformin.

Disclosures: S. Hong: None. **A. Nagayach:** None. **Y. Lu:** None. **Q.A. Duong:** None. **H. Peng:** None. **N.B. Pham:** None. **C.A. Vuong:** None. **N.G. Bazan:** None.

Digital Abstract Session

P094. Alzheimer's Disease: Neuroinflammation in Vivo Models

Program #/Poster #: P094.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Weston Brain Institute
Ontario Veterinary College

Title: Effect of continuous treatment of 5α-androstane-3α,17β-diol on ERK signalling and inflammatory protein markers in the 3xTg mouse model of Alzheimer's Disease

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Abstract: Decreased circulating levels of free testosterone have been associated with a significant increase in the risk of developing neurodegenerative diseases such as Alzheimer's Disease (AD). Treatment with testosterone, however, has shown mixed results regarding improvement in age-related cognitive dysfunction. Recent *in vitro* studies have shown that the major neurosteroid metabolite of testosterone - 5α-androstane-3α,17β-diol (3α-diol) significantly reduces extracellular signal-regulated kinase (ERK) phosphorylation associated with a reduction in programmed cell death. The role of 3α diol *in vivo* however, remains unclear. The current study tests the hypothesis that continuous long-term treatment with 3α-diol will reduce the expression of protein markers associated with AD progression in a transgenic mouse model of AD (3xTg). 3-month-old wild-type and 3xTg mice of both sexes were implanted subcutaneously with either a 1 cm long empty silastic capsule as a control, or silastic capsule releasing 3α-diol dipropionate. Brains were collected 3 months later from a subset of animals to analyze for changes in expression of hippocampal levels of phospho-ERK, phospho-tau and glial fibrillary acidic protein (GFAP) by western blotting (n=6). Three-way ANOVA was performed to test for between sex, genotype and treatment effects. Initial results from 6-month-old animals indicate sex-based differences in ERK phosphorylation with males exhibiting greater ERK 1/2 phosphorylation compared to females. No effect of 3α-diol treatment was observed. Analysis of phospho-tau (Ser 202) levels revealed a significant interaction between sex and genotype. Although no treatment effect was observed in levels of GFAP expression, transgenic mice had higher levels of GFAP compared to wild types. Transgenic males implanted with 3α-diol tended to have decreased levels of GFAP compared to sham-implanted controls; but this trend was not statistically significant. Changes in protein expression may become more apparent as the animals reach 1 year of age, when the neuropathology in the 3xTg animals fully develops.

Disclosures: S. Bhullar: None. **N. Kuwahara:** None. **K. Nicholson:** None. **L. Isaacs:** None. **N.J. MacLusky:** None. **B.D. Winters:** None.

Digital Abstract Session

P095. Mechanisms of Synaptic Dysfunction in Alzheimer's Disease – In Vitro Models

Program #/Poster #: P095.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R21 NS109750
R41 NS108895

Title: Axonal Aβ stress induces tau mislocalization and retrograde synaptic defects within a compartmentalized in vitro model system.

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Abstract: Tau protein tangles and brain atrophy are hallmarks of Alzheimer's Disease (AD). An early cellular event in the progression of AD is the mislocalization of pathological tau from the axon to the somatodendritic compartment of affected neurons. Following this mislocalization, pathological tau is released and taken up by adjacent neurons and cells. However, the molecular mechanisms that regulate tau mislocalization and how neuronal function is affected, remains unclear. To test whether exposure of axons to Aβ challenge regulates tau relocalization and leads to synaptic defects, we used microfluidic chambers to isolate hippocampal axons and treated them with Aβ conditioned media (Aβ- CM). Aβ CM is collected from Aβ oligomer-challenged microglia and contains neuroinflammatory factors that induce neuritic tau beading with exposure to non-compartmentalized neurons. Restricted exposure of Aβ CM to axons within compartmentalized chips for 24 h caused a redistribution of tau to the somatodendritic compartment in the absence of neuritic beading. In addition to tau relocalization, we found that exposure of axons to Aβ CM for 24 h was sufficient to cause a significant increase in synaptic vesicle release rate. We quantified synaptic vesicle release rate by using an FM dye based optical method in conjunction with electrical stimulation. Further we found that application of a local activity blockade (tetrodotoxin and low calcium) at the time of treatment largely inhibited the relocalization of tau puncta and synaptic vesicle release rate. These results indicate that tau mislocalization correlates with synaptic defects and these defects are ameliorated by pharmacological activity blockade. Together, our results provide confirmation that tau redistribution can be quantified in our *in vitro* model in advance of cell death, mirroring AD phenotype *in vivo* and providing a unique platform to identify therapeutic targets that may reduce the spread of AD pathology in the brain.

Disclosures: T. Nagendran: None. **J. Tseng:** None. **T.J. Cohen:** None. **A.M. Taylor:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A.M.T. is an inventor of the microfluidic chamber/chip (US 7419822 B2, EPO 1581612, EPA 2719756) and is Chief Scientist & Co-founder of Xona Microfluidics, LLC.

Digital Abstract Session

P096. Mechanisms of Synaptic Dysfunction In Alzheimer's Disease: In Vitro Models

Program #/Poster #: P096.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC 2020-04617

Title: The effects of amyloid beta protein on excitatory transmission in layer II of the entorhinal cortex

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Abstract: In Alzheimer's Disease (AD), neurodegeneration is first found in layer II of the entorhinal cortex (EC; Guo et al. 2010). Neuronal degeneration in the EC likely contributes to memory deficits and cognitive impairments that occur early in AD (Braak & Braak, 1991, 1998; Jahn, 2013; Kocahan & Doğan, 2017). Current hypotheses regarding the early stages of AD suggest this neurodegeneration begins as a result of toxic accumulation of soluble amyloid beta peptides (Aβ; Selkoe and Hardy, 2016). Elevated Aβ levels interfere with typical excitatory synaptic transmission in the hippocampus by facilitating postsynaptic calcium influx into the neurons via NMDA glutamate receptors (Findley et al., 2019; Rudy et al., 2015; Li et al., 2009; Kamenetz et al. 2003; Mucke et al., 2000). Chronically elevated calcium ion levels are thought to be a major factor that contributes to excitotoxicity and eventual cell death in both the EC and hippocampus (Findley et al., 2019; Rudy et al., 2015). Aβ mediated excitotoxicity and the effects of Aβ on excitatory transmission have been studied extensively in the hippocampus. However much less is known about the effects of Aβ on excitatory synaptic transmission in the first site of neurodegeneration, layer II of the EC. We studied the effects of incubating 400 μm horizontal slices of rat EC in 100 nM $A\beta_{(1-42)}$ (n=24) or a dimethylsulfoxide control (n=25) for 45 minutes to 3 hours on excitatory synaptic transmission in layer II EC using field excitatory postsynaptic potentials (fEPSPs) recorded in vitro. We then examined the effects of incubating slices with the addition of 50 μM of D-AP5 to the Aβ incubation (n=13) and the control (n=12). All experiments were blinded. The amplitudes of fEPSPs were increased in slices incubated in Aβ relative to control slices. The facilitation of synaptic responses induced by Aβ was blocked by constant bath application of the NMDA receptor blocker D-AP5, indicating that the activation of NMDA receptors is required for the facilitation of synaptic excitability. The facilitation of synaptic responses induced by Aβ likely results primarily from activation of postsynaptic NMDA receptors that enhance calcium influx, but activation of presynaptic NMDA receptors that enhance transmitter release in the EC may also contribute. These effects of exposure to Aβ may contribute to mechanisms of excitotoxicity that contribute to cognitive decline observed early during the progression of Alzheimer's Disease.

Disclosures: M.E. Suvanto: None. **J. Saragosa:** None. **O. Olajide:** None. **A. Chapman:** None.

Digital Abstract Session

P096. Mechanisms of Synaptic Dysfunction In Alzheimer's Disease: In Vitro Models

Program #/Poster #: P096.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC Grant 2020-04617

Title: Amyloid-beta peptide induces rapid NMDA receptor-dependent alterations at glutamatergic synapses in the entorhinal cortex.

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Abstract: Medial temporal lobe structures including the hippocampus and entorhinal cortex (EC) show early accumulation of amyloid beta peptides (Aβ) and neurofibrillary tangles that are thought to drive synaptic degeneration in Alzheimer's disease (AD). The mechanisms of Aβ-mediated synaptic degeneration have been extensively explored in the hippocampus, but surprisingly little is known about their effects in the EC. We therefore investigated the acute effects of soluble $A\beta_{1-42}$ on the molecular expression of presynaptic and postsynaptic components modulating mechanisms of glutamatergic neurotransmission in the medial and lateral EC (MEC and LEC). Acute EC slices obtained from male Long-Evans rats were exposed to solubilized $A\beta_{1-42}$ peptides (500 nM) or control solution for 3 hours. The dependence of $A\beta_{1-42}$ mediated effects on activation of NMDA glutamate receptors was tested using selective antagonists for GluN2B (Ro25-6981), GluN2A (TCN201), or the broad-spectrum NMDA receptor antagonist APV. Presynaptic effects of $A\beta_{1-42}$ in both MEC and LEC were found to include marked reduction of synaptophysin and increased VGluT2 protein expression. $A\beta_{1-42}$ also induced downregulation of synapsin-2a mRNA, which was accompanied by upregulation of Ca^{2+}-activated channel KCa2.2 mRNA levels, suggesting that dysregulation of Ca^{2+} signaling may play a role in presynaptic degeneration. Protein immunoblots further showed that $A\beta_{1-42}$ induced degeneration of postsynaptic elements PSD95 and GluN2B, although the mRNA expression of postsynaptic scaffolding elements SAP-97 and PICK-1 were unperturbed by $A\beta_{1-42}$-treatment. Also, the protein expression of astrocytes (GFAP) and microglia (IBA1) were not altered in $A\beta_{1-42}$-treated EC slices. However, we found that $A\beta_{1-42}$ induced downregulation of GluN2A and GluN2B mRNA in the EC, while GluN1 mRNA expression was unaffected. Whereas mGluR3 and mGluR5 mRNA expressions were altered by $A\beta_{1-42}$ treatment, the genetic expression of mGluR1 and mGluR8 were unchanged in slices exposed to $A\beta_{1-42}$. Interestingly, selectively blocking either GluN2A or GluN2B -containing subunits of NMDA receptors did not significantly reduce $A\beta_{1-42}$-mediated synaptic degeneration. However, coincubation of slices with both GluN2A and GluN2B blockers blocked $A\beta_{1-42}$-related degeneration of presynaptic and postsynaptic elements, consistent with our results obtained with APV. In summary, our findings indicate that $A\beta_{1-42}$ rapidly modulates synaptic components that mediate glutamatergic synaptic transmission in both the MEC and LEC through mechanisms that may involve simultaneous activation GluN2A and GluN2B containing subunits of NMDA receptors.

Disclosures: C.A. Chapman: None. **O.J. Olajide:** None. **D. Butler:** None.

Digital Abstract Session

P096. Mechanisms of Synaptic Dysfunction In Alzheimer's Disease: In Vitro Models

Program #/Poster #: P096.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AI132414

Title: Role of human cytomegalovirus infection in Alzheimer's Disease pathology

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Abstract: Alzheimer's Disease (AD) is a common, debilitating form of dementia typically characterized by a progressive decline in neuronal function that ultimately results in memory loss, unpredictable behavior, and death. To date, much remains unknown about the underlying mechanisms and potentiating factors of AD. A growing body of literature describes the potential for viral infection to play a role in AD pathogenesis and progression. Specifically, members of the viral family *Herpesviridae* have been found to induce phenotypes like those of AD, such as altered calcium signaling and amyloid beta accumulation. However, studies to investigate the direct role of viral infection in human AD pathology are lacking. Here, we capitalize on the use of human induced pluripotent stem cell (iPSC)-derived organoids generated from AD patients to directly test whether infection with human cytomegalovirus (HCMV) can worsen AD pathology. Cortical organoids are generated from a familial AD patient harboring a Presenilin-2 (PSEN2) mutation. Simultaneously, control organoids are produced from an iPSC line with no apparent AD-related mutations. We either infect organoids with the clinical HCMV strain (TB40/E-eGFP variant) or mock treat at day 30 (D30) of differentiation, with subsequent collection at D60. Samples are then processed for RNA sequencing, protein analysis, and RT-qPCR. Early data demonstrate that AD organoids exhibit increased soluble amyloid beta burden compared to control organoids, which we hypothesize will increase with HCMV infection. Also, RNA sequencing data from control organoids infected with HCMV show alterations in calcium signaling. Specifically, HCMV infected organoids exhibit upregulated expression of NMDA receptor subunit GRIN2C, a component found to confer lower ion conductance for calcium. Moreover, AMPA receptor subunits GRIA1, GRIA3, and GRIA4 show a clear downward trend in expression in infected organoids compared to mock. Alterations to these ionotropic glutamate receptors potentially indicate a detrimental impact on long-term potentiation, a key cellular mechanism underlying memory. We predict that these gene changes will be amplified in AD organoids, with the AD + HCMV infection condition demonstrating the most disruption. Interestingly, calmodulins (CALM1-3) are among the most down-regulated transcripts in control HCMV infected organoids, further highlighting the impact of HCMV on calcium signaling pathways. In sum, these experiments will directly test the role HCMV plays in AD pathology, which has important implications for viral pathogens in neurodegenerative disease.

Disclosures: J.W. Adelman: None. **M. Schumacher:** None. **B. O'Brien:** None. **R. Mokry:** None. **I. Luecke:** None. **S. Terhune:** None. **A. Ebert:** None.

Digital Abstract Session

P097. Mechanisms of Synaptic Dysfunction In Alzheimer's Disease: In Vivo Models

Program #/Poster #: P097.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSF Award #1748523
NSF Award #1826871
NSF Award #182282

Title: Mutations in microtubule associated protein UNC-33 lead to defects in autophagosome maturation in C. elegans, connecting autophagy and neurodegeneration

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Abstract: The accumulation of misfolded proteins is a shared pathology among neurodegenerative disorders such as Parkinson's and Alzheimer's. These defective proteins are degraded in a multi-step process known as autophagy. During this process, the defective proteins are first sequestered within an autophagosome. The autophagosome is then transported along microtubules from the synapses to the cell body. Microtubules and their microtubule-associated proteins (MAPs) such as CRMP2/UNC-33 are key elements supporting the transport of autophagosomes. Studies indicate that the accumulation of these autophagic vacuoles could contribute to the development of neurodegenerative diseases. Based on this information and considering that autophagy is dependent on the trafficking of autophagosomes to the cell body through motor proteins, our lab hypothesizes that trafficking of synaptic autophagosomes may be defective in unc-33 mutants. To test this hypothesis, we monitored the trafficking of double fluorescently tagged autophagosomes (dFP) in the AIY neuron using confocal microscopy. Autophagy flux was also quantified under autophagy-inducing conditions through western blotting techniques. To further understand the mechanisms by which CRMP-2/UNC-33 regulates autophagy, we analyzed autophagosome maturation during trafficking to the cell body. Images of 25 animals per strain, per condition, were obtained and quantified after randomization. Preliminary data of dFP in autophagic vacuoles shows a statistically significant build up of autophagosomes in the neurite of unc-33 mutants when compared to the control strain DCR5074. Analysis of autophagy flux through western blotting also shows the accumulation of cleaved dFP in unc-33 mutants subjected to autophagy inducing conditions. This suggests that autophagy flux is dependent upon the proper functioning of UNC-33. Taken together, this is the first report that analyzes the potential role of UNC-33 in neuronal autophagy and provides insight on its role in neurodegeneration.

Disclosures: I.I. Bazaldua: None. **J. Holechek:** None. **M. Lopez:** None. **E. Carrillo:** None. **R. Jauregui:** None. **A. Holgado:** None.

Digital Abstract Session

P097. Mechanisms of Synaptic Dysfunction In Alzheimer's Disease: In Vivo Models

Program #/Poster #: P097.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1 R15 NS101608-01A1

Title: The CHD protein, Kismet, may regulate gamma-secretase proteolysis of amyloid precursor protein-like at the Drosophila neuromuscular junction

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Abstract: Group III chromodomain helicase DNA-binding (CHD) proteins and their *Drosophila* ortholog, Kismet (Kis), catalyze nucleosome remodeling to regulate genes important for several aspects of synaptic function including morphology, vesicle endocytosis, and receptor localization. Specifically, we find that Kis negatively regulates transcription of *Appl*, the *Drosophila* amyloid precursor protein (APP) homolog. Amyloidogenic processing of APP by beta- and gamma-secretases leads to the production of toxic, insoluble Abeta fragments implicated in Alzheimer's disease (AD) progression. However, aberrant synaptic organization could leave APPL inaccessible to proteolytic processing by secretases leading to an accumulation of APPL in the central nervous system. Of the many different proteins regulating synaptic organization, Kis binds to the regulatory regions of two tetraspanins, *tsp42Ee* and *tsp42Eg*, which are expressed in motor neurons and muscle, respectively. Tetraspanins form self-organizing clusters, known as tetraspanin-enriched microdomains, and regulate the spatiotemporal distribution of proteins at the synapse. CD63, the mammalian homolog of Tsp42Ee and Tsp42Eg, binds to gamma-secretase and may represent a mechanism by which tetraspanins regulate synaptic levels of APP/APPL. Notably, both *kis* and *tsp42Ee* mutants show an increase in synaptic levels of Endophilin A (EndoA) and Dynamin (Dyn), a decrease in both *rab11* transcripts and synaptic localization, and locomotor impairments, suggesting that Kis may exert its synaptic organizational effects through regulation of tetraspanins, specifically *tsp42Ee*. Furthermore, loss of either APPL function or pharmacologic inhibition of APPL processing restores synaptic levels of Rab11, EndoA, and Dyn. While the specific cellular processes underlying Alzheimer's disease are still not well understood, these data suggest a role for Kis in the regulation of tetraspanins and promote our understanding of the cellular mechanisms underlying AD pathology.

Disclosures: E.L. Hendricks: None. **F.L.W. Liebl:** None.

Digital Abstract Session

P097. Mechanisms of Synaptic Dysfunction In Alzheimer's Disease: In Vivo Models

Program #/Poster #: P097.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NINDS 1R15NS101608-01A1
NIH NINDS R15NS101608-01A1S1

Title: Investigating a potential interaction between the chromatin remodeling protein Kismet and APP in a Drosophila model of Alzheimer's disease

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Abstract: Alzheimer's disease is characterized by an abundance of plaques formed from amyloid β (Aβ) peptides. These plaques result in a loss of neurons and synaptic connections, which leads to impaired memory and places Alzheimer's disease as a leading cause of dementia in geriatric patients. The cell adhesion molecule amyloid precursor protein (APP) can be processed by β-site APP-cleaving enzyme (BACE). BACE cleaves APP and, through a series of other enzymatic functions, ultimately causes Aβ to be released from the plasma membrane to aggregate and form plaques. Overexpression of human APP and BACE1 in *Drosophila* larval motor neurons is used as a synaptic model for late onset Alzheimer's disease. Kismet (Kis), which is the *Drosophila* ortholog of the mammalian chromatin remodeling enzymes CHD7 and CHD8, positively regulates the localization of synaptic cell adhesion molecules and BMP signaling. Expression of human *APP* and *BACE1* in motor neurons produces similar phenotypes as are observed in *kismet* loss of function mutants including impaired neurotransmission and larval locomotion, deficient endocytosis, increased synaptic levels of the BMP signaling protein pMad, and increased *neuroligin2* transcripts. In addition, *app-like* transcripts are increased in *kismet* mutant motor neurons. To further investigate a potential connection between Kis and APP, we will examine expression of *kismet* in *app-like* mutants and neuronal APPL in *kismet* mutants. These data will clarify whether interactions between Kismet and APPL may regulate synaptic function.

Disclosures: F.L. Liebl: None. **N. Linskey:** None. **E. Hendricks:** None.

Digital Abstract Session

P097. Mechanisms of Synaptic Dysfunction In Alzheimer's Disease: In Vivo Models

Program #/Poster #: P097.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant 2RF1AG008200-29
NIH grant 2R01-NS047229
NIH grant P50AG05138
Alzheimer's Association grant AARF-17-531426

Title: Ps1 familial alzheimer disease mutants inactivate factor-dependent protection against neurotoxicity by affecting neuroprotective complexes of nmda receptor.

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Abstract: Excitotoxicity is known to play key roles in brain neurodegeneration and stroke. We obtained data that neuroprotection by trophic factors such as ephrinB1(EFNB1) and brain-derived neurotrophic factor (BDNF) (called Factors) depends on presenilin1 (PS1; Barthet et al., Neurobiol Aging. 34: 499–510, 2013) and requires *de novo* formation of "survival complexes" that are Factor-stimulated complexes of *N*-methyl-d-aspartate receptor (NMDAR) with Factor receptor (FR) and PS1 (Rahim et al., https://doi.org/10.1093/braincomms/fcaa100). Using coimmunoprecipitation, western blot and *in vitro* cell survival assay we found that absence of PS1 reduces formation of survival complexes and abolishes neuroprotection. Peptides designed to disrupt formation of survival complexes also decrease the Factor-stimulated neuroprotection. Strikingly, Factor-dependent neuroprotection and levels of *de novo* Factor-stimulated survival complexes decrease dramatically in neurons expressing PS1 familial Alzheimer disease (FAD) mutants. Mouse neurons and brains expressing humanized knockin (KI) PS1FAD mutants contain increased amounts of constitutive non-functional PS1-NMDAR complexes unresponsive to Factors. In addition, the stability of the FAD PS1-NMDAR complexes differs from that of wild type (WT) complexes and using Middle Cerebral Artery Occlusion (MCAO) and immunohistochemistry of brain sections we found that brain neurons expressing heterozygous FAD mutants are more vulnerable to cerebral ischemia than neurons of WT brains. Furthermore, NMDAR-mediated excitatory postsynaptic currents (EPSCs) at CA1 synapses are altered by PS1 FAD mutants in cortical neurons in *in vitro* electrophysiology experiments. Importantly, high levels of PS1-NMDAR complexes are also found in postmortem Alzheimer disease (AD) brains expressing PS1 FAD mutants. Together, our data identify a novel PS1-dependent neuroprotective mechanism and reveal a pathway by which PS1 FAD mutants increase toxicity-induced neuronal death in the absence of AD neuropathological hallmarks. These findings have implications for the pathogenic effects of FAD mutants and therapeutic strategies.

Disclosures: **N.K. Robakis:** None. **M.A. Rahim:** None. **Y. Yoon:** None. **C. Dimovasili:** None. **N. Kezunovic:** None. **L. Chen:** None. **A. Schaffner:** None. **G.W. Huntley:** None. **I. Ubarretxena-Belandia:** None. **A. Georgakopoulos:** None.

Digital Abstract Session

P097. Mechanisms of Synaptic Dysfunction In Alzheimer's Disease: In Vivo Models

Program #/Poster #: P097.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Research Grants Council of Hong Kong (HKUST16102019, HKUST16149616, HKUST16103017, and HKUST16102717)
National Key R&D Program of China (2017YFE0190000 and 2018YFE0203600)

Area of Excellence Scheme of the University Grants Committee (AoE/M-604/16)
Theme-based Research Scheme (T13-605/18W)
Innovation and Technology Commission (ITCPD/17-9)
Guangdong Provincial Key S&T Program (2018B030336001)
Shenzhen Knowledge Innovation Program (JCYJ20180507183642005 and JCYJ20170413173717055)

Title: Roles of EphA4 signaling in the pathogenesis of Alzheimer's disease

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Abstract: Alzheimer's disease (AD), the most common form of dementia, is characterized by irreversible memory loss with no effective treatment to date. The accumulation of amyloid-beta (Aβ) is believed to be an important pathological contributor in AD, which leads to synaptic failure. Emerging evidence along with our previous findings have demonstrated that dysregulated erythropoietin-producing hepatocellular A4 (EphA4) signaling is involved in abnormal hippocampal synaptic function and impaired cognition in AD progression. However, the underlying mechanisms remain unclear. Here, we inhibited EphA4 activity using KYL peptide in an APP/PS1 transgenic AD mouse model, and examined its effect on hippocampal synaptic function. We found that blockade of EphA4 signaling by KYL treatment rescued the loss of excitatory synapses in the hippocampus of APP/PS1 mice. Moreover, KYL administration restored the impairment in hippocampal synaptic plasticity. To further delineate the downstream mechanisms by which EphA4 inhibition rescues hippocampal synaptic dysfunction in AD, we conducted single-nuclei RNA sequencing analysis. The expression of several genes and pathways associated with excitatory synapse formation and maintenance were identified to be restored in the hippocampus of APP/PS1 mice after KYL treatment. Collectively, our findings suggest that modulation of EphA4 signaling can restore excitatory synaptic functions in the hippocampus in AD.

Disclosures: **X. Yang:** None. **Y. Wang:** None. **J. Lin:** None. **K. Cheung:** None. **K. Hung:** None. **W. Fu:** None. **A. Fu:** None. **N. Ip:** None.

Digital Abstract Session

P098. Clinical and Pre-Clinical Imaging Studies In Alzheimer's Disease

Program #/Poster #: P098.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant 5T35AG029795-13

Title: Olfactory decline during aging predicts gray matter volume loss in key AD brain regions

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Abstract: Objective: To examine whether olfactory decline is associated with structural neuroimaging findings in brain regions specific to Alzheimer's dementia (AD). Rationale: Olfactory dysfunction signals increased AD risk, but whether olfactory decline predicts gray matter loss in specific brain regions is unclear. Methods: 121 older adults (mean age=77.7, SD=7.1 at baseline; 69% women) with normal cognition (NC; n=104) and mild cognitive impairment (MCI; n=17) completed a median of 4 Brief Smell Identification Tests (BSIT) over a median of 6 years prior to MRI and final cognitive diagnosis (NC, MCI, or AD) as part of the Rush Memory and Aging Project, a prospective, longitudinal study exploring the origins of dementia. Linear regression of annual BSIT scores and age for each participant yielded individual trajectories of smell function categorized as: severe decline, decreased, unchanged, or improved. Gray matter volumes (GMV) in the primary olfactory (PO), secondary olfactory (SO), temporal (T), fronto-parietal (FP), sensorimotor (SM), and visual (VS) brain regions were estimated (% of intracranial volume) using standard methods (FreeSurfer v6, gyral-based and sub-cortical segmentation) from available 3T MRI. Relationships between olfactory trajectories and regional GMVs were evaluated, along with AD risk factors (e.g. APOE status), using trend tests and multivariable linear regression. Results: The mean trajectory (slope) of olfactory change was -0.2, SD +/- 0.5. Participants with MCI (n=25) and AD (n=23) had smaller GMV in the PO, SO, and T regions ($p<0.01$, all) compared to those with NC (n=73) but not in the FP, SM, and VS regions. Confirming our main hypothesis, those with more severe olfactory decline had smaller GMV in the PO, SO, T, and FP areas ($p<0.05$, all) but not in the SM or VS. After adjustment for known AD risk factors (e.g., age, sex, education, global cognition at MRI, APOE genotype), more severe olfactory decline (β= -0.11, 95% CI -0.21, -0.00) predicted smaller GMV only in the PO region, with an effect size comparable to carrying APOE-ε4 (β= -0.36, 95% CI -0.64, -0.09). Older age was significantly associated with smaller GMV across all brain regions. Conclusion: Increasing severity of olfactory decline leads to specific GMV loss in the PO, SO, and T regions beyond that seen with increasing age and mirroring patterns seen in MCI and AD. Both severity of olfactory decline and APOE-ε4 genotype independently predict neurodegenerative changes in the PO cortex, suggesting that primary olfactory brain regions play a key role in AD pathogenesis.

Disclosures: R.R. Pacyna: None. **D. Han:** None. **K.E. Wroblewski:** None. **M.K. McClintock:** None. **J.M. Pinto:** None.

Digital Abstract Session

P098. Clinical and Pre-Clinical Imaging Studies In Alzheimer's Disease

Program #/Poster #: P098.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P30 AG066506

Title: Free water increases in cholinergic regions and along brainstem tracts with cognitive impairment severity

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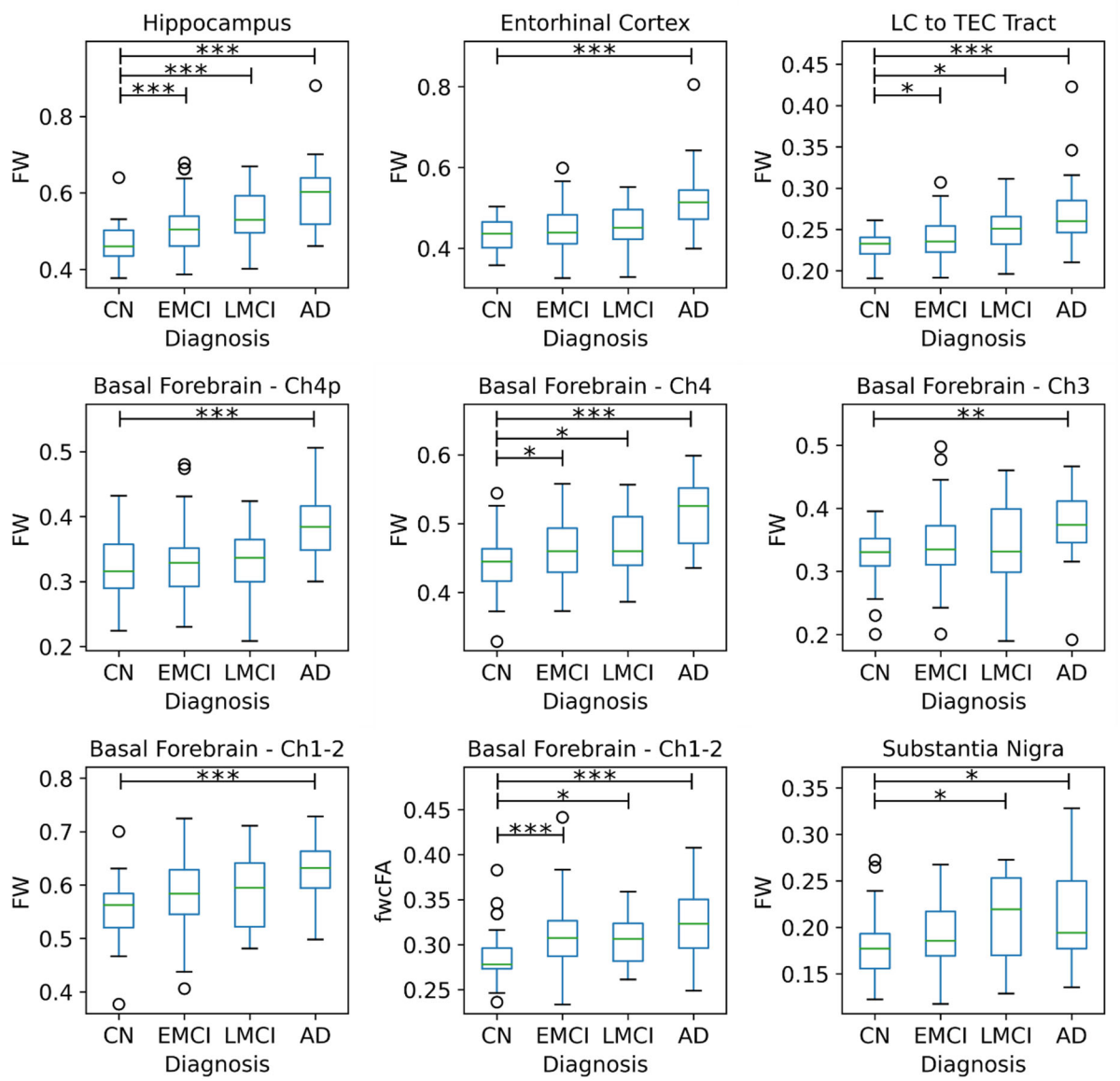
Abstract: The purpose of this study was to determine the anatomical progression of neurodegeneration in the early stages of Alzheimer's disease severity as measured by clinical diagnosis, clinical measures, and Neurofilament-light chain (NfL). To accomplish this aim, free water, a diffusion MRI metric, was calculated in the hippocampus, basal forebrain, entorhinal cortex, and a locus coeruleus (LC) to transentorhinal cortex (TEC) tract in four patient groups: cognitively normal (CN; n=31), early mild cognitive impairment (EMCI; n=77), late mild cognitive impairment (LMCI; n=24), and Alzheimer's disease (AD; n=23). Blood samples were collected for quantification of NfL levels and all patients received a battery of cognitive assessments. Our results show that free water increases with disease severity in all of the examined regions but only a subset of these regions (hippocampus, nucleus basalis of Meynert, and LC to TEC) are significantly different in EMCI compared to CN. Furthermore, free water in all of the examined regions significantly correlated with patient performance on clinical measures (Montreal Cognitive Assessment, Clinical Dementia Rating scale Sum of Boxes, Mini Mental State Exam, and Loewenstein-Acevedo Scale for Semantic Interference and Learning) and neurofilament-light chain. Together, these results suggest that neurodegeneration may occur in the hippocampus, nucleus basalis of Meynert, and LC to TEC tract in the early stages of Alzheimer's disease while other basal forebrain regions, the entorhinal cortex, and the substantia nigra are not affected until a later stage. These findings also show that free water imaging is a clinically-relevant and non-invasive marker that is sensitive to changes in cognitive impairment severity.



Disclosures: **W.T. Chu:** None. **D. Vaillancourt:** None. **S. Coombes:** None. **W. Wang:** None.

Digital Abstract Session

P098. Clinical and Pre-Clinical Imaging Studies In Alzheimer's Disease

Program #/Poster #: P098.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BMEG322151

Title: Multimodal characterization of hippocampal integrity in mild cognitive impairment: combining volumetry, diffusion, perfusion, and viscoelasticity from magnetic resonance imaging

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Abstract: Novel neuroimaging biomarkers are urgently being sought to characterize early changes to the brain in mild cognitive impairment (MCI), which is typically a precursor to Alzheimer's disease (AD). As the hippocampus is a critical site of AD pathogenesis that underlies principal symptoms of memory impairment, numerous studies have utilized multiple imaging modalities to characterize hippocampal integrity. Here we additionally include magnetic resonance elastography (MRE)[1], which has recently emerged as a sensitive measure of microstructural tissue health[2-4]. For the first time, we adopt a multimodal neuroimaging approach consisting of measures of hippocampal viscoelasticity, volume, perfusion, and diffusion - including neurite orientation and dispersion density imaging (NODDI) metrics - resulting in a total of twelve separate measures of hippocampal health. The purpose of this study was to determine which MRI measures were most sensitive in detecting differences in hippocampal integrity between cognitively healthy older adults (OA) and those with amnestic MCI. We recruited 23 OA and 7 MCI participants (demographics in Table 1). A binomial logistical regression was performed to establish the effect of the 12 hippocampal measures on the likelihood of an MCI diagnosis. Hippocampal integrity metrics for both OA and MCI groups are presented in Table 1. The full model correctly classified 72% of cases. Viscoelastic damping ratio (DR) from MRE was 32% higher in the MCI group and was the only significant predictor in differentiating between OA and MCI (p=0.013). The significant relationship between MCI status and DR (B=0.796, S.E.=0.321, after adjusting for age and sex) suggests that for every 0.01 increase in DR, the likelihood of having MCI is 2.22 times higher. No other hippocampal measures were significant predictors in the model, although hippocampal volume was close to approaching significance (p=0.090). Our results suggest that MRE measures of viscoelasticity may provide a unique and highly sensitive biophysical signature of hippocampal health relevant to microstructural changes that are expected to occur in MCI.

Table 1. Summary of demographics and hippocampal neuroimaging measures

		OA	MCI	% difference	P-Value
Demographics	N	23	7		
	Sex	13M, 9F	3M, 4F		
	Mean age	68.9 ± 5.8	72.1 ± 6.8	4.35%	0.234^
	California Verbal Learning Test	92.69 ± 12.98	82.0 ± 16.39	-11.53%	0.165^
	NIH – toolbox Cognition Fluid Composite	48.56 ± 9.2	44.29 ± 12.0	-8.79%	0.330^
Structural MRI	Volume (mm^3)	7836 ± 699	6687 ± 720	-14.67%	0.090
MRE	Shear stiffness (kPa)	2.77 ± 0.48	2.23 ± 0.29	-13.72%	0.523

	Damping Ratio	**0.205 ± 0.04**	**0.272 ± 0.05**	**+32.42%**	**0.013***
DTI	Axial Diffusivity	6.02 ± 0.27	6.20 ± 0.33	+1.87%	0.988
	Fractional Anisotropy	0.232 ± 0.03	0.220 ± 0.03	-5.17%	0.685
	Mean Diffusivity	6.02 ± 0.27	6.20 ± 0.33	+2.99%	0.768
	Radial Diffusivity	5.29 ± 0.28	5.49 ± 0.36	+3.78	0.683
NODDI	Neurite Density Index	0.508 ± 0.05	0.508 ± 0.04	0.00%	0.929
	Fractional of Isotropic Diffusion	0.228 ± 0.06	0.241 ± 0.04	+5.70%	0.680
	Orientation Dispersion Index	0.426 ± 0.03	0.426 ± 0.03	-1.17%	0.730
ASL	Perfusion	32.30 ± 7.4	33.62 ± 10.9	+4.09%	0.653
	aCBV	0.135 ± 0.07	0.150 ± 0.05	+15.38	0.329

Disclosures: E.M. Tinney: None. **L. Hiscox:** None. **P. Delgorio:** None. **C. Martens:** None. **C. Johnson:** None.

Digital Abstract Session

P098. Clinical and Pre-Clinical Imaging Studies In Alzheimer's Disease

Program #/Poster #: P098.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Western University Internal Seed Fund

Title: In vivo molecular imaging of the mouse cholinergic projection system

Authors: *K. M. ONUSKA[1], H. R. C. SHANKS[1], M. FOX[2], J. HICKS[2], J. THIESSEN[2], V. F. PRADO[3], M. A. M. PRADO[4], T. W. SCHMITZ[4];
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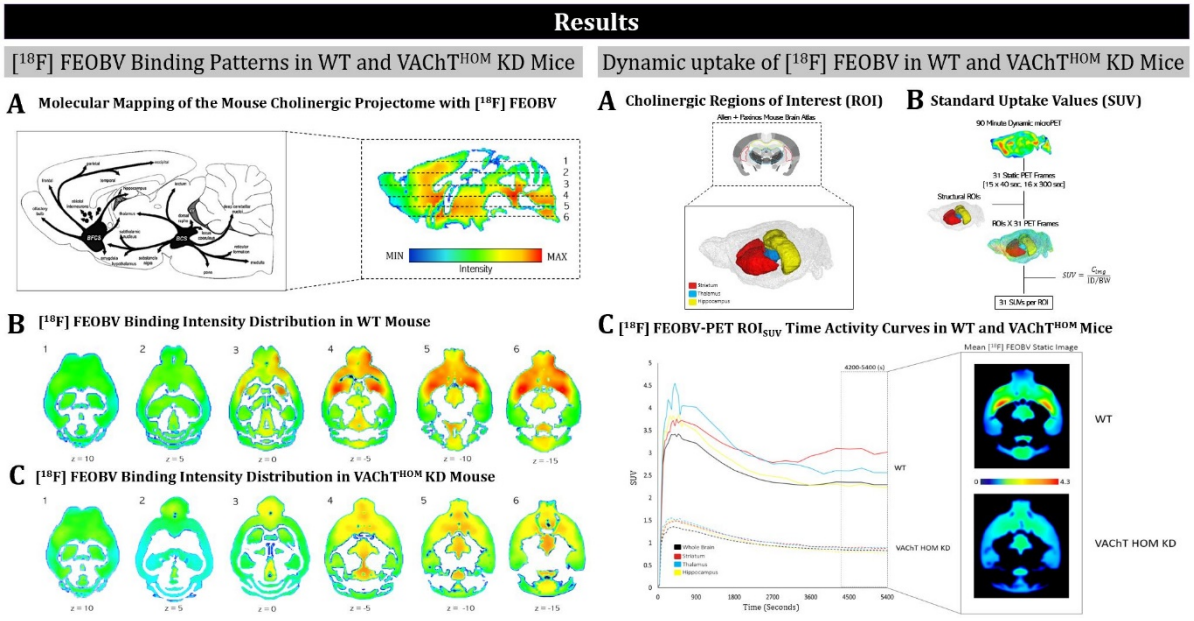
Abstract: The cholinergic neurons of the basal forebrain (BF) are among the first cells to become damaged in Alzheimer's disease (AD). Structural magnetic resonance imaging (sMRI) studies have shown that this damage occurs prior to cortical degeneration and memory loss. Although sMRI is sensitive to early volumetric losses within the BF, it cannot specifically assay degeneration of distal BF cholinergic axons, which is thought to occur earlier than degeneration

of the cell bodies within the BF. Positron emission tomography (PET) with the [^{18}F] FEOBV radiotracer overcomes this problem. [^{18}F] FEOBV binds to the vesicular acetylcholine transporter (VAChT), a protein found solely in cholinergic nerve terminals, thereby providing a cell-type specific measurement of the cholinergic projectome. However, the sensitivity and specificity of *in vivo* [^{18}F] FEOBV-PET to the endogenous expression of VAChT is unknown. This has created a major obstacle for assessing the utility of [^{18}F] FEOBV-PET as a biomarker for preclinical stages of AD. The goal of this study is therefore to calibrate the sensitivity and specificity of [^{18}F] FEOBV-PET for measuring preclinical changes in the cholinergic cortical projectome. To do this, we used mouse lines in which endogenous VAChT expression is held under genetic control. We evaluated binding intensity patterns and uptake profiles of [^{18}F] FEOBV in a wild type (WT) mouse and a knockdown (KD) mouse with 60% global reductions in VAChT expression (VAChT^{HOM} KD). First, we demonstrated that [^{18}F] FEOBV-PET binding patterns reflect the underlying anatomy of the mouse cholinergic projectome in both WT and VAChT^{HOM} KD mice (Results, *left*). Next, we observed that decreases in [^{18}F] FEOBV uptake occur in regions that receive cholinergic input in the VAChT^{HOM} KD mouse when compared to the WT mouse, consistent with the 60% global reduction of VAChT present in this mouse. (Results, *right*). Overall, these preliminary results support [^{18}F] FEOBV-PET as a sensitive *in vivo* cell-type specific tool for measuring cholinergic integrity.



Disclosures: **K.M. Onuska:** None. **H.R.C. Shanks:** None. **M. Fox:** None. **J. Hicks:** None. **J. Thiessen:** None. **V.F. Prado:** None. **M.A.M. Prado:** None. **T.W. Schmitz:** None.

Digital Abstract Session

P098. Clinical and Pre-Clinical Imaging Studies In Alzheimer's Disease

Program #/Poster #: P098.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: An anatomically and biochemically specific relationship between phosphatidylcholine and basal forebrain degeneration in Alzheimer's disease

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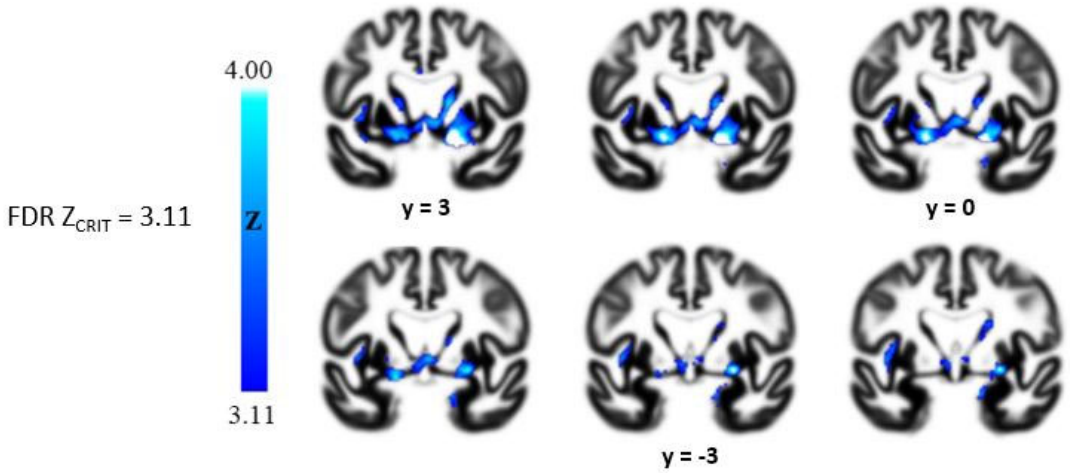
Abstract: Basal forebrain cholinergic neurons degenerate before other cell types in Alzheimer's disease (AD). The factors which contribute to cholinergic selective vulnerability are poorly understood. We hypothesized that AD-related reductions in the bioavailability of the lipid phosphatidylcholine (PC) may potentiate vulnerability of cholinergic basal forebrain neurons. All neurons use PC to maintain their cell membrane. Cholinergic neurons also use PC to make acetylcholine. Moreover, cholinergic neurons are highly plastic in adulthood which may increase demand for PC to support axonal remodeling. An AD-related bottleneck on PC may therefore selectively impact cholinergic neuronal functions earlier and more severely than other cell types. To test our hypothesis, we leveraged data from the Alzheimer's Disease Neuroimaging Initiative. Participants were divided into a normal cerebrospinal fluid (nCSF; n = 62) group and an abnormal cerebrospinal fluid (aCSF; n = 161) group based on their CSF ratios of phosphorylated tau and amyloid beta. Groups were age matched ($t = 0.59$, $p = 0.55$). Longitudinal structural magnetic resonance imaging data were used to calculate grey matter annual percent change for each participant. Partial least squares analyses assessed the multivariate relationship between serum lipids, including PC, and neuroimaging data. Of all serum lipids, PC ($p = 0.002$ on 5000 permutations) and acylcarnitine ($p = 0.003$ on 5000 permutations) were the only lipid types to exhibit a relationship with grey matter degeneration which was modified by CSF-confirmed AD pathology. In addition to biochemical specificity, the relationship between PC and longitudinal grey matter degeneration revealed a spatial pattern (Figure; blue) with high anatomical specificity to brain regions known to be cholinergic, such as the basal forebrain and striatum. Overall, this study provides *in vivo* evidence for a selective relationship between PC and basal forebrain degeneration in human AD.



Disclosures: **H.R.C. Shanks:** None. **K.M. Onuska:** None. **D.K. Barupal:** None. **T.W. Schmitz:** None.

Digital Abstract Session

P098. Clinical and Pre-Clinical Imaging Studies In Alzheimer's Disease

Program #/Poster #: P098.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Operating Grant MOP-125915

Title: Declines in cognitive-motor integration performance are correlated with lower white matter integrity in older adults with genetic risk for Alzheimer's disease

Authors: ***A. ROGOJIN**, D. J. GORBET, K. M. HAWKINS, L. E. SERGIO;
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Abstract: *Introduction:* Cognitive-motor integration (CMI) involves concurrent thought and action, which requires the interaction of large networks in the brain [1]. Previous findings have shown that CMI performance is impaired in individuals with specific dementia risk factors (family history of dementia and presence of the APOE e4 allele) [2]. These findings suggest that CMI impairments are associated with early dementia-related brain changes. The objectives of the current research study are 1) to examine changes in white-matter integrity associated with dementia family history, sex, and APOE status, and 2) to assess the relationship between white matter integrity and cognitive-motor performance. *Methods:* Participants included right-handed older adults with a high-risk (n=25, 12 female) and a low-risk (n=24, 12 female) for dementia. Participants were tested on four visuomotor tasks where reach and gaze were increasingly spatially dissociated using two linked touchscreens. These tasks included a standard condition requiring direct interaction with visual targets, and three dissociated non-standard conditions requiring CMI (visual feedback reversal, plane-change, and plane-change + feedback reversal). APOE genotyping was determined from salivary measures, and diffusion-weighted magnetic resonance images (dw-MRI) were collected to look at white matter integrity. Automated fiber quantification (AFQ) was used to characterize white matter properties in healthy aging compared to increased dementia risk. *Results:* Preliminary analysis of these data has revealed significant correlations between the mean fractional anisotropy in several major white matter tracts and CMI task performance in individuals with the APOE e4 allele. These data support our hypothesis that disruptions in CMI performance are associated with identifiable brain alterations early in disease progression. *Conclusion:* The preliminary findings provide insight into the impact of AD pathology on neural networks underlying complex visuomotor transformations, and demonstrate that the CMI paradigm discussed in this study may be used as a non-invasive, easily accessible assessment tool for dementia risk.
[1] Caminiti R, et al. (2017) eNeuro. 4(1):1-35. [2] Rogojin A, et al. (2019) JAD. 71(2):685-701.

Disclosures: **A. Rogojin:** None. **L.E. Sergio:** None. **D.J. Gorbet:** None. **K.M. Hawkins:** None.

Digital Abstract Session

P098. Clinical and Pre-Clinical Imaging Studies In Alzheimer's Disease

Program #/Poster #: P098.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Brigham Young University, College of Life Sciences, Mentoring Environment Grant
NIH/NIA 1 R21 AG037843
Brigham Young University, School of Family Life, Gerontology Program
Brigham Young University, Dr. Sarah M. McGinty Neuroscience Graduate Student Research Fellowship
Neurodar, LLC
Limitless Worldwide, LLC

Title: Optimization of relaxation times to provide the best signal intensity and resolution in MR images following exposure of Gadolinium-based Contrast Agents

Authors: *A. A. SOTELO[1], N. HUANG[2], K. S. STEED[4,5], J. A. HAMILTON[2], J. WISCO[3,5]; [1]Dept of Physiol. and Biophysics & Dept. of Anat. and Neurobio., [2]Dept of Physiol. and Biophysics, [3]Dept. of Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA; [4]Biomed. Educ., California Hlth. Sci. Univ., Fresno, CA; [5]Brigham Young Univ., Provo, UT

Abstract: Previous studies have indicated that Gadolinium-based Contrast Agent (GBCA) immersion of post mortem animal brains can enhance their MRI signal. We examined the utility of a GBCA for the enhancement of murine brains in our ongoing study of hippocampal pathophysiological differences in transgenic Alzheimer's disease (AD). Ex-vivo Presenilin-1 (PSEN1), Tau, and wild-type (WT) mice [12 (6M, 6F) in each cohort] were sacrificed at 6 months of age. One brain hemisphere was immersed in, then imaged with fomblin, then imaged again after being immersed for one week in gadolinium (Gd). We wanted to know the extent of signal gain from the Gd using ex-vivo imaging in a 11.7T MRI Bruker 500 MHz magnet. We assessed signal gain between cohorts. We acquired T1, T2, and T2* images at 11.7T throughout the volume of the hemisphere and compared the signal intensity of the body of the hippocampus between the image contrasts. We hypothesized that the T2* relaxation time, derived from the rapid acquisition with relaxation enhancement (RARE) sequence, to be the most effective due to its multiple 180 degree echos. Measurements were acquired from one axial slice at a level where both the anterior and posterior horns of the lateral ventricles were visible using the analysis software Horos (https://horosproject.org/). We did paired t-tests for Gd for each of T1, T2 and T2*. T2 normal images displayed a significant difference in the scores for Gd absence (M= 7.9, SD=4.8) and the addition of Gd (M=16.6, SD=4.4) conditions; $t(35)= -7.556$, $p = 0.000$. Our findings indicated POST-Gd T2 images as the optimal signal intensity for hippocampal clarity.

Additionally, T2* normal image SNR presented a significant difference in the scores for Gd absence (M= 28.2, SD=10.9) and the addition of Gd (M=19.8, SD=7.9) conditions; t(35)= 3.807, p = 0.001. These results suggest that the addition of Gd positively improves 11.7T image SNR. No difference in Gd enhancement was observed between transgene cohorts. An approximate linear difference in T2 and T2* signal in 11.7T following Gd exposure in 11.7T MR field strengths was observed. This study indicates post-mortem imaging following Gd exposure, at high-field strength can be added to our protocol to better assess general high-field visualization effects in human and animal models; while contributing to existing knowledge by providing further analysis and understanding of pathological changes in mice brains.

Disclosures: **A.A. Sotelo:** None. **N. Huang:** None. **K.S. Steed:** None. **J.A. Hamilton:** None. **J. Wisco:** None.

Digital Abstract Session

P098. Clinical and Pre-Clinical Imaging Studies In Alzheimer's Disease

Program #/Poster #: P098.08

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH R01AG065549

Title: Applications of volume imaging by 3D electron microscopy and advanced segmentation tools to the reanalysis of legacy biopsy samples from Alzheimer’s

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Abstract: Senile Dementia of Alzheimer’s Type (SDAT) is perhaps the most intensively studied neurodegenerative disease. Early electron microscopic (EM) studies identified pronounced hallmarks of AD, Paired Helical Filament (PHF) containing tangles and amyloid-bearing plaques. We have re-examined brain biopsy samples taken from human specimens with severe dementia and AD in the 1960s. We experiment with an array of imaging approaches with a focus on throughput and autonomous analysis. Large fields of serial-section electron microscopy (SSEM) samples are taken and registered to reveal enriched 3D scenes of autophagosomal stresses, neurofibrillary tangles, somatic lipofuscin agglomerations, and vascular-adjacent plaques. In order to scale a label-analysis of the tissue, we test deep-learning neural network (DNN) object classifiers and content-aware image restorers. Due to challenges of high-resolution imaging in pathology samples, a high-degree of effort and sophistication is required of the autonomous techniques, regardless we see initial successes by pioneering emerging machine-learning advances. Most EM DNNs classifiers use binary classification with softmax or sigmoid activation functions. We find that using multilabel classification without activation functions creates significant improvements to classifier signal, especially in the context of sparse objects, such as synapses. We also apply generative adversarial network (GAN) DNN approaches, such as to try and artificially preserve the samples, and make them appear more like canonical EM

fixation-and-preservation samples from other organisms for the purposes of enhanced acuities. After apply GAN-drive content-aware image restoration, certain neurite processes become discernable that were otherwise obfuscated by the challenges of the specimen. Resolution deficits are also addressed and boosted using GANs, using an emerging machine-learning method of EM Superresolution. We find that testing these emerging A.I. approaches produces data that bridges the divide between the difficulties of experimentation and analysis of SDAT in model species such as mouse, and directly mining pathologically-relevant quantities and qualitative observations from these clinical samples.

Disclosures: **M. Madany:** None. **E. Bushong:** None. **S. Peltier:** None. **M.H. Ellisman:** None.

Digital Abstract Session

P099. Alzheimer's Disease Models: Behavioral Effects

Program #/Poster #: P099.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the National Key R&D Program of China (2017YFE0190000 and 2018YFE0203600)
the Hong Kong Research Grants Council Theme-based Research Scheme (T13-605/18-W)
the Area of Excellence Scheme of the University Grants Committee (AoE/M-604/16)
the Innovation and Technology Commission (ITCPD/17-9)
the Lee Hysan Foundation (LHF17SC01)
the National Natural Science Foundation of China (31671047)
the Guangdong Provincial Key S&T Program (2018B030336001)

Title: Allele-specific editing of mutations in familial Alzheimer’s disease ameliorates amyloid-associated pathologies in transgenic mice

Authors: ***Y. DUAN**[1,2], T. YE[1,2,3,4], Z. QU[5], Y. CHEN[1,3,4], A. MIRANDA[1], K. CHEUNG[1,2], X. ZHOU[1,2], Y. CHEN[1,2,3,4], A. K. Y. FU[1,2,3], V. GRADINARU[5], N. Y. IP[1,2,3];
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Abstract: Familial Alzheimer’s disease (AD) is caused by autosomal dominant missense mutations in the genes encoding amyloid precursor protein (APP), presenilin 1 (PSEN1) and presenilin 2(PSEN2), which result in excessive production of amyloid-beta peptides. Given that

most patients with familial AD have a heterozygous disease mutation and humans that naturally carry one copy of *APP*, *PSEN1*, or *PSEN2* do not exhibit obvious neurological symptoms, the selective disruption of the disease-causing allele at the adult stage may be a rational and feasible treatment strategy for familial AD. While it has been demonstrated that CRISPR/Cas9-mediated genome editing disrupted familial AD mutations and reduced Aβ production *in vitro*, the inefficient delivery of CRISPR/Cas9 into the brain and limited efficiency of genome editing restrict the potential beneficial effects of allele-specific editing *in vivo*. Here, we show that a CRISPR/Cas9-based strategy can selectively and efficiently edits the *APP* Swedish (*APPswe*) mutation *in vivo* and ameliorate amyloid-associated pathology in two different familial AD transgenic mouse models via an all-in-one AAV-mediated delivery of a small Cas9 and the sgRNA. Of note, this CRISPR-mediated beneficial effect is effective in transgenic mice at an age when amyloid pathology is obvious and can persist for at least 6 months after a single virus administration. Thus, our results provide strong evidence that CRISPR/Cas9-mediated disruption of the allele that carries familial AD mutation can ameliorate amyloid-mediated pathology *in vivo*. Our findings also provide important insights for the development of disease-modifying treatments for familial AD as well as other brain diseases caused by dominant mutations.

Disclosures: **Y. Duan:** None. **T. Ye:** None. **Z. Qu:** None. **Y. Chen:** None. **A. Miranda:** None. **K. Cheung:** None. **X. Zhou:** None. **Y. Chen:** None. **A.K.Y. Fu:** None. **V. Gradinaru:** None. **N.Y. Ip:** None.

Digital Abstract Session

P099. Alzheimer's Disease Models: Behavioral Effects

Program #/Poster #: P099.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Use of deep neural network algorithm on cognitive performances to early identify Alzheimer’s disease: An animal study

Authors: S. SUTOKO[1], A. MASUDA[2], A. KANDORI[1], H. SASAGURI[3], T. SAITO[4], T. C. SAIDO[3], *T. FUNANE[1];
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Abstract: One of the most common types of dementia is Alzheimer’s disease (AD; 60-70%). The prevalence rate of AD is increasing as the population is getting older; more than 30% of elderly people (> 85 years old) suffered AD. The core AD symptoms associated with declines of memory, speech, linguistic, and executive functions that may lower quality of life and disrupt behaviors. The AD prognosis might take decades; thus, the AD studies are often done in the animal samples to accelerate findings. One of AD-modelled mice is created by using a knock-in (KI) strategy; those mice express preclinical AD symptoms caused by the overproduction of amyloid-β peptide (Aβ) plaques. These AD-modelled mice are suitable for the study of early AD

identification. In this study, we aimed to develop a practical and affordable method for early AD screening based on only the behavioral evaluation rather than the neuroimaging measurement. Wild-type (WT) and KI AD-modelled (*App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$) mice were raised, and their behaviors during cognitive tasks (*e.g.*, learning, impulsivity, attention, compulsivity) were assessed in an automated monitoring system. The behavioral parameters were quantified by correct and error rates as mice were performing tasks at phases 1 (age of 8-12 months) and 2 (age of 13-17 months). The deep neural network (DNN) algorithm was used for classifying *App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice from WT mice. The conventional threshold algorithm was also conducted to confirm the DNN method as a superior classifier. The stepwise feature selection method supported both DNN and conventional threshold algorithms to optimize uses of behavioral parameters. The classification performance was relatively high (88.0-89.3% accuracy) even at the early age (*i.e.*, phase 1) when the AD symptoms were still underdeveloped. The use of DNN algorithm offered a lower performance variability (*i.e.*, low standard deviation across cross-validation; 89.3 ± 9.8% vs. 88.0 ± 17.9% accuracy) and a better practicability (*i.e.*, fewer used parameters and required tasks; 4 vs. 11 parameters) than that of conventional threshold algorithm. The DNN algorithm can be advantageous for any purposes, such as prognostic and therapeutic monitoring. Classifying behavioral parameters were related to compulsive and learning impairments in *App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice; the importance of those behavior evaluations is frequently underestimated in the current diagnostic paradigm. This study unveil the potential of early AD screening based on only behaviors that will significantly improve translatability to human studies.

Disclosures: **S. Sutoko:** A. Employment/Salary (full or part-time):; Hitachi, Ltd.. **A. Masuda:** None. **A. Kandori:** A. Employment/Salary (full or part-time):; Hitachi, Ltd.. **H. Sasaguri:** None. **T. Saito:** None. **T.C. Saido:** None. **T. Funane:** A. Employment/Salary (full or part-time):; Hitachi, Ltd..

Digital Abstract Session

P099. Alzheimer's Disease Models: Behavioral Effects

Program #/Poster #: P099.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC

Title: Assessing the longitudinal effects of early life enrichment on multisensory integration in male wildtype and 3xTgAD mice

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Abstract: Alzheimer's Disease (AD) is associated with several cognitive deficits that interfere with an individual's lifestyle. The cognitive reserve hypothesis posits that individuals who have a high education, higher occupational attainment, and exercise regularly will be less susceptible to cognitive impairment associated with aging and dementia. Cognitive reserve can be modeled with a standard Environmental enrichment (EE) protocol, which places mice in a large cage with extra cagemates, a running wheel, and objects that can be substituted daily. Unfortunately, the standard EE procedure has a limit on quantifying the enrichment each individual mouse obtains from being in a shared enriched cage. Thus, we developed a novel EE procedure to enable better control and quantification of daily enrichment. In the present study, we used 69 male wildtype (WT) and 63 male Triple Transgenic AD (3xTg-AD) mice to evaluate the potential for modeling cognitive reserve using this procedure. Mice were assigned to each of the four following conditions immediately after weaning at 5 weeks of age: Environmental Enrichment homecage (EH), which is modeled after 'conventional' EE protocols; Enrichment Track (ET), in which mice run laps on an obstacle track 6 days/week with novel obstacles daily; Exercise Control Track (CT), in which mice run laps but are not exposed to complex obstacles; and Standard Housing (SH). Following two months in these conditions, we tested all mice on tasks assessing multisensory integration abilities, an understudied but potentially important aspect of AD cognitive impairment. These tasks were the tactile-visual Cross-Modal Object Recognition (CMOR) task with a 5-min retention delay, and the olfactory-tactile Multisensory Oddity (MSO) task, which removes the mnemonic component. At 3 months of age, only the WT ET, EH, and 3xTG-AD ET groups successfully performed the CMOR task. All mice were re-tested at 6 months, 9 months, and 12 months of age, but the results were nonsignificant and none of the mice could successfully perform the CMOR task, possible due to procedural difficulties and age-related memory impairment. We therefore tested the mice on the olfactory-tactile MSO task at 12 months of age, which also included unimodal tactile and olfactory controls. Results revealed that ET mice (both WT and 3xTg-AD) were the only groups that could perform all three tasks (MSO and unimodal tasks). Thus, our early results suggest that enrichment track training early in life may confer cognitive and perceptual benefits related to multisensory integration and that this effect can reverse deficits on the CMOR and MSO tasks that relate to AD-like pathology.Supported by NSERC.

Disclosures: **S. Weber:** None. **H.A. Collett:** None. **E.A. Huff:** None. **S.D. Creighton:** None. **S. Gattas:** None. **R. Osterlee:** None. **A. Odina:** None. **K. Vint:** None. **J. Lin:** None. **S. McCabe:** None. **B.L. McNaughton:** None. **B.D. Winters:** None.

Digital Abstract Session

P099. Alzheimer's Disease Models: Behavioral Effects

Program #/Poster #: P099.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG008796
NIH Grant AG017139

Title: Spatial memory deficits are observed in young adult and middle-aged TgF344-AD rats

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Abstract: Early-onset Alzheimer's disease (AD) has been linked to mutations in the amyloid precursor proteins (APP), giving rise to the amyloid cascade hypothesis of AD (Selkoe & Hardy, 2016). The strong hereditary nature of mutations in APP gene with early-onset AD has led to much work and focus on the amyloid hypothesis using transgenic mouse models to understand AD (Myers & McGonigle, 2019). While the mouse models of AD engineered to overexpress just Aβ peptides are extremely useful to examine the effects of Aβ peptides (e.g., 5xFAD, Tg2576, PSAPP), they fail to recapitulate the complex range of human AD pathologies without the addition of human tau mutation (e.g., 3xTg-AD). Namely two critical factors are missing from the Aβ-overproducing transgenic AD mice: (1) lack of neurofibrillary tangles (NFTs) as a measure of robust tauopathy, and (2) lack of consistent and extensive neuronal loss. In addition, these transgenic mouse models generally have amyloid pathology early in life, unlike the majority of human AD cases that show more severe symptoms with aging. Therefore, we have established a colony of a rat model of AD, TgF344-AD, that overexpress Aβ peptides and recapitulates the full range of human AD hallmarks without the introduction of additional human tau mutations (Cohen et al., 2013). Using hippocampus-dependent spatial water maze task, we found that memory of the hidden-platform location is significantly impaired in nearly 50% of young adult (6-8 month old: 11 female, 15 male) and middle-aged (12-13 mo: 6 female, 15 male) TgF344-AD rats, as compared to wild-type young adult F344 rats (6-8 mo: 9 female, 14 male). Preliminary immunohistochemical examination revealed extensive amyloid plaques detected with thioflavin S and 6E10, and increased hyperphosphorylated tau detected with AT8 in the temporal lobe regions (e.g., hippocampus, entorhinal cortex) from middle-aged TgF344-AD rats. Experiments are ongoing to continue behavioral, immunohistochemical, biophysical and proteomic experiments that will include middle-aged and aged wild-type (19-22 mo) F344 and aged TgF344-AD rats. The rats will also be tested in an additional hippocampus-dependent task, trace eyeblink conditioning.

Disclosures: M.M. Oh: None. **C. Lin:** None. **C. Spaeni:** None. **N. Vilcek:** None. **C. Hicheri:** None. **J.F. Disterhoft:** None.

Digital Abstract Session

P099. Alzheimer's Disease Models: Behavioral Effects

Program #/Poster #: P099.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA grant AG061831

Title: The APP23 mouse model of Alzheimer's disease exhibits early disruption of circadian rhythms that progress with developing pathology

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Abstract: Circadian rhythm disturbances impact nearly all Alzheimer's disease (AD) patients. The most common disturbances include daytime sleepiness, sundowning, insomnia and increasing sleep fragmentation. Early, preclinical changes in circadian rhythms and sleep in AD support the role of circadian disfunction as a driver of the disease and suggest that circadian disruption may be an important target for treatment. Therefore, there is a fundamental need to identify research models that recapitulate these key facets of the disease and permit investigation of therapeutic approaches. Notably, circadian disruptions have not been well characterized in AD mouse models. APP23 mice (transgenic mice with the Thy1 promoter driving expression of the human APP gene containing the Swedish mutation) have been reported to exhibit disrupted behavior resembling the sundowning seen in AD patients; however, detailed circadian phenotyping has not been performed. We hypothesized that circadian disruption in APP23 mice manifests early and progresses with advancing neuropathology. To assess circadian rhythms in APP23 mice and littermate controls (n=3-5 per sex and genotype), we measured behavioral sleep and ongoing cage locomotor activity from presymptomatic animals (starting at 7 mo). To assess circadian function, we measured responses to phase shifting of light (6-hr phase advance), and to negative masking (1-hr light pulse in the dark phase) and positive masking (1-hr dark pulse during the light phase). Our analysis showed that APP23 transgenic mice (Tg) exhibit progressive disruptions in sleep and circadian activity patterns, including the emergence of excessive wakefulness and activity in the active phase accompanied by excessive sleep fragmentation. In addition, at 10 mo of age APP23 Tg mice manifest an exceptionally fast entrainment to a 6-hour phase shift when compared to NTg, and display altered behaviors in response to negative and positive light masking. Further, analysis of older Tg mice with full symptomatic neuropathology (>15 mo) reveals that these mice do not present the age-related decline in activity behaviors we observe in age-matched NTg mice; instead, old APP23 Tg continue to manifest disrupted circadian activity patterns, including excessive agitation with elevated activity onset imprecision. Taken together, our findings show that APP23 Tg mice present early circadian disruptions that are progressive and advance with developing neuropathology. This suggests that the APP23 mouse may be a good model in which to study the interaction of circadian disruption and AD neuropathology.

Disclosures: D.S. Whittaker: None. **L. Akhmetova:** None. **J. Mott:** None. **C.S. Colwell:** None. **P.A. Desplats:** None.

Digital Abstract Session

P100. Alzheimer's Disease: Cellular Function

Program #/Poster #: P100.01

Topic: C.08. Ischemia

Support: CIHR grant "pannexin-1 opening in neurons"
CIHR grant "Mechanisms of suppression of excitotoxicity by amyloid beta"

Title: Pre-symptomatic levels of amyloid beta are neuroprotective against stroke

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Abstract: During Alzheimer's disease (AD) progression, the amyloid β (Aβ) protein is pathologically produced and deposited amongst the brain parenchyma. Only 5% of AD is familial, thus environmental risk factors and co-morbidities play a key role in disease development. For instance, ischemic stroke increases in the risk of developing AD by five-fold. Hypoxia, a product of ischemia, upregulates Aβ production with unknown functional outcome. Due to the link between amyloid burden and AD severity, we hypothesized that Aβ would enhance excitotoxicity during hypoxia. We recently demonstrated that ischemia triggers metabotropic NMDAR-dependent activation of PANX1 channels, contributing to the anoxic depolarization (aDP), a large inward NMDAR-dependent current that occurs in response to reversed glutamate uptake and elevated glutamate release during stroke, and eventually causing cell death. Since NMDAR signaling is known to be modulated by Aβ, we hypothesized that Aβ could modify stroke evoked PANX1 activity, the resultant aDP, and associated neuronal death. To our surprise, we found that low concentrations of Aβ are protective and reduce PANX1 opening during the aDP. Using whole-cell patch clamp electrophysiology and 2-photon microscopy on hippocampal slices from young 5xFAD or 5xFAD/Thy1-GCaMP6f mice, we determined that 5xFAD mice had altered aDP severity and calcium dysregulation compared to wild-type littermates, which was dependent on Aβ load. Low concentrations (pM to nM) of oligomeric Aβ attenuated the aDP, while reducing endogenous Aβ levels using a γ-secretase inhibitor increased aDP severity. Aβ potently blocked PANX1 currents in response to elevated NMDA and hypoxia, however, Aβ failed to block PANX1 currents directly (as determined in PANX1-expressing HEK cells). These findings were further explored using a novel model of photothrombotic stroke in awake and behaving mice. The molecular mechanisms of Aβ-dependent regulation of metabotropic NMDAR signaling were also investigated. These data reveal that Aβ production could be increased as a neuroprotective mechanism during hypoxia to reduce PANX1 activation, thereby attenuating the aDP and downstream cell death pathways. However, with prolonged/repeated ischemic events, Aβ could reach toxic levels and coincide with hallmark pathophysiology of AD.

Disclosures: A.K.J. Boyce: None. **L.A. Palmer:** None. **A.W. Lohman:** None. **R.J. Thompson:** None.

Digital Abstract Session

P100. Alzheimer's Disease: Cellular Function

Program #/Poster #: P100.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KAKENHI JP (22221004,17H01391)

Title: High-fat diet aggravates the hippocampal Alzheimer disease pathology in the App$^{NL-F/NL-F}$ mouse model

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Abstract: Insulin resistance, a major symptom of type-2 diabetes mellitus (T2DM), is a known risk factor for Alzheimer's disease (AD). Emerging evidence has shown that both diseases share similar pathophysiology which includes amyloidogenesis and cognitive decline. In the present study, we aimed to understand how T2DM or related conditions contribute to AD pathogenesis. We used the *App*$^{NL-F/NL-F}$ knock-in mouse model of AD, which contains a humanized Aβ region along with two pathogenic mutations, the Swedish "NL" and the Iberian "F" to express APP at wild-type levels while producing elevated pathogenic Aβ. *App*$^{NL-F/NL-F}$ mice recapitulate several AD-associated pathologies, including amyloid plaques in the cortex and hippocampus, and show signs of very mild cognitive impairment at 18 months of age, but avoid potential artifacts introduced by the *APP* transgene. For this study, 6-month-old *App*$^{NL-F/NL-F}$ and wild-type male mice were fed with a regular diet (RD) or high-fat diet (HFD) for 12 consecutive months. We then compared the effects of the diet on the AD pathogenesis and hippocampal gene expression profiles between the two mouse lines. HFD treatment successfully increased body weight and impaired glucose tolerance levels in both mouse lines at 18 months of age. However, behavioral analysis by Morris water maze revealed an impaired cognitive function only in HFD-fed *App*$^{NL-F/NL-F}$ mice, accompanied by marked increases in both Aβ deposition and microgliosis. Moreover, HFD-fed *App*$^{NL-F /NL-F}$ mice exhibited a significant decrease in the volume of the granule cell layer in the dentate gyrus and an increased accumulation of 8-oxoguanine, an oxidized guanine base in nuclei of the granule cells. Gene expression profiling by microarray revealed that populations of cell types in hippocampus were not significantly changed between the two mouse lines regardless of the diet. Interestingly, we found that HFD treatment decreased expression of the amyloid binding protein, transthyretin (TTR) mRNA, in *App*$^{NL-F/NL-F}$ but not wild-type mice. We further confirmed the decreased protein levels of TTR by western blotting and immunofluorescence microscopy suggesting that the decreased levels of TTR may be a cause of the increased amyloid deposition in the hippocampus of HFD-fed *App*$^{NL-F/NL-F}$ mice. It is likely that the chronic exposure to HFD increased oxidative stress in the brain of *App*$^{NL-F/NL-F}$ mouse, thereby aggravating the AD pathology through altered gene expression. Further studies using the HFD-fed *App*$^{NL-F/NL-F}$ mice will help us to understand the exact mechanisms underlying the effects of T2DM on the AD pathogenesis.

Disclosures: **G. Mazzei:** None. **N. Abolhassani:** None. **R. Ikegami:** None. **N. Haruyama:** None. **K. Sakumi:** None. **T. Saito:** None. **T. Saido:** None. **Y. Nakabeppu:** None.

Digital Abstract Session

P100. Alzheimer's Disease: Cellular Function

Program #/Poster #: P100.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U54 AG054349
Harry Bubb through Cure Alzheimer's Fund CAF-50997
NIH/NIA Grant AG027544
NIH Grant AG00538
NIH Grant AG54884
NIH Grant OD010420
BrightFocus Foundation Grant A2015535S

Title: Humanized amyloid-beta expression drives accumulation of Periodic Acid-Schiff granules in the hippocampus of a knock-in mouse strain

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Abstract: Most Alzheimer's disease (AD) cases are sporadic and late-onset, yet nearly all existing mouse AD models harbor pathogenic mutations, rendering them better representations of familial autosomal-dominant forms of the disease. As a foundational step to model late-onset AD (LOAD), we generated and analyzed knock-in mice that express wildtype human amyloid beta (hAβ-KI) under control of the mouse *App* locus in exon 14 flanked by *loxP* sites (FL). Using confocal microscopy, we performed immunofluorescence stains on coronal brain slices and quantified the appearance of astrocyte-associated clusters of granules, which stained positive via Periodic Acid-Shiff (PAS) stain. To correlate the appearance of PAS granules with hAβ expression, we crossed hAβ-KI-FL mice with UBC-Cre^{ERT2} mice to enable Tamoxifen-inducible cre recombinase-mediated inactivation of hAβ expression. Finally, we generated hAβ-KI; PS1^{M146V} mice to investigate whether co-expression of familial-linked AD mutations affect the hAβ-induced presence of PAS granules. Changing 3 amino acids in the mouse Aβ sequence to its wild-type human counterpart produced age-dependent accumulation of PAS granules in the hippocampus of hAβ-KI mice by 10 months of age. Additionally, we observed impairments in cognition and synaptic plasticity in hAβ-KI mice. Remarkably, ablating hAβ expression using inducible cre reduced the formation of PAS granules and rescues cognition. Co-expression of familial-linked AD mutations with hAβ exacerbated the accumulation of PAS granules compared to homozygous hAβ-KI and WT mice. In conclusion, substituting mouse Aβ with the wild-type human isoform produces significant accumulation of PAS granules and impairments in cognition

and synaptic plasticity, highlighting the usefulness of this hAβ-KI mouse strain in investigating risk factors of LOAD.

Disclosures: **D.I. Javonillo:** None. **K.M. Tran:** None. **D. Baglietto-Vargas:** None. **J. Phan:** None. **S. Forner:** None. **C. Da Cunha:** None. **S. Kawauchi:** None. **A.J. Tenner:** None. **F.M. La Ferla:** None. **G.R. MacGregor:** None. **K.N. Green:** None.

Digital Abstract Session

P100. Alzheimer's Disease: Cellular Function

Program #/Poster #: P100.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CAPES/PNPD
CNPq
FAPERJ
ISN / CAEN

Title: An oxidative mechanism for cholinergic dysfunction in neurons exposed to Aβ-oligomers

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Abstract: An oxidative mechanism for cholinergic dysfunction in neurons exposed to Aβ-oligomers
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The neurotransmitter acetylcholine is synthesized by the enzyme choline acetyltransferase (ChAT), which is also a phenotypic marker of cholinergic neurons. Defective cholinergic transmission may underlie the onset and development of several CNS pathologies, including Alzheimer's disease (AD). Previous work by our group, using the avian retina as a CNS model, showed that ChAT activity in cultured or ex vivo neurons is markedly and specifically down-regulated by excitotoxic stimuli. This effect precedes any changes in cell viability or enzyme levels and requires calcium influx and nitric oxide (NO) production. More recently, we reported similar results in a more specific pathological context, using amyloid-β peptide oligomers (AβOs). These diffusible toxins accumulate in the AD brain and may be central to the disease. Exposing cultured cholinergic neurons to AβOs inhibited ChAT activity before any detectable loss of neurons or enzyme expression. The effect was linked to excitotoxicity and reactive oxygen species production, being likely caused by oxidative damage to the enzyme. Here, we expand those observations to a mammalian model, using cultured neurons of the rat septal region, and attempt to identify oxidative modifications involved in ChAT inhibition. Using S-nitrosothiol resin-assisted capture and thiol labeling, we show that cysteine modification is not central to the mechanism of inhibition. Tyrosine nitration, however, was found to be induced in cultures exposed to glutamate, AβOs and NO donors, and correlated well with loss of ChAT

activity. Results suggest a novel mechanism of cholinergic dysfunction that precedes neuronal death and may be relevant in early-stage AD pathology.

Disclosures: **L.E. Santos:** None. **S.T. Ferreira:** None. **F.G. de Mello:** None.

Digital Abstract Session

P100. Alzheimer's Disease: Cellular Function

Program #/Poster #: P100.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIRG-12-241456
NIA 1K01AG042500
NIH-NIGMS: 5P20GM103446

Title: The Effects of Calmodulin Kinase IIA driven TDP-43 expression in 24 month-old APP/PSEN1 background mice.

Authors: *A. ANDERSON, S. AREZOUMANDAN, M. GITCHO, S. DAVIS;
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Abstract: TDP-43 functions as a heterogeneous nuclear ribonucleoprotein involved in mRNA transport, mRNA stability, transcription, and mitochondrial metabolism. TDP-43 is the major pathological protein in frontotemporal dementia and ALS. Previously, TDP-43 pathology has been described in up to 50% of those with Alzheimer's disease. Recent evaluation of this cohort revealed a distinct pathological staging of TDP-43 proteinopathy in an aged population, which overlaps frontotemporal lobar degeneration (FTLD-TDP) and Alzheimer's disease. This overlapping pathological cohort is named limbic-predominant age-related TDP-43 encephalopathy (LATE). Through a model of cortical/hippocampal expression in an APP/PSEN1, we have extended our previously reported characterization of 9-month old mice to determine how age contributes to neurodegeneration. This TDP-43 proteinopathy is present in 20-50% of AD cases in those 80 and over. The mice characterized for this study are 24 months of age, which may be comparable to approximately 70 years of age in humans. Both human TDP-43 and nuclear localization signal defective (ΔNLS) TDP-43 in an APP/PSEN1 background were evaluated. This model shows severe neuronal loss in the hippocampus, a change in plaque deposition, aggregated tau, and a decrease in survival. This aged model of TDP-43 proteinopathy may be of use in understanding how TDP-43 contributes to neurodegeneration. **Conclusions:** hTDP-43 expression in APP background shows pathological neuronal loss with emphasis on the dentate gyrus. Further research is needed to elucidate the specific mechanisms.

Disclosures: **A. Anderson:** None. **S. Arezoumandan:** None. **M. Gitcho:** None. **S. Davis:** None.

Digital Abstract Session

P100. Alzheimer's Disease: Cellular Function

Program #/Poster #: P100.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Screening in iPSC derived neurons for modifiers of Alzheimer's related phenotypes

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Abstract: Alzheimer's disease (AD) is a complex disorder with increasing prevalence and socio-economic burden. However, majority of strategies aimed at identifying therapies for AD have been focused on targeting Aβ or TAU, which make up the plaques and tangles commonly found in people with AD. Continued failure of the drug discovery process and the accompanying trials against these targets has necessitated more and better options for therapeutic intervention. Using a multi-parametric high content phenotypic readouts with neurons derived from human differentiated iPSCs with familial AD mutations, we aim to optimize a platform for CRISPR based rescue screens for the various phenotypes associated with the mutations, such as endo-lysosomal transport, synaptic dysfunction and neuronal toxicity. The multiple-phenotypic rescue approach will enable identification novel key pathways and/or targets which could serve has drug candidates for the treatment of AD

Disclosures: S. Jain: None. **M. BsiBsi:** None. **N. Heulvemans:** None. **J. deGroot:** None. **D.F. Fischer:** None.

Digital Abstract Session

P100. Alzheimer's Disease: Cellular Function

Program #/Poster #: P100.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association IIRG-09-134220
University of Catania intramural funds
Università Politecnica delle Marche PSA PJ040046_2018
NIH R01-AG034248

Title: A failure of Amyloid-β physiological function due to deletions of α7 nicotinic acetylcholine receptors triggers an Alzheimer's disease-like pathology

Authors: *M. TROPEA[1], W. GULISANO[1], D. D. LI PUMA[2,3], M. MELONE[4,5], O. ARANCIO[6], C. GRASSI[2,3], F. CONTI[4,5], D. PUZZO[1,7];
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Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder affecting the elderly, but its intricate pathophysiology remains unclear. The increase of amyloid-beta peptide (Aβ) and hyperphosphorylated tau protein, and the failure of cholinergic transmission, are considered key events in AD. However, previous works have demonstrated that, in the healthy brain, low physiological concentrations of Aβ are necessary for synaptic plasticity and memory processes, acting through α7 subtype nicotinic acetylcholine receptors (α7nAChRs). Thus, we hypothesized that α7nAChRs deletion might induce a compensatory increase of Aβ production that, in turn, would trigger an AD-like pathology. To validate this hypothesis, we evaluated whether α7nAChR KO mice might develop alterations typical of AD. 3xTg models of AD and wild type animals were used as controls. We found that α7 KO mice presented an age-dependent impairment of synaptic plasticity and memory, starting at 12 months of age. In particular, we evidenced an impairment of Long Term Potentiation and Paired Pulse Facilitation at the CA3-CA1 hippocampal synapses. Furthermore, Novel Object Recognition and Location, and Fear Conditioning behavioral studies, demonstrated a reduction of recognition, spatial and contextual fear memory in α7nAChR KO mice.The onset of the impaired phenotype was paralleled by an increase of Aβ levels and Amyloid Precursor Protein (APP) expression. This was accompanied by hyperphosphorylation of tau at different residues known to be involved in AD, i.e., Thr205, Ser199 and Ser396. Consistently, α7nAChR KO showed a decrease of GSK-3β (Ser9), an important regulator of tau phosphorylation. An increase of PHF-1 immunoreactivity and the presence of paired helical filaments and neurofibrillary tangles confirmed that α7nAChR deletion induced tau pathology. In conclusion, our findings suggest that α7nAChRs malfunction might precede the increase of Aβ and tau in the cascade of events leading to AD. These results provide a different perspective to interpret the failure of previous therapeutic approaches against AD. Furthermore, α7 KO mice could represent an interesting model to study sporadic AD and revise the role of Aβ, tau and cholinergic transmission in AD pathophysiology.

Disclosures: M. Tropea: None. **W. Gulisano:** None. **D.D. Li Puma:** None. **M. Melone:** None. **O. Arancio:** None. **C. Grassi:** None. **F. Conti:** None. **D. Puzzo:** None.

Digital Abstract Session

P100. Alzheimer's Disease: Cellular Function

Program #/Poster #: P100.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Prevent Blindness Ohio
Ohio Lions Eye Research Foundation

Title: Alzheimer's disease pathology accelerates age-related visual changes in the 3xtg mouse model

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Abstract: While Alzheimer's disease (AD) is often considered a disease of cognition, the earliest changes involve sensory systems, and thus serve as an attractive method for early diagnosis. This study aimed to temporally characterize the decline of retinal ganglion cell (RGC) function in the 3xtg AD mouse model using pattern electroretinogram (PERG) recordings. Sex and age-matched 3xtg and control C57BL6 mice were compared across three ages that represented early, emerging, and progressing disease states in 3xtg mice. Axonal transport along the retinofugal projection was also assessed in mice using cholera toxin subunit beta (CTB) which was injected into the intravitreal chamber of the eye and was then anterogradely transported to the superior colliculus. Synaptic structural markers (i.e. vesicular glutamate transporter 2 and estrogen-related receptor beta) were labeled immunohistochemically in the superior colliculus, and RGC densities in retina were labeled with RbPMS. Results indicated that while C57 mice did demonstrate age-related decreases in PERG amplitude beginning at 12 months, the 3xtg mice exhibited significant reductions in PERG amplitude earlier, beginning at 8 months of age, and a further decrease in PERG amplitude by 12 months of age. These results suggest that while age-related changes in RGC function are expected, the presence of AD-related pathologies exacerbates these deficits. Percent area fraction (PAF) analysis of the superior colliculus revealed that within 3xtg mice, females tended to have a lower PAF of CTB coverage than males, though this did not reach significance. By 12 months of age, the females exhibited a significant decrease in CTB coverage throughout the superior colliculus, indicating that axonal transport was deficient in these animals. Surprisingly, in male 3xtg mice, no age-related changes were noted. In both female and male 3xtg mice, no age-related changes for either synaptic marker (VGlut2 or ERRβ) was noted, suggesting that functional deficits precede structural changes in the 3xtg mouse model. Sections throughout the retina were sampled and the results revealed that in C57 mice, there was again an age-related decline in RGC density beginning at 12 months of age. In 3xtg, however, there was a significant decrease in RGC density at 8 months of age, with a further significant decrease at 12 months of age. The difference in RGC density between C57 and 3xtg was significantly different at 12 months of age with 3xtg having a much lower RGC density. Collectively, these results suggest that the presence of AD-related pathologies exacerbates age-related deficits in the visual system.

Disclosures: G. Frame: None. **C.M. Dengler-Crish:** None.

Digital Abstract Session

P101. Alzheimer's Disease: Targeting A-beta

Program #/Poster #: P101.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACYT A1-S-16282

Title: Eternal sunshine of a spotless worm mind: in vivo effects of peptide inhibitors of amyloid beta aggregation

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Abstract: Deposition of amyloid beta peptide (Aβ) in the brain is one of the major hallmarks in Alzheimer´s disease (AD) pathogenesis. The amino acid sequence of the Aβ peptide is essential for its self-assemble properties, a complex process that involves the production of soluble intermediates that lead to amyloid plaque deposition. Currently, soluble Aβ oligomers formed in early aggregation are being considered as primary neurotoxic agents and prevention of Aβ aggregation has emerged as a therapeutical target for AD. An approach in the development of peptide inhibitors of aggregation is the use of Aβ as a starting point. Previously, we generated three Aβ peptides variants by introducing the point mutations M35C, A30W or K28A in each one of two Aβ peptides of different length, 40 and 42 amino acids, respectively. All these substitutions lie in key regions that participate during the aggregation process. Cell culture assays showed a decrease in reactive oxygen species production correlated with a reduction in cytotoxicity but not with the aggregation properties of the variants. Furthermore, the 1-40 mutants were able to prevent *de novo* aggregation of the WT peptide while 1-42 mutants prevented and disorganized pre-formed WT aggregates. *Caenorhabditis elegans* is an *in vivo* model that allows for the screening of Aβ aggregation inhibitors and the evaluation of their associated effects on behavior. In this work, samples of around 30 age-synchronized worms were treated with each peptide. Lifespan and chemotaxis assays were performed in a wildtype strain to evaluate toxicity and associative memory. For chemotaxis assays, *C. elegans* was conditioned with benzaldehyde beginning at the egg stage. The conditioned worms associated the odor cue with the presence of food and were capable to remember the association, allowing the testing of memory recall in the worms. Additionally, the progression of an Aβ-dependent paralysis phenotype was assessed in transgenic worm strains expressing Aβ (CL4176 and CL2006). Only A30W mutant 1-40 variant showed a slightly reduced toxicity at 40μM with 20% of worms surviving up to 19 days, while the remaining peptides had little to non-effect in toxicity in comparison to wildtype Aβ. However, treatment with K28A peptide 1-42 variant was able to prevent loss of associative memory, while M35C and K28A mutants caused a delay of up to 48 hours of the paralysis phenotype onset. Although further studies need to be conducted to analyze the effectiveness of the peptides, these results suggest that some of the mutants could improve Aβ related phenotypes in *C. elegans* and, therefore, have a putative potential for the design of future drugs against Alzheimer's Disease.

Disclosures: D.P. Quijano: None. **A.E. Estrada:** None. **M. González:** None. **V.J. Valdés:** None. **R. Vidaltamayo:** None. **V. Zomosa:** None.

Digital Abstract Session

P101. Alzheimer's Disease: Targeting A-beta

Program #/Poster #: P101.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01DA038635-S1

Title: Identification of Novel Delta Opioid Receptor(DOR) Antagonists for the Treatment of Alzheimer's disease

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Abstract: Alzheimer's disease (AD) is the most common form of dementia, which affects 47 million people worldwide. There are over five million AD patients over the age of 65 in the U.S. which is predicted to increase to 16 million by 2050. There are currently no approved disease-modifying therapies for AD and attempts to prevent or slow the progression of the formation of beta-amyloid plaques by targeting both the beta-secretase 1 (BACE1) and gamma-secretase enzymes have not yet achieved clinical success. Studies suggest that indirect modulation of the function of these enzymes via G-protein coupled receptors (GPCRs) may provide a novel strategy to reduce A-beta peptide production with potentially less side effects. Among GPCRs that influence amyloidogenesis, the delta opioid receptor (DOR), in particular, has been shown to play an important role in the trafficking and function of BACE1 and gamma-secretase and in the production of A-beta peptide. DOR activation increases BACE1 and gamma-secretase activity *in vitro* and in a mouse model of AD, and antagonism of DOR specifically blocks the amyloidogenic pathway and efficaciously prevents AD progression in mice. These effects were demonstrated using a known DOR antagonist, naltrindole. However, the potential of DOR antagonists as therapeutic agents for AD is yet to be explored. The aim of this project is to create novel DOR antagonists via medicinal chemistry and identify the most promising lead compound based on binding, selectivity, and functional profile *in vitro*. Further, select a small set of the most promising compounds *in vivo* and evaluate the compound's ability to mitigate AD-like pathology using APP/PS double-transgenic mice. We report here the creation of multiple selective DOR antagonists with low nanomolar affinity/potency, and we have demonstrated the ability of these antagonists to block BACE1 activity *in vitro*. Lead compounds showed systemic stability and blood brain barrier penetration using the *in vivo* tail flick assay in CD-1 mice. We will further report our progress in testing these compounds *in vivo* using APP/PS AD model double-transgenic mice.

Disclosures: P. Tanguturi: None. **S. Ananthan:** None. **V. pathak:** None. **P.K. Venukadasula:** None. **C.E. Augelli-Szafran:** None. **J.M. Streicher:** None.

Digital Abstract Session

P102. Amyloid Beta: Mechanisms of Tissue Extraction or Depletion

Program #/Poster #: P102.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Field Neurosciences Institute, Ascension St. Mary

Title: Tetrahydrocurcumin showed greater anti-amyloid properties than other curcumin-derivatives in turmeric extract: a comparative molecular docking and in vitro studies

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Abstract: Decreasing amyloid beta protein (Aβ) levels using anti-amyloid compounds is a promising strategy to prevent Alzheimer's disease (AD). We and others have demonstrated that curcumin (Cur) is a potent anti-amyloid compound. Unfortunately, Cur comprises only a small percentage of the compounds comprising tumeric extract and it has limited bioavailabilty. Therefore, investigating the anti-amyloid properties of other Cur-derivatives has sparked considerable interest in AD research. In this study, we compared the anti-amyloid and neuroprotective properties of different Cur-derivatives, such as bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC) and tetrahydrocurcumin (THC) using molecular simulation, *in-silico* and *in vitro* studies. We measured binding energy, torsion angle, and hydrogen bonding capabilities of different Cur-derivatives with Aβ42. Dot blot assays, photo-induced cross linking of unmodified protein (PICUP) and transmission electron microscopy (TEM) were performed to compare the Aβ aggregation inhibition using these derivatives. Neuroprotective effects of these derivatives were evaluated in N2a and SH-SY5Y cells using Aβ42 (10 μM) as a toxin. Finally, Aβ-binding capabilites were compared in the brain tissue derived from 5xFAD, a mouse model of AD. Molecular dynamic studies revealed that THC had greater binding capability to Aβ than other Cur-derivatives. In addition, the enol form of Cur (ECur) showed stronger binding to Aβ in comparison to keto form of Cur (KCur). THC showed greater inhibition of Aβ oligomers and fibrils than other Cur-derivatives as revealed by dot blot and PICUP methods. In addition, all of these derivatives showed a similar degree of neuroprotection *in vitro,* and labeled Aβ-plaques *in vivo*. Overall, THC is much more active in binding and inhibiting Aβ aggregation *in vitro* in comparions to other Cur-derivatives. Our findings suggest that THC may provide a more potent prophylactic therapeutic for inhibiting Aβ pathologies in AD than other Cur-derivatives.

Disclosures: P. Maiti: None. **J. Manna:** None. **N. Koli:** None. **L. Rakesh:** None. **G.L. Dunbar:** None.

Digital Abstract Session

P102. Amyloid Beta: Mechanisms of Tissue Extraction or Depletion

Program #/Poster #: P102.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: K08AG065463
P30AG013854-25
Alzheimer's Disease Discovery Fund Diagnostics Accelerator Program

Title: Soluble protein extraction from paraformaldehyde (PFA) and neutral buffered formalin (NBF) fixed paraffin embedded human brain tissue

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Abstract: Introduction Human brain banking is an essential examination of neurodegenerative proteinopathies, such as amyloid-β (Aβ), a primary pathologic culprit in Alzheimer's Disease (AD). Studies have demonstrated correlations between cognitive impairment and soluble Aβ (sAβ) levels. Furthermore, sAβ from frozen human brain tissue has shown threefold higher levels in AD when compared to healthy controls. However, the majority of human brain banked tissue is fixed and embedded in paraffin blocks. We have optimized and validated an efficient reproducible procedure for extraction of soluble proteins from paraformaldehyde (PFA) and neutral buffered formalin (NBF) fixed paraffin embedded human brain tissue which can then be quantified using assays for multiplexing such as Meso Scale Discovery (MSD). **Methods**: Human brains from the Northwestern University Alzheimer's Disease Center brain bank, with a range of Consortium to Establish a Registry for Alzheimer's disease (CERAD) scores, were fixed with either PFA or NBF, and blocks were embedded in paraffin. Blocks of neocortex were then cut into 3 curls, each 14 microns thick, and deparaffinized in xylene. Two different protein extraction techniques were attempted: one using the Qiagen QProteome FFPE Tissue Kit and the other using one of five homebrew extraction buffers. Homebrew extraction buffers contained 2% sodium dodecyl sulfate with increasing concentrations of tris (20mM, 100mM, 300mM, 400mM, and 500mM). BCA was used to assess total protein concentration. Samples were analyzed for total protein concentration via BCA. Western blots were performed for validation to confirm presence and amount of proteins of interest. **Results**: BCA findings revealed all extraction techniques to yield detectable protein, with the 300mM tris homebrew extraction buffer yielding the best results. Western blots confirmed extraction of soluble proteins including; Aβ, glial fibrillary acidic protein (GFAP), ionized calcium binding adaptor molecule 1 (Iba1), and human tar-DNA binding protein 43 (hTDP-43). Findings across cases with increasing CERAD scores were consistent with traditional semi-quantitative methods. Western blotting showed increasing Aβ with increasing CERAD scores. **Conclusions**: Effective extraction of soluble proteins from

PFA and NBF fixed paraffin embedded human tissue provides a useful approach for quantitative comparative studies of neurodegenerative disease associated proteins and other biomarkers.

Disclosures: **J. Lilek:** None. **K. Ajroud:** None. **C. Spencer:** None. **I. Ayala:** None. **A. Kawles:** None. **T. Gefen:** None. **A. Feldman:** None. **M. Mesula:** None. **Q. Mao:** None. **C. Geula:** None. **R. Vassar:** None. **E.H. Bigio:** None. **M.E. Flanagan:** None.

Digital Abstract Session

P103. Tau Aggregation and Phosphorylation

Program #/Poster #: P103.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1R41AG057274
NIH 1R21AG051820
NIH 1R01AG062435

Title: Hyperphosphorylated tau-based drug discovery for Alzheimer's disease and tauopathies

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Abstract: Alzheimer's disease (AD) is affecting 5.8 million Americans and more than 30 million people worldwide. There is currently no treatment or prevention for this devastating disease. AD is one of several neurodegenerative tauopathies that share a common pathology in the brain, that is, the deposition of hyperphosphorylated tau protein in selective neurons and other brain cells. AD patients suffer from progressive decline of cognitive and additional neurological functions. These clinical manifestations correlate with the spatiotemporal distribution of tau deposits. The pathological roles of abnormally phosphorylated tau have been recapitulated by intracranial injection experiments on mice. Accordingly, molecules that inhibit or enhance the pathological activities of hyperphosphorylated tau are potential therapeutics and risk factors, respectively, for AD. Identification of such molecules will likely lead to efficacious treatment or prevention. One of the formidable hurdles of tau-centric AD drug discovery has been the lack of a means to produce pathophysiologically relevant hyperphosphorylated tau for in vitro uses. We have developed the PIMAX system to synthesize hyperphosphorylated tau (p-tau) in *E. coli* that enables disease mechanism and drug discovery studies. We showed that p-tau produced by PIMAX possesses a core phosphorylation pattern highly relevant to the disease, and that the aggregates of p-tau causes cell death in vitro. Using a suite of different assays, we conducted a chemical library pilot screen from which we identified both p-tau aggregation inhibitors (apomorphine and raloxifene) and enhancers (certain benzodiazepines) that had been linked to cognitive impairments. These are active prescription drugs treating different clinical conditions, and they have previously been linked to cognitive integrity. Our discoveries suggest a molecular

mechanism underlying these prior findings, and that these drugs are excellent candidates for further development structure-activity relationship studies. Medicinal chemistry refinement of these compounds, as well as a larger-scale screen of novel compounds are ongoing.

Disclosures: **M. Kuo:** None. **M. Liu:** None. **T. Dexheimer:** None. **D. Sui:** None. **S. Hovde:** None. **R. kwok:** None. **K. Wang:** None. **H. Chien:** None.

Digital Abstract Session

P103. Tau Aggregation and Phosphorylation

Program #/Poster #: P103.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Johns Hopkins University Woodrow Wilson Undergraduate Research Fellowship

Title: Tau-related neurodegeneration in circadian clock neurons of drosophila

Authors: ***S. WANG**, M. KELES, K. DEIBEL, I. BLUM, M. WU;
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Abstract: Tau protein aggregation is one of the neuropathological hallmarks of Alzheimer's disease (AD), one of the most prevalent form of dementia worldwide. Accumulation of tau has been shown to lead to abnormal functioning and ultimately the death of neurons. Recent research suggests that pathological tau seeds spread synaptically and recruit native tau in connected neurons, leading to the spread of abnormal tau throughout the brain. However, how pathological tau induces toxicity and spreads between neurons remains unclear. Since many AD patients suffer from multiple sleep disorders and exhibit weakened circadian rhythms and degeneration of their suprachiasmatic nuclei, we used the power of Drosophila genetics to investigate the molecular mechanisms mediating tau toxicity. The experiments conducted used a model in Drosophila expressing human pro-aggregate mutants of tau to examine its morphological and behavioral effects in circadian clock neurons. Human tau was expressed in clock neurons using the pdf-GAL4 driver. We found that the expression of human tau leads to the elongated and thinner axon terminals that exhibit extensive branching compared to the controls. This morphological phenotype was observed for different variants of tau. We then looked at the effects of tau expression on the circadian behavior. Activity monitoring of 20-day old flies expressing tau revealed circadian arrhythmicity. In contrast, 3-day old flies had intact circadian rhythms suggesting an age dependent mechanism for tau related disruption of clock neuron physiology. Using this behavioral assay, we are currently planning to conduct an unbiased large-scale RNAi screen to identify novel genes that are required for tau toxicity.

Disclosures: **S. Wang:** None. **M. Keles:** None. **K. Deibel:** None. **I. Blum:** None. **M. Wu:** None.

Digital Abstract Session

P103. Tau Aggregation and Phosphorylation

Program #/Poster #: P103.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the Grant-in-Aid for Scientific Research on Innovative Areas “Brain Protein Aging and Dementia Control” (T.M. 26117004)

Title: Phosphorylation of microtubule-associated protein tau regulates its ability to bind and polymerize microtubules in mice brains.

Authors: *A. HAGITA[1,2], T. MIYASAKA[1,2];
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Abstract: Tau is a microtubule-associated protein (MAP) that has abilities to bind on microtubules (MTs) and stabilize them. Tau is also identified as a framework of insoluble inclusions, formed in the affected neurons in the brains of dementia, tauopathy. Because the abnormal tau purified from NFTs is hyperphosphorylated and lost its functions, it is considered that the phosphorylation of tau may affect its functions onto MTs. However, in fact, the effects of the phosphorylation on the physiological functions of tau in vivo remain obscure. Firstly, to analyze the MT formation in mice brains, we optimized the procedure that enables us to quantify not only stable but also labile MTs and free tubulins, and evaluate the dynamics of MAPs in vivo. Using this method, we found that approximately 35% of total tubulin forms stable MTs and 51% of it forms labile MTs. About 70% and 22% of total tau bound on stable and labile MTs, respectively. Therefore, a large part of tau and tubulin are incorporated into MTs. Tau recovered in soluble-free tubulin fraction was also associated with tubulin and still had an ability to assemble to labile MTs, but not stable MTs. Secondary, to examine the dynamics of tubulin and tau in response to phosphorylation, we analyzed anesthesia-induced hypothermia model, in which tau was hyperphosphorylated. About 10% of labile MTs-bound tau became free and lost its functions of MT polymerization, although neither dissociation of tau from stable MTs nor MT disruption was induced by tau phosphorylation. This result indicates that the functions of stable MTs-bound tau was more resistant to phosphorylation than that of labile MTs-bound or MTs-unbound tau. We further analyzed the MT formation in the postnatal stages in mice brains, in which the function of tau for MTs is suppressed by phosphorylation. Tau was highly phosphorylated in MTs-unbound fraction in the first week of life, then dephosphorylated until P14. According to its dephosphorylation, tau came to assemble into MTs. Despite of unchanged proportion of free tubulin in development, stable MTs were gradually increased according to the days after birth inversely with labile MTs. Interestingly, both of these tau hyperphosphorylation models share the effective phosphorylation site of tau detected by AT8. Finally, we found that phosphorylated and MTs-unbound tau was increased in P301S mutant tau transgenic (tauopathy’s model) mouse brain. These findings suggest that phosphorylation regulates the

functions of tau on MTs in mice brains under physiological condition, and also suggest that the phosphorylation determine abnormal behavior of tau in pre-pathological step.

Disclosures: A. Hagita: None. **T. Miyasaka:** None.

Digital Abstract Session

P104. Alzheimer's Disease: Tau Animal Models

Program #/Poster #: P104.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Boehringer Ingelheim Fonds (BIF) PhD Fellowship
Science, Technology, and Research Scholars (STARS) II Fellowship Program, sponsored by the Yale College Dean's Office

Title: Effects of Fyn kinase inhibition with AZD0530 on Tau pathology and Tau-induced phenotypes in mouse models of tauopathy

Authors: *S. TANG, S. NIES, H. TAKAHASHI, S. STRITTMATTER;
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Abstract: Alzheimer's disease (AD), one of many progressive neurodegenerative tauopathies characterized by the misfolding and aggregation of hyperphosphorylated Tau, has no known cure. Recent studies demonstrate that overactivation of Fyn, a Src family tyrosine kinase that can phosphorylate Tau, causes excitotoxicity and neuronal death via Tau and Fyn mis-localization in the post-synaptic area. We aimed to assess the utility of Fyn inhibition in preventing development of tauopathy with the Fyn kinase inhibitor AZD0530. The effects of Fyn inhibition were examined in a transgenic model of tauopathy expressing human 1N4R Tau with the P301S mutation (strain PS19) and in *in vitro* and *in vivo* models of Tau spreading using human AD brain-derived Tau fibrils (AD-Tau). We first treated PS19 mice and wild-type (WT) littermates with chow formulated with either AZD0530 or a vehicle control (n=11-15 per group). Treatment (10 mg/kg of AZD0530 per day *ad libitum*) began at two months of age, prior to the onset of Tau pathology or memory impairments. At eight months old, vehicle-treated PS19 mice exhibited a spatial memory deficit in the Morris water maze that was prevented with AZD0530 treatment. Immunohistochemistry revealed a reduction in Tau hyperphosphorylation, gliosis, and synaptic loss in the hippocampus of AZD0530-treated PS19 mice, although AZD0530 had no significant effect on phosphorylation at Tyr18 of Tau. Proximity ligase assay in HEK-293T cells expressing human Fyn and Tau suggested that AZD0530 treatment also decreases the Fyn/Tau interaction. To examine whether Fyn inhibition affects Tau spreading, we next treated primary neuronal cultures with AD-Tau in the presence or absence of AZD0530. We found that AZD0530 treatment significantly suppresses mouse Tau pathology induced by AD-Tau in cultures of primary neurons. To further investigate the effects of Fyn inhibition on Tau spreading *in vivo*, we injected AD-Tau into the hippocampus and cortex of the right hemisphere of 3-month-old WT (C57BL/6) mice and then treated the mice with AZD0530 for nine months starting at fourteen

days post-injection. In this setting, we failed to observe any effect of AZD0530 treatment on formation of Tau inclusions induced by AD-Tau. These data demonstrate that the accumulation of intracellular Tau and its spreading to other cells is differentially sensitive to Fyn inhibition in different model systems. The cellular and molecular determinants of Fyn-dependence for Tau pathology require further exploration based on neuronal subpopulation, glial cell participation, and endogenous Tau substrate for accumulation to define the potential role for Fyn inhibition as a therapeutic intervention.

Disclosures: S. Tang: None. **S. Strittmatter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Allyx Therapeutics. **S. Nies:** None. **H. Takahashi:** None.

Digital Abstract Session

P104. Alzheimer's Disease: Tau Animal Models

Program #/Poster #: P104.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Development of a neuronal microfluidic cell culture model for screening inhibitors of tau propagation

Authors: K. TITTERTON, N. VENKAT, K. YANAMANDRA, X. LANGLOIS, J. WU; AbbVie, Cambridge, MA

Abstract: The aggregation and propagation of the microtubule associated protein tau are key events in the pathophysiology of Alzheimer's disease (AD). Tau is a natively unfolded and soluble neuronal protein, which under pathological conditions, starts to aggregate and propagates between neurons. Recapitulating tau propagation using *in vitro* model systems has proven challenging. Here, we cultured transgenic murine neurons expressing all 6 isoforms of human tau on a mouse tau KO background in a microfluidic system. Using such devices allows neurons grown in two separate chambers to communicate between each other via axonal projections that extend through microgrooves and to establish synaptic connections between them. By seeding hTau neurons in one chamber only, we could show that tau aggregation was propagating from chamber to chamber, demonstrating unambiguously the trans-neuronal propagation of pathological forms of tau. Adding anti-tau antibodies to the receiving neuronal population(s) reduces tau propagation, suggesting that tau antibodies can stop inter-neuronal pathological tau spread. The *hTau* neuronal microfluidic platform can be used to identify anti-tau antibodies and other inhibitors of tau propagation.
Disclosure: All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Disclosures: K. Titterton: A. Employment/Salary (full or part-time):; AbbVie. **N. Venkat:** A. Employment/Salary (full or part-time):; AbbVie. **K. Yanamandra:** A. Employment/Salary (full

or part-time):; AbbVie. **X. Langlois:** A. Employment/Salary (full or part-time):; AbbVie. **J. Wu:** A. Employment/Salary (full or part-time):; AbbVie.

Digital Abstract Session

P104. Alzheimer's Disease: Tau Animal Models

Program #/Poster #: P104.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: George Mason University Psychology Department

Title: Traumatic brain injury increases tau tangle formation and cell loss in double transgenic APP/tau Alzheimer's type mice

Authors: *R. E. BARKEY[1], K. M. CRAVEN[1], N. T. COSCHIGANO[1], C. L. NEELY[1], D. D. CERRI[1], J. M. FLINN[2];
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Abstract: This study examined the effects of repetitive mild TBI (rmTBI) paradigm duringadolescence on the deposition of amyloid and tau and neuronal loss in dual tg AD mice at 8months of age. Alzheimer's disease (AD) is characterized by the formation of neuritic plaquescomposed of amyloid and neurofibrillary tangles composed of tau protein in the brain.Traumatic brain injury (TBI) increases the risk of developing AD. Increases in tau protein andamyloid plaques as well as neuronal degeneration are seen in TBI and may play a role in theprogression of AD, as these changes overlap with AD neuropathology. Adolescents are at anincreased risk of sustaining TBI and are then more vulnerable to a second concussive impact,with the majority of reported TBIs being mild. In this study, a dual transgenic (Tg) mouse modelthat expresses both amyloid and tau pathology (J20 hAPP bred with rtg4510 (tau) mice fromJackson Laboratories) underwent a novel rotational rmTBI paradigm to determine the effects ofrmTBI on the progression of AD pathology. Adolescent dual Tg mice sustained 5 closed-headinjuries with 48 hours between each hit, using a Controlled Cortical Impact (CCI) device on adropping platform to produce rotational effects that mimic the acceleration and decelerationforces seen in human TBI. Infralimbic (IL), cortical, and hippocampal brain regions wereassessed for tau pathology with Thioflavin-S, Congo Red, and Luxol FastBlue/Cresyl-Violetstains when mice were 8 months of age. There were significantly more tau tangles in Tg micethat sustained rmTBI compared to Tg mice who did not receive rmTBI seen in the IL (p =0.011), parietal association cortex (p = 0.002), entorhinal and perirhinal cortex (p< 0.001), visualassociation cortex (p = 0.015), mid dentate gyrus (DG) (p = 0.035), mid CA1 (p = 0.007), lateDG(p<0.001), and late CA1 (p< 0.033) In the temporal cortex, mice who sustained rmTBI hadlarger tau tangles than SHAM mice (p = 0.049). There were no significant differences in numberof amyloid plaques in any of the brain regions assessed (p> 0.05). There was significantly lower
cell density in the primary and secondary motor cortex (p = 0.044) and in the visual associationcortex (p = 0.024) in mice who received rmTBI compared to SHAM mice. There

were alsoseveral genotype effects were AD type mice had significantly lower cell density in hippocampalregions ($p< 0.001$), compared to wt mice, but AD mice also had significantly greater cell densityin the secondary somatosensory and primary and secondary motor cortical regions ($p< 0.05$)compared to wt mice. This study demonstrated that rmTBI during adolescence progresses theneuropathology of AD later in life.

Disclosures: **R.E. Barkey:** None. **K.M. Craven:** None. **N.T. Coschigano:** None. **C.L. Neely:** None. **D.D. Cerri:** None. **J.M. Flinn:** None.

Digital Abstract Session

P104. Alzheimer's Disease: Tau Animal Models

Program #/Poster #: P104.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ARC-NL Graduate Fellowship to ST
Dr. Howard Strong Memorial Fellowship in Aging to ST
CIHR Project Fund PJT-162124 to QY

Title: Understanding differential roles of stress and enrichment in pathogenesis of Alzheimer's Disease in a novel rat pre-tangle tau model

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Abstract: Tau pathology is a major hallmark of Alzheimer’s Disease (AD). Clinical studies have shown that tau pathology in humans is initiated in the locus coeruleus (LC), indexed by the appearance of hyperphosphorylated, pre-tangle tau. Pre-tangle tau occurs early in humans (in childhood or puberty) and evolution from pre-tangle to neurofibrillary tangles and symptomatic AD takes decades. We have established a pre-tangle tau model in rats by infusing pseudo-phosphorylated human tau in the LC, mimicking human origin of abnormal tau. This study aims to assess whether environmental stress or enrichment, either early or late in life, influences the general behaviour and cognitive functions in our pre-tangle tau model. Tyrosine hydroxylase-Cre rats were bilaterally infused in the LC with AAV9-rEF1a-DIO- htauE14-EGFP, or a control EGFP virus at 2-3 months old. Stress and enrichment paradigms were applied at either post-natal days 2-10 (early-life), or from 5-7 months old (adulthood). Cage controls did not have any behavioural interventions. Behavioural testing was carried out 7 months post-infusion, including measures of stress, anhedonia, spatial recall, and olfactory discrimination. Preliminary results show differential effects of environmental factors interacting with pre-tangle tau. While pre-tangle infused rats showed higher stress in elevated plus maze, late, but not early enrichment, reduced stress level. However, early enrichment improved spatial discrimination in the pre-tangle tau rats, while late stressed pre-tangle tau rats showed a trend of more severe deficiency in

a difficult odour discrimination learning. All together, these results suggest environmental factors may have brain region-specific effects in pre-tangle tau pathophysiology.

Disclosures: **S. Torraville:** None. **A. Ghosh:** None. **C. Reinhardt:** None. **T. Omoluabi:** None. **C. Crossley:** None. **L. MacGowan:** None. **Q. Yuan:** None.

Digital Abstract Session

P104. Alzheimer's Disease: Tau Animal Models

Program #/Poster #: P104.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Rna-binding proteins musashi and tau soluble aggregates initiate nuclear dysfunction

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Abstract: Oligomeric assemblies of tau and RNA-binding proteins (RBPs), Musashi (MSI) are reported in Alzheimer's disease (AD). However, the role of MSI and tau interaction in their aggregation process and its effects are nor clearly known in neurodegenerative diseases. Here, we investigated the expression and cellular localization of MSI1 and MSI2 in the brains tissues of Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Frontotemporal dementia (FTD) as well as in the wild-type, tau knock-out and P301L tau mouse models . We observed that the formation of pathologically relevant protein inclusions was driven by the aberrant interactions between MSI and tau in the nuclei associated with age-dependent extracellular depositions of tau/MSI complexes. Furthermore, tau and MSI interactions induced impairment of nuclear/cytoplasm transport, chromatin remodeling and nuclear lamina formation. Our findings provide mechanistic insight for pathological accumulation of MSI/tau aggregates providing a potential basis for therapeutic interventions in neurodegenerative proteinophaties.

Disclosures: **M. Montalbano:** None. **S. McAllen:** None. **N. Puangmalai:** None. **U. Sengupta:** None. **N. Bhatt:** None. **O.D. Johnson:** None. **M.G. Kharas:** None. **R. Kayed:** None.

Digital Abstract Session

P104. Alzheimer's Disease: Tau Animal Models

Program #/Poster #: P104.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R01NS087142-01
NIH Grant P50 AG025688

Title: Pathological Tau Induces Neurodegeneration by Sequestering and Inhibiting LSD1/KDM1A

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Abstract: Alzheimer's disease (AD) is an irreversible, progressive brain disorder caused by neuronal dysfunction in the cortex and hippocampus. AD is characterized by the aberrant accumulation of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated tau (NFTs). However, the molecular mechanism by which NFTs lead to neuronal cell death remains unclear. Surprisingly, we found that the histone demethylase LSD1/KDM1A is mislocalized to NFTs in AD cases and is completely depleted from the nucleus in the degenerating cortical neurons of PS19 Tauopathy mice. To determine if this interaction is functional, we deleted LSD1 in adult mice. Loss of LSD1 systemically in adult mice is sufficient to recapitulate many aspects of AD, including widespread neuronal cell death in the hippocampus and cortex, learning and memory defects, and global gene expression changes that match AD cases. This suggests that NFTs may interfere with LSD1 function by sequestering LSD1 in the cytoplasm. If Tau is functioning through the sequestration of LSD1, then reducing LSD1 in PS19 Tauopathy mice should make these mice more sensitive to pathological tau. Consistent with this, we find that reducing LSD1 in PS19 mice accelerates the depletion of LSD1 from the nucleus. This results in decreased survival, exacerbated paralysis, and increased neurodegeneration. Reducing LSD1 also exacerbates the genome-wide expression changes induced by the PS19 Tau transgene. Based on these data, we propose the following model: pathological tau leads to neuronal cell death in AD by sequestering LSD1 in the cytoplasm and interfering with the continuous requirement for LSD1 to epigenetically repress transcription associated with alternative cell fates. If this model is correct, then overexpressing LSD1 should make it more difficult for pathological tau to deplete LSD1 from the nucleus. This would be expected to delay the ability of tau to kill neurons. Strikingly, we find that viral overexpression of LSD1 in hippocampal neurons of PS19 mice at 8.5 months, when pathological tau is already present, is sufficient to suppress tau-induced neurodegeneration and block the Tau induced immune response through 11 months. In addition we find that overexpression of LSD1 specifically counteracts tau-induced gene expression changes genome-wide. This work establishes LSD1 as a major downstream effector of Tau mediated neurodegeneration and a highly promising target for therapeutic intervention in AD.

Disclosures: D.J. Katz: None. **A.K. Engstrom:** None. **A.C. Walker:** None. **R.A. Moudgal:** None. **D.A. Myrick:** None. **S.M. Kyle:** None. **Y. Bai:** None. **M.A. Christopher:** None.

Digital Abstract Session

P104. Alzheimer's Disease: Tau Animal Models

Program #/Poster #: P104.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH K12GM081266-11
JPB 194610-02
RCF 203805-01 Project 1

Title: Cellular and molecular mechanisms underlying the disease-enhancing effects of cyclic GMP-AMP synthase in tauopathy

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Abstract: Immune hyperactivation is a prominent feature of neurodegenerative diseases such as Alzheimer's disease (AD) and other tauopathies. Studies from our lab and others have demonstrated that immune dysregulation is not merely a bystander effect, but rather actively drives disease pathogenesis in various neurodegenerative disease models. Nonetheless, it is not completely understood what mechanisms specifically mediate these disease-enhancing effects of immune dysfunction in neurodegenerative diseases. Recently, aberrant activation of nucleotide sensing pathways has emerged as drivers of immune dysfunction and neurodegeneration in models of Parkinson's, Huntington's disease and amyotrophic lateral sclerosis. Here, we investigate the roles of cyclic GMP-AMP synthase (cGAS), a cytosolic DNA sensor implicated in antiviral interferon signaling, in disease pathogenesis in the P301S tauopathy model. To do this, we crossed P301S transgenic mice to *Cgas* knockout mice to generate transgenic and non-transgenic littermates expressing two, one or no copies of *Cgas,* and analyzed the effects of *Cgas* deletion on the molecular, cellular and behavioral changes associated with tauopathy. We utilized single nuclei RNA sequencing of aged P301S and non-transgenic hippocampi to generate robust molecular and cellular profiles of neurons and glial cells to ascertain genotype specific effects. Interestingly, our analyses revealed that *Cgas* deletion significantly reduced microglial and astrocyte transformation to interferon-enriched disease signatures, and mitigated pyramidal neuron loss in P301S mice. Further immunohistochemical analyses showed that *Cgas* deletion abolished tauopathy-associated synapse loss in CA1 neurons. Lastly, we performed Morris water maze tests to analyze spatial learning and memory. Striking, *Cgas* deletion completely rescued spatial learning, as well as short and long term memory impairments in P301S mice. Altogether, our results identify cGAS signaling as a disease enhancing mechanism that drives adverse cellular, synaptic, and cognitive changes in tauopathy.

Disclosures: J.C. Udeochu: None. **L. Fan:** None. **Y. Huang:** None. **H. McGurran:** None. **Y. Zhou:** None. **I. Lo:** None. **J. Holtzman:** None. **M. Gill:** None. **L. Gan:** None.

Digital Abstract Session

P104. Alzheimer's Disease: Tau Animal Models

Program #/Poster #: P104.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Institutes of Aging: Zhen Yan AG064656
National Institutes of Aging: Zhen Yan AG056060

Title: Epigenetic Treatment of Behavioral and Physiological Deficits in a Tauopathy Mouse Model

Authors: *W. WANG, C. QING, T. TAN, F. YANG, J. WILLIAMS, Z. YAN;
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Abstract: Epigenetic abnormality is implicated in the cognitive decline associated with neurodegenerative diseases, such as Alzheimer's disease (AD). Accumulation of neurofibrillary tangles composed of hyperphosphorylated tau is a common feature of AD. Transgenic mice that express mutant P301S human tau protein develop AD-like progressive tau pathology and cognitive impairment. Here, we show that the euchromatic histone-lysine N-methyltransferase 2 (EHMT2) is significantly elevated in the prefrontal cortex (PFC) of P301S Tau mice (5-6 months old), leading to the increased repressive histone mark, H3K9me2, which is reversed by treatment with the selective EHMT inhibitor UNC0642. Behavioral assays show that UNC0642 treatment induces the robust rescue of spatial and recognition memory deficits in P301S Tau mice. Concomitantly, the diminished PFC neuronal excitability and glutamatergic synaptic transmission in P301S Tau mice are also normalized by UNC0642 treatment. In addition, EHMT inhibition dramatically attenuates the level of hyper-phosphorylated tau in PFC of P301S Tau mice. Transcriptomic analysis reveals that UNC0642 treatment of P301S Tau mice has normalized a number of dysregulated genes in PFC, which are enriched in cytoskeleton and extracellular matrix organization, ion channels and transporters, receptor signaling and stress responses. Together, these data suggest that targeting histone methylation enzymes to adjust gene expression could be a potential therapeutic strategy for cognitive and synaptic deficits in neurodegenerative diseases associated with tauopathies.

Disclosures: W. Wang: None. **C. Qing:** None. **T. Tan:** None. **F. Yang:** None. **J. Williams:** None. **Z. Yan:** None.

Digital Abstract Session

P104. Alzheimer's Disease: Tau Animal Models

Program #/Poster #: P104.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The role of sleep in the progression and treatment of Alzheimer's Disease

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Abstract: Sleep is an essential physiological behavior that supports brain health and cognitive function. In Alzheimer's disease (AD), a devastating neurodegenerative disorder, patients experience accelerated sleep loss which can be correlated with AD onset and contribute to AD

progression. Tau is an axonal microtubule stabilizing protein that forms aggregates in AD and contributes to the cognitive decline, synapse loss and neuronal death. Tau mislocalization and aggregation at synapses may impair sleep and restorative sleep-dependent homeostatic plasticity. I hypothesize that sleep disruption occurs early in AD progression and subsequently drives further tau mislocalization and aggregation, and cognitive decline. Preliminary sleep behavior data using P301S (PS19) transgenic mice shows that differences in sleep behavior arise as early as 3 months in females and 6 months in males. Accumulation of tau becomes apparent between 6-9months. These results highlight sex differences in the onset of sleep disruption and support sleep disruption as an early-stage symptom. Endocannabinoids provide an intriguing avenue for therapeutic intervention because of their role in promoting sleep and anti-inflammatory signaling. Preliminary work shows that sleep disruption in PS19 mice can be acutely reversed by increasing the endocannabinoid anandamide. The objectives of this work are to investigate the link between sleep loss and tau aggregation and to identify the therapeutic window targeting endocannabinoid signaling during sleep in advance of tau pathology and cognitive decline. These studies will provide a deeper understanding of the behavioral and molecular changes that occur during abnormal sleep in AD and highlight endocannabinoids as a suitable signaling pathway for enhancing the restorative benefits of sleep.

Disclosures: S. Martin: None. **L. Sharek:** None. **J. Lord:** None. **G. Diering:** None.

Digital Abstract Session

P105. Therapeutic Strategies: Preclinical Cellular Models

Program #/Poster #: P105.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant DGAPA/PAPIIT number IA210620

Title: Characterization of signaling pathways associated with neurodegeneration in skin fibroblasts from patients with familial Alzheimer's (FAD-PS1 M146L/A246E)

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[2]Physiol., Sch. of Medicine, UNAM, Mexico City, Mexico

Abstract: *Introduction:* Alzheimer's disease (AD) is a neurodegenerative disorder characterized by memory loss. There are two types of AD: sporadic and familial Alzheimer's. Mutations in the genes of amyloid precursor protein (APP), presenilin1 (PS1), and presenilin 2 (PS2) cause the early onset familial AD contributing to APP processing, Amyloid Beta peptide generation and tau protein hyperphosphorylation. It is known that the identification of early biomarkers for the diagnosis of AD represents a challenge. It has been suggested that the use of peripheral cells other than neuronal cells such as mesenchymal cells from bone marrow or dental pulp, blood cells, and fibroblasts derived from patients with familial or sporadic AD represent an opportunity

to detect related molecular changes within neurodegenerative pathways that have been previously studied in the brains of Alzheimer's patients and neuronal cultures from murine Alzheimer's models that together with other diagnostic clinical criteria, could allow the early identification of AD patients. *Methods:* We cultured skin fibroblasts from patients with familial Alzheimer's disease with a Presenilin 1 mutation (M146L or A246E) and apparently healthy individuals obtained from the Coriell Institute (New Jersey) cell repository in Earl MEM salts medium with 15% non-inactivated Fetal Bovine Serum. These cells were characterized by immunodetection of the markers Vimentin and S100A4 and their chromosomal stability through karyotyping. From an in silico analysis of the data reported by Antonell's group in 2016 from brain samples from patients with FAD-PS1, we identified pathways associated with neurodegeneration, which we confirmed using Western blot techniques and a proteomics study performing 2-DE gels and mass spectrometry analysis. *Results:* We identified differences in the expression of proteins related to the autophagic-lysosomal pathway (LC3-II, LAMP2, and CATD) in affected individual´s fibroblasts. We also identified changes in kinases related to hyperphosphorylation of tau protein (ERK1/2, GSK3 and ptau231), and proteins related to cellular stress (HSP60 and HSP70), changes that have been identified in nerve tissue samples from patients with neurodegenerative diseases such as AD. *Conclusion:* In summary, our results indicate that samples derived from peripheral tissue from patients with FAD-PS1, such as fibroblasts, show altered pathways associated with neurodegeneration, giving evidence that fibroblasts can be useful in the search and modeling of pathways related to neurodegeneration as well as for identification of early biomarkers related to AD.

Disclosures: G. Lopez-Toledo: None. **M. Silva-Lucero:** None. **J. Arias-Montaño:** None. **M. Cardenas-Aguayo:** None.

Digital Abstract Session

P105. Therapeutic Strategies: Preclinical Cellular Models

Program #/Poster #: P105.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant EY005121

Title: Elovanoids (ELVs) and Neuroprotectin D1 (NPD1) counteract senescence programing by inverting the amyloidogenic pathway.

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Abstract: *Purpose:* Aβ42, a product of the amyloidogenic pathway, is a component of drusen in age-related macular degeneration (AMD) and of senile plaques in Alzheimer’s disease (AD). In AMD, Aβ triggers inflammation, perturbs retina function and activates senescence signaling. We showed recently that Elovanoids (ELVs) counteract Aβ-peptide-induced senescence programing (Do KV et al., PNAS, 2019). In this study, we aim to overexpress the amyloidogenic pathway.

We hypothesize that ELVs and NPD1 reverse the formation of Aβ towards the pro-survival sAPPα and downregulate the senescence signaling in primary human retinal pigment epithelial cells. *Methods:* Plasmid construct containing Swedish double mutant APP protein (APPsw), which dominantly produces sAPPβ (a precursor of amyloid beta), was transfected into primary human RPE cells. After 24h, ELVs alone, NPD1 alone were added at 500nM concentrations in sAPPβ overexpressed cells. After 24h and 48h of treatment, the medium were collected, precipitated and subjected to the Western Blots for holo-APP precursor, sAPPβ for amyloidogenic pathway, and sAPPα for non-amyloidogenic pathway. The cellular samples are also collected for α-secretase (ADAM10), β-secretase (BACE1) and γ-secretase (PS1) measurement. eGFP transfected cells were used as negative controls. The mRNA was extracted and analyzed the senescence gene expression using qPCR. *Results:* The senescence in the sAPPβ overexpressed cells was confirmed by qPCR at 24h, consistent with the activation of amyloidogenic pathway. At 24h, higher sAPPα production was observed in the ELV treatment. At 48h, higher sAPPα production was observed in the NPD1 treatment. In contrast, reverse production of sAPPβ was observed, lower level with ELV treatment 24h and with NPD1 at 48h. An equal amount of holo-APP, regarded as total of all sAPP forms, was detected in all studied conditions. The mechanisms involve the increase of α-secretase (ADAM10) and decrease of β-secretase (BACE1) by ELVs and NPD1. The senescence signaling is also inhibited by the treatment with ELVs and NPD1 in the sAPPβ overexpressed cells. *Conclusions:* ELVs and NPD1 shift the amyloidogenic pathway to the non-amyloidogenic sAPPα and protect the human RPE cells from senescence, which is activated by overexpression in Swedish double mutant APP. However, the ELVs have earlier protection, at 24h, when compared with NPD1.

Disclosures: K.V. Do: None. **M.I. Kautzmann:** None. **B. Jun:** None. **N.G. Bazan:** None.

Digital Abstract Session

P105. Therapeutic Strategies: Preclinical Cellular Models

Program #/Poster #: P105.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Review: vitamin k_2 shows promise as a novel therapy for alzheimer's disease

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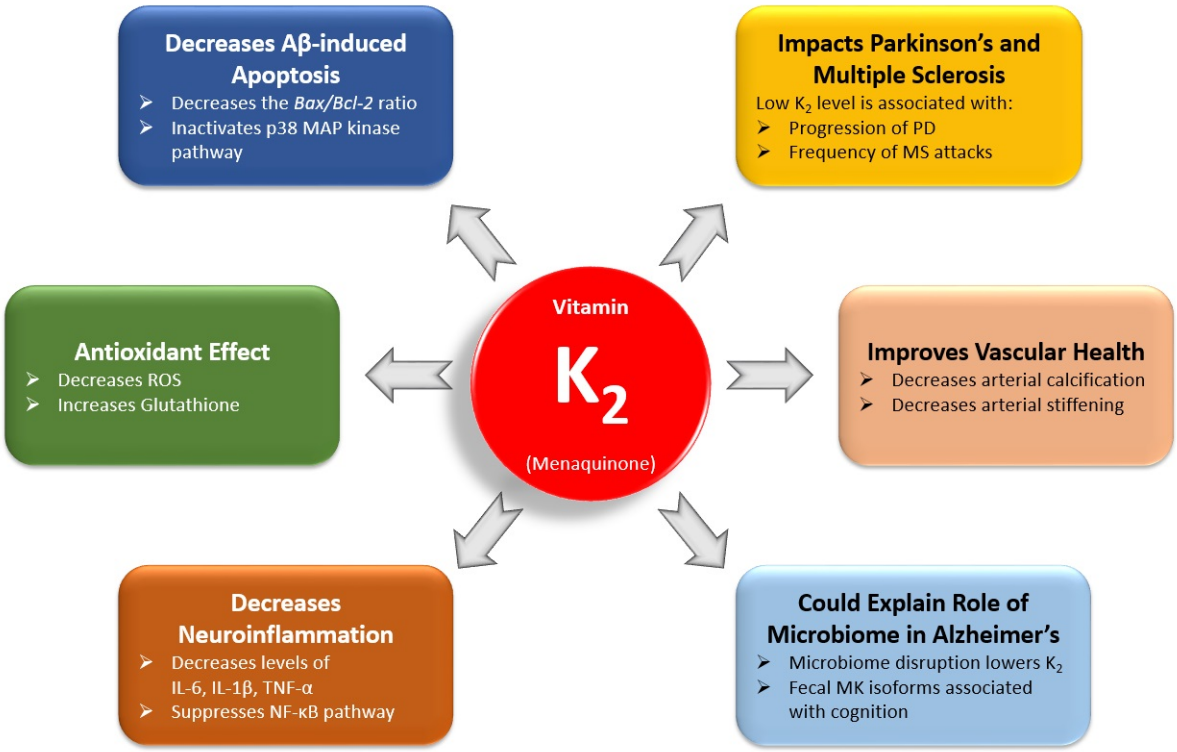
Abstract: We review the evidence for a relationship between vitamin K_2 and Alzheimer's disease, emphasizing the need for further research. Because K_2 has emerged as an essential nutrient for human health, we propose that it is of critical importance to investigate its role in Alzheimer's, a debilitating disease for which no cure has been developed. K_2, also known as menaquinone (MK), is one of two forms of vitamin K, and it has been shown to impact neuroinflammation, cardiovascular health, and cognition. K_2 is essential for activating Gla proteins such as the matrix Gla protein and growth arrest-specific protein 6 (Gas6). The matrix Gla protein is important for reducing arterial calcification, and Gas6 regulates cell growth and is

widely expressed in the nervous system. The hallmark of Alzheimer's is the extracellular deposits of neurotoxic beta-amyloid (Aβ) plaques in the brain. Two different studies used cell cultures to demonstrate that K_2 and Gas6 suppress Aβ cytotoxicity and strongly suggest the antiapoptotic effects of K_2 (Yagami et al., 2002; Hadipour et al., 2020). Two other studies that used different types of glial cells (microglia and astrocytes) and distinct forms of K_2 (MK-4 and MK-7) investigated the role of K_2 in glial activation and both found that K_2 inhibited the production of proinflammatory cytokines (Saputra et al., 2019; Yang et al., 2020). Three European population studies provide evidence that K_2 improves vascular health by reducing arterial calcification, arterial stiffness, and the risk of cardiovascular events (Geleijnse et al., 2004; Beulens et al., 2009; Knapen et al., 2015). We propose that K_2 can influence the development of Alzheimer's through its role in vascular health. Although low levels of K_2 have been associated with the progression of other neurodegenerative diseases such as Multiple Sclerosis and Parkinson's disease, there is a lack of research on the role of K_2 in Alzheimer's. We conclude that it is necessary to perform clinical studies that directly explore the role of K_2 in Alzheimer's.



Disclosures: A. Popescu: None.

Digital Abstract Session

P105. Therapeutic Strategies: Preclinical Cellular Models

Program #/Poster #: P105.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant DGAPA/PAPIIT IA210620

Title: Three-dimensional organoid-like cultures promotes the differentiation of human dental pulp mesenchymal stem cells into neuronal phenotype

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Abstract: Introduction: Dental pulp stem cells (DPSCs) are capable of both self-renewal and multi-lineage differentiation, which play a positive role in dentinogenesis. Dental pulp is a vital structure present in the inner core of tooth containing mesenchymal stem cells (MSCs). Gronthos *et al*., 2000, isolated the stem cells from dental pulp and termed it as DPSCs. DPSCs are one of the widely researched tissues for its easy availability without morbidity to the human organs or tissues. It is considered as an organic waste, which can be retrieved easily from dental clinic trash bin. Teeth extracted for orthodontic purposes, and impacted third molars in young adult people constitute good source of stem cells. Various studies have proved DPSCs can differentiate into osteogenic, neurogenic myogenic, vasculogenic, chondrogenic, and adipogenic lineages. DPSCs unlike other stem cells have distinction, that is, it is derived from neural crest cells and has better lineage potential to differentiate into neural tissues than bone marrow-derived stem cells. In neurodegenerative diseases, there is an important neuronal loss, thus neuronal differentiation from DPSCs could be a suitable source of stem cells with potential to differentiate into neural cells. Methods: We isolated DPSCs from a first molar donated from a healthy female of 46 years old. We used an enzymatic mix of collagenase-dispase to disaggregated the tissue, incubated at 37o C for 45 min, shaking it every 15 min, to obtain the cellular suspension that was then seeded on a T25 culture bottle. Cells were cultured at 37oC and 5% of CO2 in an incubator. Culture medium was DMEM-F12 + 10% inactivated FBS. Cells were banked in a Nitrogen tank or further storage.Results: Cells were characterized by Western blot and RT-PCR for the detection of phenotype markers such as CD73, CD105, CD166, Oct-4, SOX-2, Nanog and BIII-Tubulin, and Nestin, and they were negative to hematopoietic markers (CD14, CD34 and CD45). To form the organoid like structures we followed Lancaster et al 2014, Natures´s protocol, in brief, cells were cultured for 24-48 h without substrate and then for 35 days in Vitro (DIV) in different surfaces and scaffolds. After 35 DIV cultured on Geltrex scaffold, DPSCS, formed organoid like structures, that were positive to BIII-Tubulin, MAP-2, tau and NeuN, indicating its neuronal differentiation.Conclusion: This 3D culture approach for DPSCs, has an important therapeutic implication for the treatment of neurodegenerative disease, such as Alzheimer´s disease, Huntington disease and Parkinson´s disease. And for modeling the neuropathologies to better understanding the molecular process involve an to test potential new drugs.

Disclosures: **A.I. Gutiérrez-Malacara:** None. **M. Silva-Lucero:** None. **G. López-Toledo:** None. **M. Cardenas-Aguayo:** None. **C. Torres-Rosas:** None.

Digital Abstract Session

P105. Therapeutic Strategies: Preclinical Cellular Models

Program #/Poster #: P105.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Emory Goizueta Alzheimer's Disease Research Center Funding Award
Department of Veterans Affairs Career Development Award IK2RX000870
NIH-NIA grants: K01AG042498

Title: A Pilot Clinical Trial of Adapted Tango to Improve Negative Health Impacts in Middle Aged African-American Female Caregivers of Persons with Alzheimer's Disease

Authors: *L. JEONG[1], N. LIANG[2], R. SHIN[1], A. A. BAY[2], L. E. MCCULLOUGH[3], W. HU[2], A. R. HART[2], M. HACKNEY[2], W. WHARTON[4];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder resulting in memory loss and a reduction in the ability to perform activities of daily living. The role of caring for someone with AD in an African American (AA) family often falls on daughters, and the burden of caregiving can increase stress and depression, which are independent risk factors for AD. Furthermore, older AAs and women have an increased risk of developing AD than older whites and men. Taken together, these differences in risk suggest that AA women who are caregivers of a parent with AD are at high risk for developing AD. This phase I study compared the cognitive, motor, and psychosocial benefits of a well-established 12 week, 20-lesson adapted Argentine Tango intervention (N=24) to a no-contact control group (N=10) in middle-aged (45-65 years) AA women who are most at risk for the development of AD from parental history. Out of the thirty-four participants, some women (n=17) were familial caregivers; therefore, the impact of caregiving burden in AA women was examined in this subset. Participants were tested for inflammatory biomarkers in their blood and on their cognition, physical/motor ability, and mood before and after the intervention or control. Participants in the study were on average 60 years old, had a BMI of 30, and were highly educated. Significant effects were noted in the Tango group after the intervention, which showed reduced levels of tumor necrosis factor-alpha ($p=0.011$), interleukin-7 ($p=0.003$) , and interferon-gamma ($p=0.011$); improved performance on the maintenance of 30 s chair stand task ($p=0.018$), the Fullerton Advance Balance Scale ($p=0.023$), and inhibition of the color word interference test ($p=0.031$). Additionally, large effects were noted in Trails Making Test, as well as small-moderate effects in body position spatial task, and TOL move accuracy for the Tango group. Further studies should be conducted with this dance intervention in larger groups, as the trial resulted in substantial cognitive and motor improvements and reduction in inflammatory biomarkers through a non-pharmacologic and affordable solution that may decrease the risk of developing AD among AA familial caregivers most at risk.

Disclosures: **L. Jeong:** None. **N. Liang:** None. **R. Shin:** None. **A.A. Bay:** None. **L.E. McCullough:** None. **W. Hu:** None. **A.R. Hart:** None. **M. Hackney:** None. **W. Wharton:** None.

Digital Abstract Session

P105. Therapeutic Strategies: Preclinical Cellular Models

Program #/Poster #: P105.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: U01 AG057562
Handleman Emerging Scholar Program
Robert E. Nederlander Sr. Program for Alzheimer's Research

Title: Assessing cell survival and immunosuppression efficacy in intracranial human neural stem cell transplantation

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Abstract: Highly developed and effective immunosuppression (IS) protocols to prevent graft rejection are key to clinical translation of cellular therapies for the treatment of Alzheimer's disease (AD). Although Tacrolimus is a frequently used immunosuppressive agent for xenotransplantation applications, transplanted human cell survival was unreliable in our preliminary studies, with few grafts present 6 weeks post-transplant. Graft survival is typically not evaluated until terminal histology, making it challenging to efficiently assess IS *in vivo*. Bioluminescence Imaging (BLI) is a molecular imaging tool that uses light generated from a Luciferase enzyme-substrate for signal detection specific to live cells. Integrating BLI with cell transplantation would address these technical challenges and provide crucial insight into graft survival. Therefore, we set out to establish a BLI-based method of tracking cell viability *in vivo* to simultaneously assess graft survival and compare IS regimens in real time.
Human neural stem cells (hNSCs) were modified to express dual Firefly Luciferase and green fluorescent protein reporters (hNSC-FLuc+/GFP+). hNSCs were administered to male C57BL/6J mice at 8 weeks of age (360k total cells; 3 bilateral fimbria fornix injections). IS groups included Tacrolimus (Tac, 5mg/kg IP), Tac plus monoclonal antibodies (Mab) to CD40L and CD4 (20 mg/kg IP), Mab alone, and no IS. BLI validation controls included 180k unlabeled hNSC or dead hNSC-FLuc^{+}/GFP^{+}, with contralateral 180k live hNSC-FLuc^{+}/GFP^{+}. Serial BLI was performed on postoperative day (POD) 2, 7, 10, and weekly for 8 weeks using the IVIS Spectrum *In Vivo* imaging system.
Dead and unlabeled cell controls validated that BLI signal resulted only from viable, labeled cells. BLI signal correlated histologically with viable hNSC grafts. Robust BLI signal was visible in all mice at POD2. The no-IS group lost BLI signal after POD7 and the Tac group after

POD28. However, robust signal was seen in 100% of the Tac+Mab and Mab alone groups at POD56, indicating robust hNSC graft survival. Quantification showed significantly increased total flux in both Mab-receiving groups compared to no IS and Tac groups. Therefore, Mabs enable hNSC survival for 8+ weeks in BL6 mice. Further study of Mab IS has demonstrated robust hNSC survival through 6 months post-transplant. An additional study in 5XFAD mice is ongoing to assess Mab-based IS and hNSC survival in an AD mouse model.
In conclusion, CD4 and CD40L IS without Tacrolimus is sufficient for robust graft survival. Tacrolimus is not always well-tolerated in mice nor patients, so a transition toward Mab-based IS may be advantageous in cellular therapies.

Disclosures: **S.N. Mason:** None. **O.N. Kashlan:** None. **K.S. Chen:** None. **A.W. Stebbins:** None. **D. Rigan:** None. **G.G. Murphy:** None. **E.L. Feldman:** None. **L.M. McGinley:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R00AG044469
NIH R01 AG055581
NIH R01 AG056622
BrightFocus A2017457S

Title: Memory impairments in Tg19959 and APP/PS1 AD model mice are alleviated by eEF2K inhibitor A-484954

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Abstract: Mounting evidence indicates synaptic failure as an early and key event in Alzheimer's Disease (AD) pathophysiology. Maintenance of long-term memory and synaptic plasticity requires *de novo* protein synthesis. Phosphorylation of mRNA translation factor eukaryotic elongation factor 2 (eEF2) by its kinase eEF2K results in inhibition of general protein synthesis. Previous studies have shown elevated levels of eEF2 phosphorylation in *post mortem* AD human brain tissue and in AD mouse models. Here we investigated whether suppression of eEF2 phosphorylation via eEF2K inhibitor A-484954 can alleviate AD-associated synaptic failure and memory impairments. Aged Tg19959 mice (6-9 months), APP/PS1 (12-16 months) and age-matched controls were treated with a subcutaneous pellet containing A-484954 or vehicle. The pellet continuously releases treatment over 30 days. Starting two weeks after pellet placement, the mice underwent cognitive assessment via the Novel Object Recognition (NOR) and Morris Water Maze (MWM) tasks. We found that cognitive impairments displayed in aged Tg19959 and APP/PS1 mice were alleviated with treatment of A-484954. Brain tissue from these mice also underwent high performance liquid chromatography (HPLC) to assess drug concentration.

In addition, Golgi-Cox stain was done to assess dendritic spine density and morphology. Spine analysis indicates a rescue in levels of mature spines in the CA1 region of the hippocampus in APP/PS1 mice treated with A-484954. Taken together, our results suggest that treatment with a eEF2K inhibitor, A-484954, alleviates cognitive impairments and restores synaptic morphology in a mouse model of AD.

Disclosures: **N. Kasica:** None. **W. Yang:** None. **X. Zhou:** None. **T. Ma:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF MSIT 2016M3C7A1904391
NRF MSIT 2020M3E5D9080660

Title: Mitochondrial dysfunction by lactate dehydrogenase B deficiency causes oxidative stress and neurodegeneration via suppressing AdipoR1/PGC1-alpha signaling.

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Abstract: Age-dependent decrease of mitochondrial function and oxidative stress play critical role in neurodegeneration. Lactate dehydrogenase B (LDHB) is a glycolytic enzyme that catalyzes the interconversion of pyruvate and lactate in brain. Osmotin, a homolog of mammalian adiponectin is known to regulate AdipoR1/ PGC1-alpha signaling. Of note, PGC1-alpha downstream signal pathway known to regulate mitochondria biogenesis and antioxidative stress. In this study, To elucidate the role of Osmotin in mitochondrial function and to test the hypothesis that mitochondrial dysfunction induced by LDHB deficiency plays an significant role in neurodegeneration, we conducted an experiment using LDHB knockout ($LDHB^{-/-}$) mice. Depletion of LDHB proteins from the $LDHB^{-/-}$ mice, resulted in a dramatic increase oxidative stress in cortex and hippocampus and elevated the expression of apoptosis mediators such as Bax (Bcl-2-associated X protein), Caspase-3, Cytochrome C in the cortical and hippocampal regions. Moreover, we assessed the LDHB knockout-induced oxidative stress-mediated neuroinflammation, neuronal apoptosis, and cognitive deficits in $LDHB^{-/-}$ mice. Treatment with osmotin markedly attenuated LDHB knockout-induced oxidative stress, synaptic dysfunction, and learning and cognitive deficit via upregulating AdipoR1/ PGC1-alpha signaling. In briefly, these findings reveal that osmotin rescues oxidative stress-mediated neuroinflammation, neurodegeneration via upregulating AdipoR1/ PGC1-alpha signaling in $LDHB^{-/-}$ mice. This research suggests that suppression of LDHB may be closely related with the oxidative stress or progression of neurodegeneration and osmotin might be potential antioxidant and mitochondria biosynthesized agent via upregulating AdipoR1/ PGC1-alpha signaling.

Disclosures: **J. Park:** None. **K. Saeed:** None. **M. Jo:** None. **M. Jo:** None. **M. Kim:** None. **H. Lee:** None. **M. Kim:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Effects of tart cherry extract and omega fatty acids on behavioral outcomes and amyloid beta pathology in the 5xFAD animal model of Alzheimer's disease

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Abstract: Background: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder. The two cardinal protein aggregations observed in the AD brain are β-amyloid peptide (Aβ) and subsequently hyper phosphorylated tau. Multiple diseases can cause dementia, but AD is the most common accounting for nearly 70% of cases. Nutraceuticals or foods identified as giving health benefits have demonstrated progress in animal models and humans. There are currently multiple clinical trials looking at treating patients with exogenous antioxidants and omega fatty acids. This study tested TBR, a proprietary blend of tart cherry extract and omega fatty acids in the 5xFAD model of Alzheimer’s disease.
Methods: Six and 12-month, 5xFAD mice and age matched WT controls were orally treated every other day for two months. Treatment was with 60mg/kg of TBR or the equivalent dose of vehicle (.05% methylcellulose). All animals were tested pre and post treatment using the open field (OF) and novel object recognition (NOR) test. Post treatment the animals were tested using the Morris water maze (MWM). Twenty-four hours post treatment the animals were euthanized, and the tissue was collected to analyze differences in neuronal morphology, neurodegeneration, amyloid plaque load and glial activation.
Results: TBR has been demonstrated to reduce deficits in this model of AD. 5xFAD animals that were treated with TBR had reduced anxiety as measured by boli in the open field test and latency to find the escape platform during the probe and reverse trial of MWM. Additionally, GFAP was decreased in the AD+TBR vs AD+VEH when measured by western blot. It is essential to understand the separate effects by which amyloid beta and tau disturb the cognitive and the molecular functioning of the brain.**Support for this study was provided by the Field Neurosciences Institute and the John G. Kulhavi Professorship in Neuroscience at CMU*

Disclosures: **Z. Bowers:** None. **P. Maiti:** None. **L. Paladugu:** None. **G. Dunbar:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Jean Perkins Foundation
Scully Initiative
Taube Philanthropies

Title: Modulating $p75^{NTR}$ signaling with the small molecule LM11A-31 prevents and reverses parvalbumin interneuron degeneration in a mouse model of Alzheimer's disease

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Abstract: Cognitive deficits prevalent in Alzheimer's disease (AD) patients and mouse models can be partially attributed to neural network hyperexcitability due to inhibitory GABAergic interneuron (IN) dysfunction. The parvalbumin (PV)-expressing sub-type of these INs is vulnerable to amyloid toxicity and shows functional deficits and degeneration early in AD progression. PV-INs are ensheathed by extracellular matrix structures termed perineuronal nets (PNN), which support their homeostasis and are depleted in AD. This depletion is driven by proNGF, which can induce degeneration via the p75 neurotrophin receptor ($p75^{NTR}$). The small molecule $p75^{NTR}$ ligand, LM11A-31 (C31) inhibits degenerative and promotes trophic signaling, ameliorating cognitive deficits and AD-related pathology in mouse models of the disease. C31 also inhibits proNGF binding to $p75^{NTR}$. Here, we examined the effects of C31 on PV-IN atrophy in the $hAPP^{Lond/Swe}$ ($APP^{L/S}$) AD mouse model. C31 (25 mg/kg) or vehicle (water) was administered by oral gavage twice daily (5 days/week) to male $APP^{L/S}$ and non-transgenic (nTg) mice for 3 months starting at either 4 - 5.5 or 10.5 - 12 months of age (*n*=6-10 mice/group). Immunostaining demonstrated $p75^{NTR}$ expression in PV-INs in hippocampus and cortex of $APP^{L/S}$ mice. There was no difference in hippocampal (CA1/2/3) PV-IN number between $APP^{L/S}$ and nTg mice at either age examined, however marked neurite degeneration was evident. At 7-8.5 months-old, area occupied by PV-containing neurites was reduced by 37% and length by 18% in $APP^{L/S}$ mice; branching complexity was also diminished. C31 prevented these deficits. At 13.5-15 months-old $APP^{L/S}$ mice had PV neurites that were 23% shorter than those in nTg mice along with reduced area and complexity. C31 treatment, initiated after degeneration was already well established, restored PV neurite area, length and complexity to nTg levels, representing an actual element of pathology reversal. Protective effects of C31 could occur, in part, by blocking proNGF-induced PNN dysfunction. Fewer PV neurons were encompassed by PNNs in $APP^{L/S}$ versus nTg hippocampus at both ages, and PV immunostaining intensity in PV/PNN-labeled cells was diminished. C31 did not prevent decreased PV-PNN number but PV

staining intensity per PV/PNN-labeled neuron was greater in treated $APP^{L/S}$ mice, suggesting that C31 may help maintain PV-IN homeostasis. In all, these results indicate that C31 can prevent and reverse PV-IN atrophy in AD mice with appreciable pathology. Most AD patients begin treatment at advanced pathological stages, thus C31's restorative effects may have particular clinical relevance.

Disclosures: T.L. McHugh: None. **D.A. Simmons:** None. **N. Belichenko:** None. **S. Seeman:** None. **S.M. Massa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of California, San Francisco, Dept. of Veteran's Affairs. **F.M. Longo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of North Carolina, University of California, San Francisco, Dept. of Veterans Affairs, PharmatrophiX.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: OSU CCVM Pilot program
Oregon Royal Center for Aging and Technology Pilot Program

Title: Ibuprofen induces differences in NMDA and AMPA receptor functions between males and females in 5xFAD mice

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Abstract: Characteristics of Alzheimer's disease include memory deficits and amyloid plaques. Interactions between amyloid and N-methyl D-aspartate receptors (NMDAR) appear to contribute to declines in synaptic plasticity. Our lab discovered that the 5xFAD mouse model of amyloid overexpression showed hyperactive NMDAR responses at 4 months of age, which could lead to excitotoxicity or increased amyloid interactions. Previously, our lab determined that ibuprofen (IBU), a non-steroidal anti-inflammatory drug (NSAID) can decrease NMDAR expression in male mice, so we treated 5xFAD mice with ibuprofen for 2 months, in order to try to reduce the increased synaptic NMDAR responses and improve spatial memory. At 2 months of age, hemizygous 5xFAD or wildtype (WT) mice were fed either regular chow or chow with 375 ppm of ibuprofen. At 4 months of age, mice underwent testing in the Morris water maze (MWM) and then were used for electrophysiology. The MWM involved acclimation (2 days), place & probe trials (4 days), reversal trials (1 day) and cued trials (1 day). For electrophysiology, mice were transcardially perfused with calcium-free cold artificial cerebral spinal fluid (aCSF). Brain slices (300μm) in normal aCSF were placed on a multielectrode probe (MED64) to examine the Schaffer collateral to CA1 synapses in the hippocampus. The field

excitatory post synaptic potential (fEPSP) was analyzed in a drug study with the use of AMPA (DNQX), GluN2A (PEAQX), GluN2B (Ro25-6981) and all NMDA receptors (AP5) antagonists. Following equilibration, an input (fiber volley)/output (fEPSP) curve was generated and analyzed by comparison of linear curve fit.All groups showed improved performance across place trials (p=.04-<.0001) except female WT IBU fed mice, but there was no significant effect of treatment or genotype on long term memory or reversal trials with the hidden platform. IBU did improve performance in probe trials in hemizygous females and IBU-fed mice overall showed more perseveration in reversal probe trials. Based on curve fit differences for electrophysiology, both male and female 5xFAD showed increased I/O responses for NMDAR and GluN2A (p=.007-<.0001). IBU increased responses for NMDAR, GluN2A and AMPAR in both WT (p<.0001) and 5xFAD (p=.02-.0009) females, but decreased responses to GluN2B and AMPAR in 5xFAD males (p=.045-.0002) and had no effect on WT males (p=.35-.94). Overall, IBU improved memory in 5xFAD females, potentially by further increasing glutamatergic signaling in the hippocampus, but reduced cognitive flexibility. In addition, it appeared that IBU had opposite effects on NMDARs and AMPARs in females versus males.

Disclosures: E.P. Sackinger: None. **K.R. Magnusson:** None. **J. Rauenhorst:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG056998

Title: Early assessment of metabolic alterations under calcineurin inhibition using ^{1}H magnetic resonance spectroscopy in a canine model of preclinical Alzheimer's disease

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Abstract: Background: In Alzheimer's disease, increased expression and activity of the protein phosphatase calcineurin (CN) appears at early stages of cognitive decline and has been shown to exacerbate Aβ pathology, synaptic dysfunction and chronic neuroinflammation (Norris et al., 2005). A wealth of evidence for the amelioration of these changes following CN inhibition suggests that CN inhibitors (CNI) may be a promising therapeutic for preventing or slowing AD pathogenesis (Reese & Taglialatela 2011). The current study is investigating the efficacy of chronic treatment with Tacrolimus, an FDA-approved CNI, as an anti-AD treatment in a canine model of preclinical AD, where interim findings are presented after 1 year of treatment.
Methods: Fifteen middle-aged beagles (5-8 years old) are treated with Tacrolimus (0.075 mg/kg) twice daily and are compared to age-matched beagles maintained under placebo. The dogs undergo extensive cognitive behavioral testing at baseline as well as throughout the course

of treatment for the next two years and undergo MRI scanning at 3T every year following baseline. ^{1}H magnetic resonance spectroscopy (^{1}H-MRS) was used to probe neuronal integrity and astrocyte metabolism of the hippocampus and posterior cingulate gyrus (PCG). **Results:** At baseline, dogs demonstrated an age-dependent decline of spatial memory abilities, consistent with prior evidence of spatial impairment in beagles beginning at middle age (Cotman & Head 2008). ^{1}H-MRS measures in the PCG at baseline showed levels of N-acetylaspartate (NAA), a marker of neuronal integrity, that were positively correlated with baseline spatial memory performance. Furthermore, levels of myo-inositol, an astrocyte marker, were positively correlated with age, in line with age-related increases of myo-inositol commonly observed in aging and AD (Wang et al., 2015). In the hippocampus, an increase in myo-inositol levels was found one year later only among placebo-treated dogs. **Conclusions**: Measurements of *in vivo* metabolism using ^{1}H-MRS offer novel insight into the metabolic alterations that underlie cognitive abilities in middle-aged beagles and provide early evidence for the efficacy of Tacrolimus in protecting against age-related metabolic alterations.

Disclosures: J.N. Lingad: None. **C.E.L. Stark:** None. **C.M. Norris:** None. **E. Head:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG054349

Title: Development of new mouse strains containing alleles of loci associated with increased risk of late-onset Alzheimer’s disease (LOAD)

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Abstract: Efforts to develop therapy for Alzheimer’s disease have consistently failed, despite success in preclinical trials in animal models. This suggests that current animal models do not recapitulate human AD in cellular mechanisms and/or physiological conditions. Development of new predictive animal models of LOAD are required to advance the field. The mission of MODEL-AD is to develop, characterize, and distribute Next-Gen preclinical models for LOAD using open science. Our approach is to engineer mouse models to express combinations of genetic variants identified as risk factors in human LOAD populations in genome-wide association studies (GWAS). To assess the role of LOAD risk alleles, homozygous mutations of GWAS-risk factors are introduced on several AD platform models (such as a 5xFAD, and a humanized Aβ allele). We have developed 10 risk models so far (ABCA7, BIN1, PICALM, ABI3, TREM2, CLU, EPHA1, SPI1). Prior to deep-phenotyping, new models are analyzed by

RNA-seq from hippocampus and cortex. This approach enables us to capture early responses to the risk factors and correlate transcription profiles in the mouse models to key human disease processes and pathways. Analysis is being performed at 4 and 12 months of age and the candidate models are analyzed for pathological phenotypes to 24 months of age. Deep phenotyping is performed using clinically relevant measures including metabolomics, biomarkers, neuropathology, electrophysiology, imaging and cognitive assays. This comprehensive approach will determine key phenotypes and the therapeutic time window for each model. All models are made available without restriction from the Jackson Laboratory, and all data will be shared via the AD Knowledge Portal. For more information see www.model-ad.org

Disclosures: **S. Kawauchi:** None. **S. Forner:** None. **J. Neumann:** None. **A. Walker:** None. **J. Alcantara:** None. **G. MacGregor:** None. **K. Green:** None. **F. LaFerla:** None. **A. Tenner:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG053150
AG029777
AG033474
AG066384
AG057325
AG062021

Title: Efficacy of a small molecule tau inhibitor in young and aged htau mice

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Abstract: The premise of this program is that tau oligomers are the acutely toxic species of tau and that their reduction will modify the course of AD. We have shown that tau oligomers cause disruption of neuronal signaling and inhibit the formation of memory in mice (Fá et al., Sci Rep. 2016 Jan 20;6:19393), and that certain forms of tau oligomers are toxic when applied to cultured neurons (Tian et al., Int J Cell Biol. 2013;2013:260787). The discovery of small molecule inhibitors was performed with assays targeting tau self-association, the initial step in the tau aggregation cascade. Preventive efficacy studies were performed in htau (Davidowitz et al., J Alzheimers Dis. 2020, 73:147-161) and JNPL3 mice that demonstrated that the lead compound reduced self-association of soluble tau and inhibited formation of insoluble tau aggregates. The

overall goal of this program is to discover and develop small molecule therapeutics targeting tau self-association for the treatment of AD and ADRD. Here, we present results from a therapeutic study comparing efficacy of the lead compound in the htau mouse model of tauopathy relative to a preventive study in htau. Measurements of therapeutic efficacy include reduction of insoluble and hyperphosphorylated tau, and amelioration of behavioral deficits. Therapeutic studies were independently performed in male htau mice. Mice were aged to 7 months (baseline) and treated for 5 months. Each study had 4 groups including baseline (n=20), vehicle (n=25), and two treatment groups (n=25, each) that were administered 40 or 80 mg/kg dose of lead compound formulated in feed. Working memory of htau mice (baseline group) was analyzed using Barnes maze. Biochemical analyses of levels of total tau, phosphorylated tau and insoluble, aggregated tau were performed by ELISA. Immunocytochemical examination was performed with 4 tau antibodies (MC1, PHF1, CP13 and RZ3), as well as with Iba1 and GFAP for microgliosis and astrocytosis, respectively, as time permitted under restricted access related to the pandemic. There were no adverse effects due to the compound during the course of the study. There was a 5.5.-fold difference in exposure between the 40 and 80 mg/kg treatment groups. There was dose dependent reduction to baseline levels of insoluble tau phosphorylated at Serine 202 in the cortex and of phospho-tau396,404 in the heat stable fractions from the forebrain and hippocampus. Results of this therapeutic study in conjunction with the results from the preventive study suggest that this small molecule may have potential for developing a drug targeting tau in ADRD.

Disclosures: **E.J. Davidowitz:** A. Employment/Salary (full or part-time):; Oligomerix, Inc. **P. Lopez:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. **H. Jimenez-Bravar:** None. **J. Koppel:** None. **L. Adrien:** None. **J. Eun:** None. **A. Wolin:** None. **P. Davies:** None. **J.G. Moe:** A. Employment/Salary (full or part-time):; Oligomerix, Inc..

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A Novel DDR1 inhibitor improves memory and reduces pathology in a model of Alzheimer’s Disease

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Abstract: Proteinopathies represent a subset of neurodegenerative diseases characterized by aggregated, structurally abnormal neurotoxic proteins. Despite the clear societal burden posed by proteinopathies, a complete understanding of the mechanisms by which these disorders exert their pathological effects remain elusive. A number of these diseases, such as Alzheimer’s Disease (AD), have been demonstrated to exhibit elevated expression of various tyrosine kinases (TKs) in the brains of postmortem patients. TKs such as Discoidin Domain Receptor 1 (DDR1)

are in part responsible for regulating autophagy, the cell's ability to clear waste, in healthy cells, and it is believed that overexpression of TKs may be a culprit of protein aggregation in proteinopathies. Silencing of the genes encoding tyrosine kinase receptors using shRNA constructs has been shown to alleviate levels of amyloid-beta in mice, identifying TKs as potential targets of investigation when examining the pathological mechanisms behind these conditions.Recently, we have begun investigating the use of novel tyrosine kinase inhibitors as a means of mitigating neurotoxic protein burden across animal models of disease. Following four weeks of treatment with BK40197, a potent inhibitor of DDR1, 4 month old male and female transgenic APP mice overexpressing human amyloid precursor protein scored significantly better on the novel object recognition test, a measure of memory impairment, than equal cohorts of mice treated with a vehicle (n=8). We hypothesize that this impairment is associated with early-stage accumulation of amyloid precursor protein, and that inhibition of DDR1 attenuates this process and associated neurodegeneration. We are currently performing western blot and ELISA analysis and immunohistochemistry to confirm that BK40197 treatment does in fact improve behavioral outcomes by alleviating protein burden in an DDR1-mediated manner. We will also probe for levels of amyloid-beta and phosphorylated tau, phosphorylated DDR1, and markers of autophagy such as LC3 and LAMP2A to demonstrate that activating autophagy by inhibiting DDR1 results in decreased levels of neurotoxic protein. These results will provide insight into how tyrosine kinase activity contributes to protein aggregation across disease states, elucidating a potential target for future therapeutic intervention.

Disclosures: **M. Stevenson:** A. Employment/Salary (full or part-time):; Georgetown University. **B. Kalavu:** A. Employment/Salary (full or part-time):; Georgetown University. **A. Fowler:** None. **M. Hebron:** A. Employment/Salary (full or part-time):; Georgetown University. **W. Shi:** None. **C. Wolf:** A. Employment/Salary (full or part-time):; Georgetown University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Georgetown University. **C. Moussa:** A. Employment/Salary (full or part-time):; Georgetown University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Georgetown University. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); KeiferRx LLC. F. Consulting Fees (e.g., advisory boards); KeiferRx LLC.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Weston Brain Institute
Ontario Veterinary College

Title: Effects of5α-androstane-3α,17β-diol on age-related cognitive decline in the 3xTg mouse model of Alzheimer's disease

Authors: *N. R. KUWAHARA[1], S. BHULLAR[1], K. TANG[1], K. H. JARDINE[2], B. D. WINTERS[2], N. J. MACLUSKY[1];
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Abstract: Age-related decreases in gonadal steroid hormone levels have been associated with cognitive decline and an increased risk of developing Alzheimer's disease (AD). AD is characterized by marked sex differences, with women being more likely to develop AD, and show worsened cognitive deterioration at the same stage of the disease. This may in part, reflect the neuroprotective effects of testosterone, since men with high free testosterone levels maintained into old age have a reduced risk of developing AD. Recent studies have suggested that steroid hormone-mediated neuroprotection, may be augmented through the conversion of primary gonadal steroid hormones into metabolites that may act through distinct cellular mechanisms. Previously, we demonstrated that 5α-reduced metabolites of testosterone including 5α-androstane-3α,17β-diol (3α-diol) may play an important role in protecting the brain against AD and may contribute to the observed sex differences. Inhibiting the synthesis of testosterone-derived neurosteroids impaired object recognition memory (ORM) and exacerbated AD-like neuropathology in 6-month-old male triple transgenic AD (3xTg-AD) mice. However, the possible protective effects of 3α-diol itself *in vivo* remain unknown. To determine whether treatment of 3α-diol might protect against the development of cognitive dysfunction, male and female wildtype and 3xTg-AD mice were implanted subcutaneously with 3α-diol diproprionate-filled or empty silastic capsules at 3 months of age (n=15-16 per treatment group). After 3 months of treatment, 3α-diol differentially impaired object recognition memory (ORM) in 3xTg-AD males after short term (5 min) and in females after long-term (3h) retention delays. After 6 months of treatment, 3α-diol improved ORM in 3xTg-AD males and females after the long-term (3h) retention delay. Ongoing experiments will determine the effects of 3α-diol on cognitive performance and AD-like neuropathology as the disease progresses to 12 months of age (Supported by the Weston Brain Institute and the Ontario Veterinary College).

Disclosures: **N.R. Kuwahara:** None. **S. Bhullar:** None. **K. Tang:** None. **K.H. Jardine:** None. **B.D. Winters:** None. **N.J. MacLusky:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

Title: Assessing the longitudinal benefit of two environmental enrichment methods in a triple transgenic mouse model of Alzheimer's disease

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Abstract: Cognitive reserve (CR) refers to the resilience to Alzheimer's disease (AD) pathology that may be developed through complex life experiences such as higher education and mentally stimulating leisure activities. Experiences thought to create CR in humans can be modeled through environmental enrichment (EE) in rodents. However, standard home cage EE may poorly reflect the complex environments that are thought to be responsible for CR in humans. In a previous study, we validated a novel 'obstacle course' (OC) enrichment paradigm which exposed mice to daily EE over 2 mo on a running track in which they had to navigate a variety of obstacles to a receive a reward. This increased and controlled for the amount of complexity and novelty each animal experienced. The OC conferred a stronger cognitive benefit than home cage enrichment on the spontaneous object recognition task (SOR) and complex variations of the task which require feature integration, including the object category recognition task (OCR). Human AD patients display deficits in both visual recognition and feature integration. AD model mice also show visual recognition deficits with the SOR task, whereas tasks that require feature integration have not been previously evaluated in an AD mouse model. In this study, we evaluated the potential for CR-type outcomes with two different enrichment protocols in the triple transgenic mouse model (3xTG) of AD by evaluating mice longitudinally on both the SOR and OCR task after early-life exposure to EE. Post-weaning age male 3xTG transgenic (n=65) and wild-type (WT) mice (B6129SF2/J) (n=67) were divided into 4 different groups (n = 12-21/group): obstacle course (OC), home cage enrichment (EH); exercise control course (CC); or standard housing control (SH). After 2 mo of EE, all groups were longitudinally assessed at 3-4, 6, 9 and 12-mo on the SOR and OCR tasks. We found a progressive impairment on both tasks and that early-life EE was able to 'rescue' task performance. Starting at 6-mo, all non-enriched 3xTg groups were impaired on the SOR task with a 24-h retention delay and by 12-mo were also impaired with a 3-h delay. Both OC and EH enrichment ameliorated this impairment. Furthermore, we characterized a longitudinal OCR phenotype for 3xTG mice, which showed that 3xTG mice start showing task-related impairments at 9 months of age. These impairments were mitigated only by OC enrichment. Additionally, OC enriched 3xTG mice showed enhanced OCR task performance, which was observed up to 12-mo. These results emphasize the role of early-life experiences in the creation of CR and importance of individual animal control in EE methods for accurately assessing CR-type outcomes in mice.

Disclosures: H.A. Collett: None. **S.E. Weber:** None. **M. Odina:** None. **K.M. Vint:** None. **A.M. Tyler:** None. **A.E. Huff:** None. **K. Kania:** None. **R. Osterlee:** None. **M.T. Sullivan:** None. **B.L. McNaughton:** None. **B.D. Winters:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: This work was supported by the Roskamp Institute and by a Sponsored Research Agreement between The Roskamp Institute and Enzymedica, Inc.

Title: Candida rugosa lipase enhances Alzheimer's disease-like pathology in mice

Authors: *A. MENDEN[1,2], D. HALL[1], C. HAHN-TOWNSEND[1], C. A. BROEDLOW[3], F. CRAWFORD[1,2], S. CRYNEN[1,2], N. KLATT[3], M. MULLAN[1,2], G. AIT-GHEZALA[1,2]; [1]Roskamp Inst., Sarasota, FL; [2]Open Univ., Milton-Keynes, United Kingdom; [3]Univ. of Minnesota, Minneapolis, MN

Abstract: The gut microbiome is an important symbiont with functions in and beyond the gut. Diet displays the greatest impact on gut microbial composition as well as the gut metabolome. It has been determined that microbial-derived metabolites can alleviate neuroinflammation and neurodegeneration in the brain emphasizing its potential role in Alzheimer's disease (AD).Therefore, it was investigated whether *Candida rugosa* lipase (CRL) impacts AD-like pathology in APP/PS1 transgenic mice as compared to wildtype littermates by altering microbial and metabolomic gut compositions. APP/PS1 (8mo, n=12/group) and C57BL/6J (Wt) mice (8mo, n=14/group) were treated with 5000 FiP/kg CRL in their drinking water for two months and compared to littermates receiving regular drinking water. At 0, 1 and 2 months fecal samples were collected. Furthermore, behavioral testing was conducted two weeks before euthanasia. Using 16S rRNA sequencing, we investigated microbial changes, while gas-chromatography mass-spectrometry was used to quantify the gut metabolome. Additionally, peripheral changes such as cytokine levels and metabolomic profiles were analyzed. Finally, in addition to behavioral analyses, brain pathology was examined by immunohistochemistry and cortex transcriptomics performed. To show transferability of altered brain pathology due to gut-related changes, a fecal matter transplant (FMT) study was conducted from the first study into Wt mice.Our data suggested significant differences in both genotypes post CRL treatment. Furthermore, both cecal and plasma metabolites such as tryptophan could be correlated to the microbial differences. In addition, CRL treatment enhanced significantly memory and AD-like pathology. Finally, the FMT study suggested transferability of cognitive differences between treated and untreated animals into Wt animals. In conclusion, the results of this pre-clinical study highlight CRL' s and its associated metabolites' potential as new treatment agents for AD patients.

Disclosures: A. Menden: None. **D. Hall:** None. **C. Hahn-Townsend:** None. **C.A. Broedlow:** None. **F. Crawford:** None. **S. Crynen:** None. **N. Klatt:** None. **M. Mullan:** None. **G. Ait-Ghezala:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1R01AG066489-01A1

Title: Sex differences in early impairment of fear memory extinction in the TgF344AD rat model of Alzheimer's disease.

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Abstract: Alzheimer's disease (AD) is accompanied by cognitive impairments affecting several memory domains. One such domain, emotional memory, is supported by a vast network of medial temporal lobe structures which are negatively impacted by AD. Human studies suggest the inability to acquire aversive emotional (fear) memories may be an early sign of AD that occurs prior to measurable cognitive impairments. In women, aversive emotional memories are associated with a greater incidence of neuropsychiatric disorders (e.g., anxiety and PTSD), and furthermore, AD is more likely to occur in women. However, whether sex interacts with fear memory as an early predictor of AD is not currently known. The transgenic Fischer344 AD (TgF344AD) rat model recapitulates many aspects of AD in humans. The current study used a behavioral approach in TgF344AD rats to better define the contributions of sex and genotype to early signs of memory impairments in AD. Prior studies have shown pathology in this rodent model is evident at 6 months of age. Young adult (6 mo.) male and female wild-type (WT) Fischer 344 and male and female TgF344AD rats were trained on a four-day contextual fear conditioning paradigm. Day 1 consisted of fear acquisition in context A in which rats were given a conditioned stimulus (CS, 20s tone) and unconditioned stimulus (US, 2s 1mA shock) pairing. Each pairing was delivered four times separated by a 20s intertrial interval (ITI). Day 2 consisted of CS extinction in a different context. Rats were placed in the conditioning chambers and given 20 CS trials with no US pairings separated by a 5s ITI. On day 3, extinction retrieval in context B was tested. Finally, on day 4, rats were placed in the same conditions as in context A to test fear renewal of the CS in the absence of US. On day 1, a mixed factor ANOVA (sex*genotype*trial) revealed all rats acquired fear (operationally defined as freezing) to the CS in context A, irrespective of sex or genotype. On day 2, there was a main effect of genotype on CS extinction such that AD rats were unable to extinguish fear expression relative to WT. On day 3, AD rats were less able than WT to retrieve extinction memory. On day 4, all rats showed continued freezing to the CS in context A, and this response was greater in AD females than other groups suggesting increased fear generalization. Furthermore, all females showed a renewal of fear to context A that was attenuated in males. Interestingly, AD females were unable to extinguish any fear expression during the post-CS period. These data suggest TgF344AD rats

show emotional memory impairments as early as 6 months of age, and these impairments may be greater in females than males.

Disclosures: C.M. Hernandez: None. **L.L. McMahon:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1T32HD071866
NIH Grant 1R01AG060977

Title: Alzheimer'S disease-inflicted microbiome alterations may be ameliorated by a ketogenic, time restricted diet in rats

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Abstract: While the devastating effects of Alzheimer's disease (AD) on patients and their caregivers are well known, all intervention approaches thus far seeking to prevent or reverse AD have been ineffective. Furthermore, the pathology of AD reaches far beyond the central nervous system, including metabolic impairments and gut-brain-axis perturbations. Therefore, it is imperative that the scientific community broaden their approach to include those beyond the central nervous system. The gut microbiome is compositionally distinct in individuals with AD relative to age- and sex-matched controls, making it a promising avenue for potential interventions. Furthermore, age-related changes in gut microbiome composition may reciprocally interact with several physiological systems that are impaired with both age and AD. While caloric restriction has been highlighted as a strategy for increasing healthspan, time restricted feeding (TRF) and changes in dietary macronutrient composition may be more feasible alternatives with similar health outcomes and are both currently prescribed to individuals with metabolic impairment. To begin to investigate the potential utility of these interventions, fully mature young (5 mo) and older (22 mo) adult male Fischer Brown Norway Hybrid rats were placed on a TRF regimen of a ketogenic or micronutrient and calorically matched control diet for 7 months. A third group of rats was permitted to eat standard chow *ad libitum*. Fecal samples collected at the conclusion of the study were submitted for 16S microbiome analysis, which revealed significant differences across age and diet groups, as well as across feeding paradigms. Beta diversity analysis demonstrated distinct microbiome composition across the diet groups regardless of age, indicating changing caloric content and/or timing of consumption can drive changes in gut health. Furthermore, these changes are possible in aged rats at a comparable rate to the young, suggesting microbiome alterations are a feasible target in individuals with AD. Additionally, alpha-diversity was significantly lower in all TRF-fed rats, regardless of diet. At

the phylum level, diet significantly impacted relative abundance of several microbiota, including *Verrucomicrobia, Cyanobacteria, Actinobacteria* and *Patescibacteria*. Of particular note is the species *Akkermansia*, which others have shown to be lower in humans with metabolic deficits and AD mouse models. In our study, TRF-fed rats had significantly more *Akkermansia* than did ad lib fed rats. These results indicate the value of altered macronutrient composition and feeding methodology for therapeutic interventions targeting the gut microbiome.

Disclosures: A.R. Hernandez: None. **S.N. Burke:** None. **T.W. Buford:** None. **C.S. Carter:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS Grant R01 NS083704
BBHI minigrant 2019-20-001
NIA Grant P20 AG068077

Title: qß Virus-like particle (vlp)-based vaccine against phospho-threonine 181 tau shows robust immune response and functional efficacy in the p301s mouse model of tauopathy

Authors: *N. M. MAPHIS[1], J. PEABODY[1], S. JIANG[1], Y. YANG[2], D. N. LINSENBARDT[3], D. S. PEABODY[1], B. CHACKERIAN[1], K. BHASKAR[1];
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Abstract: Background: Tauopathies are progressive neurodegenerative disorders characterized by the hyperphosphorylation of tau and development of intracellular neurofibrillary tangles, collectively referred to as pathological tau (pTau). It is becoming increasingly recognized that the development and spread of pTau accurately predicts and correlates with clinical progression in human tauopathy patients, however there are no FDA-approved drugs that target and reduce pTau. Previously, our lab has developed a virus-like particle (VLP)-based vaccine using a Qß (RNA bacteriophage) platform to multivalently display a 13-mer pTau peptide, with phospho-Threonine 181 (pT181, a well-established CSF biomarker for tauopathies). Previously, we observed that pT181-Qß generated a robust and targeted immune response against pTau in a model of tauopathy, rTg4510, resulting in reduced insoluble tau, decreased neurodegeneration and improved delay-dependent and spatial working memory. Here, we sought to expand the effects of this vaccine in an additional model of tauopathy, at a more clinically relevant intervention. **Methods:** We immunized P301S (and non-transgenic) mice twice, at 3 and 4 months of age. Next, we assessed anti-pT181 antibody titers at 1-week after the first injection and 5-months following the last injection (i.e. 9 months of age) followed by behavioral and biochemical analyses. **Results:** Anti-pT181 antibodies were significantly elevated in all mice

treated with pT181-Qß, regardless of genotype, 1 week after the first dose and remained elevated five months after the second injection. Behavioral analysis suggest that pT181-Qß-vaccinated P301S mice showed improved delay-dependent memory (as measured by novel object recognition test), and gait improvement (as assessed with Catwalk). Finally, P301S mice vaccinated with pT181-Qß had significantly downregulated transcripts involved in inflammasome activation and significantly reduced tau hyperphopsphorylation in the hippocampus, suggesting that reduction of pathological pT181-Qß can prevent pTau-driven inflammatory activation. **Conclusions:** pT181-Qß represents an efficacious and cutting-edge tool to target and reduce tau pathology in multiple animal models of tauopathy which may have substantial effects against neuroinflammation besides ameliorating tau pathology.

Disclosures: **N.M. Maphis:** None. **J. Peabody:** None. **S. Jiang:** None. **D.S. Peabody:** None. **B. Chackerian:** None. **K. Bhaskar:** None. **Y. Yang:** None. **D.N. Linsenbardt:** None.

Digital Abstract Session

P106. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics

Program #/Poster #: P106.16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Deutsche Forschungsgemeinschaft (SFB 870) to AK
European Research Council Advanced Grant to AK

Title: Scavenging β-amyloid monomers prevents early neuronal dysfunction in Alzheimer's Disease models

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Abstract: Accumulating evidence from observations in mouse models and humans indicates that one of the earliest brain changes in Alzheimer's disease (AD) is an amyloid-β (Aβ)-dependent excessive neuronal activity, i.e. hyperactivity. In mouse models of AD, elevated levels of soluble Aβ but not the emergence of amyloid plaques cause neuronal hyperactivity. Conversely, the application of soluble Aβ in wild type mice is sufficient to sustain this neuronal dysfunction. As neuronal hyperactivity is implicated in brain circuit dysfunction and cognitive decline, its treatment could have beneficial effects in AD. Here, we introduced a novel approach of preventing neuronal hyperactivity in young AD mice *in vivo* by the application of an Aβ-binding Anticalin protein to the brain.We used the Aβ-specific Anticalin H1GA, a small engineered antibody-like protein, to scavenge Aβ in a mouse model of AD and evaluated its effect on neuronal activity using *in vivo* two-photon calcium imaging. We demonstrated that the

application of H1GA to the hippocampal CA1 area stopped neuronal hyperactivity in young pre-depositing APP/PS1 mice but had no effect in wild-type mice. Using size exclusion chromatography, we next determined that H1GA binds Aβ monomers but not larger aggregates. This observation is remarkable as earlier work has shown that neuronal hyperactivity and synaptic dysfunction is predominantly caused by Aβ dimers and oligomers but not monomers. To solve this conundrum, we designed a combined *in vitro-in vivo* assay. First, we verified that synthetic Aβ monomers applied to the brains of wild type mice *in vitro* and *in vivo* did not affect the neuronal activity levels. Next, we incubated Aβ monomers for 1-2 hours and confirmed the emergence of aggregates using a Thioflavin T fluorescence assay. The application these aggregates, containing Aβ dimers and oligomers, reliably induced neuronal hyperactivity *in vivo* and *in vitro*. Finally, incubation of Aβ monomers in H1GA-containing medium prevented the hyperactivating Aβ effect. In contrast, the application of synthetic Aβ dimers potently induced neuronal hyperactivity alone or in the presence of H1GA. Together, this data indicates that the removal of Aβ monomers from the brain is sufficient to prevent neuronal hyperactivity, most likely trough the prevention of Aβ aggregation. In summary, in this study we established that scavenging of functionally inert Aβ monomers can prevent early neuronal dysfunction in mouse models of AD *in vivo*, most likely through reducing the formation of toxic Aβ dimers and oligomers. These results identify Aβ monomer scavenging agents, such as the Anticalin H1GA, as promising therapeutic agents at early stages of AD.

Disclosures: B. Zott: None. **L. Nästle:** None. **C. Grienberger:** None. **M. Simon:** None. **A. Keskin-Dargin:** None. **F. Unger:** None. **A. Skerra:** None. **A. Konnerth:** None.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A systematic review and qualitative evidence synthesis of the role of transthyretin in Alzheimer's disease

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Abstract: Introduction: The protein prealbumin or transthyretin (TTR) is found in many organs, including the brain. Studies have found a conflicting role of TTR in Alzheimer’s Disease (AD), with some finding it protective and others reporting it as part of the pathophysiologic process. No systematic review (SR), which is the highest quality of evidence, currently exists to clarify this issue. Therefore, we aimed to clarify the role of TTR in AD through a SR.
Methods: We conducted SR as per the PRISMA guidelines (Fig. 1). We searched the PubMed database on 1st July 2020 using the function: '((transthyretin) OR (prealbumin)) AND (Alzheimer's disease)'. Duplicate removal, abstract screening, and full-text review were done.
Results: We obtained 95 eligible articles (Fig. 1). Only qualitative synthesis could be performed

due to heterogeneity in study methods, precluding a meta-analysis. Majority of studies, especially ones in last two decades, find that TTR has neuroprotective role in AD. TTR has been found to clear amyloid-beta (Aβ) and inhibit Aβ aggregation. TTR influences Aβ levels gender-specifically, its action affected by sex hormones. While previous studies were conflicted about the source of TTR in cerebrospinal fluid (CSF), there is growing evidence to suggest that CSF TTR comes from 3 sources: (1) neuronal synthesis, (2) choroid plexus synthesis; and (3) diffusion from serum. CSF studies report that TTR levels here are decreased in AD and oxidized TTR forms are detectable. Serum studies also report that TTR levels inversely correlate with AD progression. Both CSF and serum studies of TTR have found it to be a good AD biomarker. Limitations: We did not utilize SCOPUS or EMBASE for searching. There is also a lack of large observational or interventional studies in clinical practice, resulting in a lack of reporting of the true utility of TTR from our SR.
Conclusions: A rapidly accumulating, significant body of evidence for a neuroprotective role of transthyretin in AD exists. CSF and serum levels of TTR can act as good biomarkers of AD. Large clinical studies are needed to show TTR's clinical utility.

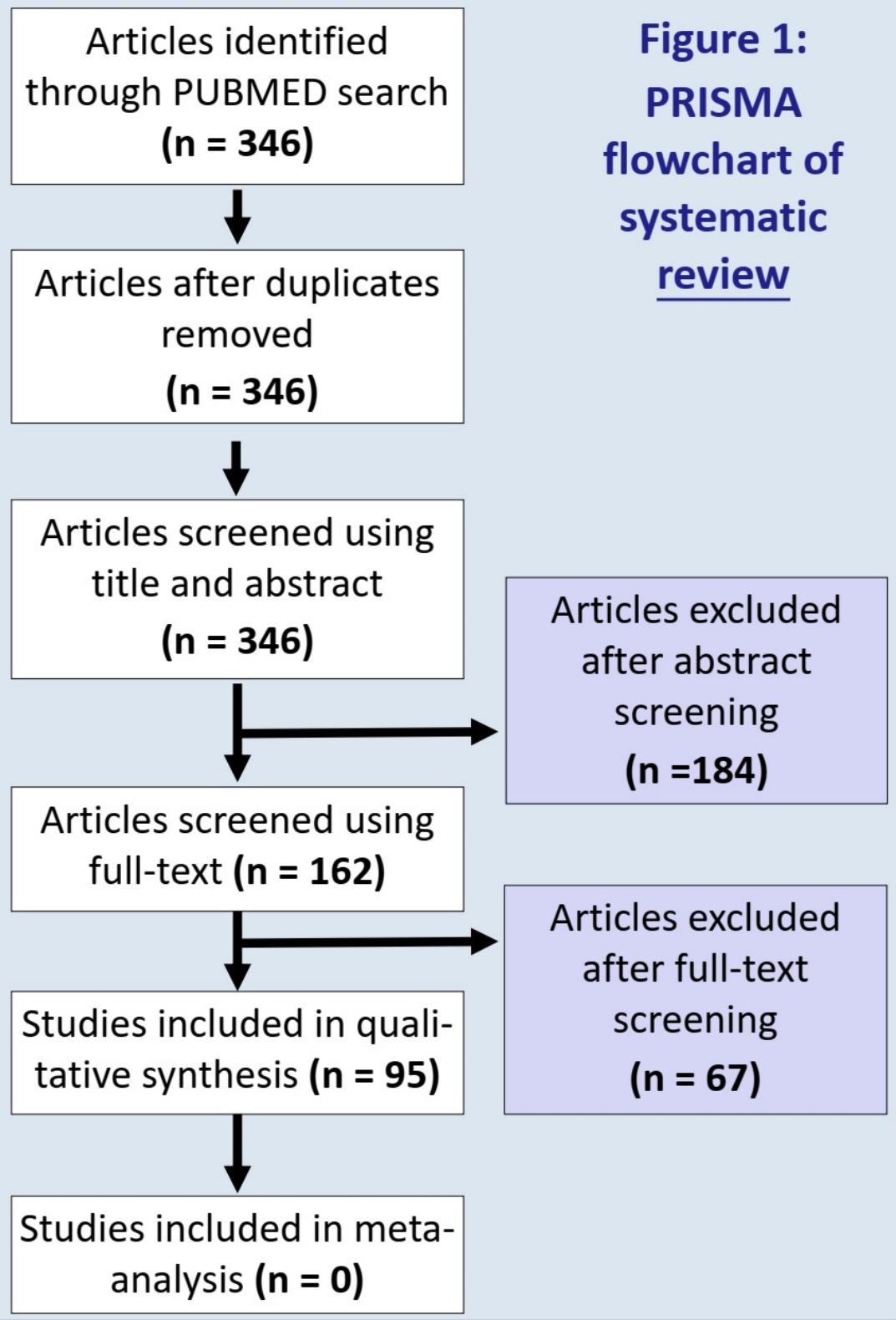

Figure 1: PRISMA flowchart of systematic review

Disclosures: **A. Ozair:** None. **A. Faruqi:** None. **A. Bajaj:** None.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant 2R01-NS047229
NIH grant P50AG05138
NIH grant AG-008200

Title: Presenilin1 Familial Alzheimer's Disease mutants decrease eprhinB2-regulated angiogenic functions, ischemia-induced brain neovascularization and neuronal survival.

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Abstract: Cerebral microvasculature abnormalities, such as degeneration of the capillary endothelium, are implicated in the genesis of Alzheimer's disease (AD) neuropathology. In addition, ischemic lesions that cause neuronal damage are often found in AD brains. The brain responds to ischemia by stimulating tissue neovascularization via sprouting angiogenesis; impairment of this function renders the brain vulnerable to the insult. It has been hypothesized that decreased angiogenesis in AD leads to insufficient blood flow and neuronal dysfunction in affected areas.The EphB4/ephrinB2 (efnB2) ligand-receptor system is known to regulate brain angiogenesis in response to ischemia. Using *in vitro* angiogenesis assays and co-immunoprecipitations we show that Presnilin1 (PS1), an important factor in familial AD (FAD), regulates angiogenic functions of EphB4/efnB2 including sprouting, tube formation and stimulation of VE-cadherin angiogenic complexes in brain endothelial cells (ECs) and these functions are impaired by PS1 FAD mutants. In addition, using kcnockin mice (KI) expressing PS1 FAD mutants, MCAO, spPLA, MRI and immunohistochemistry we found that ischemia stimulates formation of the same angiogenic complexes in the brain and that this function is attenuated by PS1 FAD mutants together with ischemia-induced neovascularization and cerebral blood flow while neuronal death is increased. The PS1 FAD KI mice are "humanized", the heterozygous FAD mice have the same genotype as human FAD patients, one PS1 FAD allele and one WT PS1 allele. We also found that PS1 FAD mutants decrease the γ-secretase processing of efnB2 and production of the angiogenic peptide efnB2/CTF2 thus impairing the angiogenic response of brain ECs. Finally, we found that a small peptide, which derives from efnB2/CTF2 rescues angiogenic functions of PS1 FAD ECs.Together, our data support the hypothesis that PS1 FAD mutants inhibit ischemia-induced angiogenic functions of ECs by decreasing the γ-secretase processing of efnB2 and impairing the EphB4/efnB2 signaling, thus decreasing neovascularization in the adult brain and leading to increased vulnerability to this toxic insult, cognitive decline and neuronal death (Yoon et al., 2020 Mol. Psychiatry,

doi:10.1038/s41380-020-0812-7). Furthermore, our data reveal a novel brain angiogenic mechanism targeted by PS1 FAD mutants and a potential therapeutic target for ischemia-induced neurodegeneration. Importantly, FAD mutant effects occur in absence of neuropathological hallmarks of AD, supporting such hallmarks may form downstream of mutant effects on neoangiogenesis and neuronal survival.

Disclosures: A. Georgakopoulos: None. **Y. Yoon:** None. **G. Voloudakis:** None. **C. Dimovasili:** None. **L. Chen:** None. **Z. Shao:** None. **C. Tang:** None. **P.R. Hof:** None. **N.K. Robakis:** None.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 2R01-NS047229
NIH grant P50AG05138
NIH grant AG-008200

Title: Gamma-secretase regulates VEGF-induced angiogenic functions of brain endothelial cells : Role of presenilin1 FAD mutants

Authors: *R. PANDEY, N. K. ROBAKIS, A. GEORGAKOPOULOS;
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Abstract: Alzheimer’s disease (AD) is one of the major neurodegenerative disorders that leads to age-dependent cognitive impairment and death. There are currently no preventative or therapeutic agents for AD. Preclinical studies have shown cerebrovascular perturbation as well as decreased vascular density in AD, which precede and accompany cognitive dysfunction and neurodegeneration. One of the factors that could contribute to cerebrovascular pathology in AD is decreased neovascularization in the brain. Neovascularization is a process regulated by angiogenesis, the formation of new blood vessels from existing ones. Endothelial cells (EC) of the blood vessels play a crucial role in angiogenesis. Vascular Endothelial Growth factor (VEGF), is a potent angiogenic factor that regulates angiogenesis via binding to its receptor VEGFR2, a type I transmembrane protein, in EC. Presenilin 1 (PS1) is an important factor for AD as its mutants are responsible for most cases of Familial Alzheimer’s Disease (FAD). PS1 is the catalytic subunit of γ-secretase, a proteolytic complex, which regulates the processing of several type I transmembrane proteins. We hypothesize that mutants of PS1 found in FAD, may affect VEGF-induced processing of VEGFR2 and VEGF-induced angiogenesis. Using in vitro angiogenesis assays we found that inhibition of γ-secretase decreases the VEGF-induced angiogenic functions of brain EC including sprouting, tube formation and migration, all important steps of sprouting angiogenesis. Using western blot and co-immunoprecipitations we also found that VEGFR2 is processed by γ-secretase and that γ-secretase promotes VEGF-

induced angiogenic complexes, which include VE-cadherin and Rok-α kinase. Finally, we observed that VEGF-induced sprouting of ECs expressing PS1 FAD mutant M146V decreases significantly compared to induced sprouting of WT controls. EC expressing PS1 M146V mutant were prepared from brains of knockin mice (KI) expressing this mutant. These mice are "humanized", the heterozygous FAD mice have the same genotype as human FAD patients, one PS1 FAD allele and one WT PS1 allele. These data suggest that PS1/γ-secretase regulate VEGF-induced angiogenic functions of EC and that PS1 FAD mutants impede these functions. This could decrease the ability of the brain to recover from toxic insults and that might lead to increased neuronal death and cognitive decline. Targeting brain angiogenesis could have therapeutic value for neurodegenerative diseases such as AD.

Disclosures: R. Pandey: None. **N.K. Robakis:** None. **A. Georgakopoulos:** None.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01-NS109260
P01-AG066597
P30-AG10124
P01-AG017586-01
R01-AG054519-02
R01-AG038490
U01-AG052943

Title: Tauopathies and TDP-43 proteinopathies display signature patterns of cortical layer pathology independent of clinical phenotype

Authors: *D. T. OHM[1], C. PETERSON[2], K. COUSINS[2], R. LOBROVICH[2], E. XIE[2], C. MCMILLAN[1], D. WOLK[1], V. M. VAN DEERLIN[3], L. ELMAN[2], M. SPINDLER[2], A. DEIK[2], A. SIDEROWF[2], J. Q. TROJANOWSKI[3], E. B. LEE[3], M. GROSSMAN[1], D. IRWIN[1];
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Abstract: Frontotemporal lobar degeneration (FTLD) is a group of distinct proteinopathies associated with predominant degeneration of frontotemporal networks that result in overlapping frontotemporal dementia (FTD) clinical syndromes. While tau inclusions (FTLD-tau) and transactive response DNA binding protein of 43kDa inclusions (FTLD-TDP) often accrue in the same gross anatomical regions, pathology within cytoarchitectonic subfields of gray matter is not well understood in the full FTLD spectrum due to lack of large comparative studies quantifying microstructural changes. The current study examined 152 FTLD patients with either FTLD-tau (n = 67) or FTLD-TDP (n = 85) to determine if the relative distribution of pathology across cortical layers is a distinguishing feature of FTLD groups and to identify which factors may

predict signature patterns of pathologic change. Paraffin-embedded sections from up to 10 neocortical and paralimbic regions per patient were immunostained for phosphorylated TDP-43 (1D3) and hyperphosphorylated tau (AT8). We digitally measured the % area occupied by tau and TDP-43 inclusions in upper cortical layers (I-III), lower cortical layers (IV-VI), and white matter adjacent to layer VI in all regions. A ratio of upper-to-lower layer pathology (layer ratio) calculated the relative distribution of pathologic burden. To compare proteinopathy groups, linear mixed effect models tested associations between layer ratio and proetinopathy groups, and between layer ratio and white matter pathology within groups while adjusting for demographics. We found a smaller layer ratio (greater pathology in lower layers) in FTLD-tau compared to FTLD-TDP (beta=-0.75, SE=0.08, $p<0.05$) which remained consistent when examined by clinical subgroup (i.e., dementia and motor syndromes). Post-hoc comparisons showed that a lower layer ratio was consistent among pathologic and clinical subgroups of FTLD-tau, whereas a higher layer ratio was shared among pathologic and clinical subgroups of FTLD-TDP. In FTLD-tau, not FTLD-TDP, greater white matter pathology was associated with relatively greater pathology in lower layers (beta=-0.048, SE=0.02, $p<0.05$). Our findings suggest that relatively high pathologic burden in lower layers and adjacent white matter is a common feature of FTLD-tau and distinct from more isolated upper layer pathology in TDP-43 proteinopathies. Examination of laminar architecture in FTLD provides new anatomical evidence that distinct layer pathology, and therefore changes to separate populations of neurons and/or glia, may contribute to the degeneration of similar if not identical cognitive networks associated with FTD.

Disclosures: D.T. Ohm: None. **C. Peterson:** None. **K. Cousins:** None. **R. Lobrovich:** None. **E. Xie:** None. **C. McMillan:** None. **D. Wolk:** None. **V.M. Van Deerlin:** None. **L. Elman:** None. **M. Spindler:** None. **A. Deik:** None. **A. Siderowf:** None. **J.Q. Trojanowski:** None. **E.B. Lee:** None. **M. Grossman:** None. **D. Irwin:** None.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS (NS085770)
NINDS (NS104160)
NIA (AG056258)
NIA (AG13854)

Title: Isolation of Exosomes from Frozen Postmortem Human / Mouse Brain and Primary Human Microglia Culture Media

Authors: *I. A. AYALA[1], C. A. ESTRADA[2], R. SHAHIDEHPOUR[2], T. D. GEFEN[2], M. E. FLANAGAN[2], M. M. MESULAM[2], C. GEULA[2];
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Abstract: Exosomes are extracellular vesicles produced in the endosomal compartment and released by most eukaryotic cells. They are the smallest extracellular vesicle and have been implicated in cell-to-cell communication under normal conditions and propagation of pathology in neurodegenerative disorders. In particular, microglia-derived exosomes have been implicated in pathologic tau propagation in Alzheimer's disease. The goal of the present experiment was to isolate exosomes from various sources and determine whether they are of microglia origin. Frozen postmortem human tissue from the middle frontal gyrus of three participants with frontotemporal dementia and two cognitively-normal controls, frozen frontal cortex from three wildtype C57/Bl6 mice, and pooled media from primary human microglia cultures were used for this purpose. Employing established high speed ultracentrifugation and fractionation protocols (Théry C et al., Curr Protoc Cell Biol, Chapter 3: Unit 3.22, 2006; Perez-Gonzalez, R et al., JBC, 287: 43108-15, 2012), we isolated exosomes from the above sources and used Western blot analysis to determine presence of known markers. 500 mg of brain tissue, and 50 ml of human microglia conditioned medium was necessary to successfully isolate and identify exosomes. Exosomes isolated from the three sources contained immunoreactivity for the exosome markers flotillin-1 and CD63. To investigate microglia as a potential origin of the isolated exosomes, we performed Western blot analysis using the microglia marker CD68. Isolated exosomes from all three sources displayed CD68 immunoreactivity. Our results demonstrate that exosomes originating in microglia can be isolated, either in a pool of exosomes from frozen brain tissue, or as pure microglia derived exosomes from cultures, and show appropriate presence of expected markers. Isolated exosomes will be useful for studies aimed at determining the role of microglia-derived exosomes in propagation of pathology in frontotemporal dementia, including FTLD-TDP-43 and FTLD-tau proteinopathies.

Disclosures: I.A. Ayala: None. **C.A. Estrada:** None. **R. Shahidehpour:** None. **T.D. Gefen:** None. **M.E. Flanagan:** None. **M.M. Mesulam:** None. **C. Geula:** None.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 NS084817
NIH Grant R01 DA044552
NIH Gratnt R01 DA044552-03S1
NIH Grant R01 DA033966
NIH Grant R01 NS060632

Title: Increased excitability and calcium influx characterize medial prefrontal cortex pyramidal neurons during early stage of Alzheimer's disease in 3xTg-AD mouse model

Authors: *N. CHOUDHURY, L. CHEN, V. T. NGUYEN, L. AL-HARTHI, X.-T. HU; Microbial Pathogens and Immunity, Rush Univ. Med. Ctr., Chicago, IL

Abstract: β-amyloid plaques and hyperphosphorylated tau tangles are hallmarks of Alzheimer's disease (AD) in several brain regions which regulate cognitive abilities, including the cortex and the hippocampus. The medial prefrontal cortex (mPFC) plays a critical role in cognition including, but not limited to, forming multisynaptic connections with the hippocampus to regulate learning/ memory-related tasks, but it is understudied in AD neuropathophysiology. We assessed the functional status of mPFC pyramidal neurons in the context of AD, using male 3xTg-AD mouse model, and electrophysiology (patch-clamp recording) in acute brain slices. We found that at the age of 3-5 months (equivalent to 20-30 years in humans), pyramidal neurons in layer 5-6 of the mPFC show abnormally increased firing followed by overactivation-induced failure to fire. These neurons are also marked by significantly more depolarized resting membrane potential, lower rheobase, higher input resistance, and lower firing threshold for evoked firing. We also assessed Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs) in these neurons, which was indicated by Ca^{2+} plateau potentials (spikes) evoked under blockade of voltage-gated Na^{+} and K^{+} channels. We show that evoked Ca^{2+} spikes in mPFC neurons have significantly increased duration and area as compared to non-AD mice, indicative of enhanced VGCC activity/ expression and elevated $[Ca^{2+}]_{in}$ as a result of AD neuropathology. Collectively, these findings demonstrate that hyperexcitability and excessive increase of Ca^{2+} influx via overactivated/overexpressed VGCCs occur in mPFC pyramidal neurons of 3xTg-AD mice at early stages of AD and suggest that alterations in neuronal Ca^{2+} homeostasis and neurophysiological properties may precede classical neuropathology of AD. Understanding early stages of AD and its impact on mPFC may provide early clues of pathways that mediate CNS dysregulation and inform therapeutic interventions prior to onset of neuropathology.

Disclosures: **N. Choudhury:** None. **L. Chen:** None. **V.T. Nguyen:** None. **L. Al-Harthi:** None. **X. Hu:** None.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA/NIH Grant P30 AG013854
NIA/NIH Grant R01 NS085770
NIA/NIH Grant R01 NS075075
NIA/NIH Grant R01 AG056258
NIA/NIH Grant R01 AG062566
NIDCD/NIH Grant R01 DC008552
NACC Grant U01 AG016976

Title: Putative Pathologic Correlates of Neurodegeneration in an Autopsy Series of FTLD-TDP Type C

Authors: *A. KAWLES, Y. NISHIHARA, N. GILL, A. FELDMAN, H. ZHANG, M. E. FLANAGAN, M. .-M. MESULAM, C. GEULA, E. H. BIGIO, Q. MAO, T. GEFEN; Mesulam Ctr. for Cognitive Neurol. & Alzheimer's Dis., Northwestern Univ., Chicago, IL

Abstract: Background. The TDP-43 type C pathologic form of frontotemporal lobar degeneration (FTLD-TDP-C) is characterized by the presence of immunoreactive TDP-43 short and long dystrophic neurites (DNs), neuronal cytoplasmic inclusions (NCIs), and neuronal loss and gliosis (NL/G). Autopsy data show a tight correlation between FTLD-TDP-C cases and either behavioral variant frontotemporal dementia (bvFTD) or the semantic variant of primary progressive aphasia (PPA-S), offering the opportunity to study the putative correlates of neurodegeneration in clinical dementia syndromes. Here, we report regional distributions of pathologic TDP-43 and the extent of NL/G in cortical and subcortical regions in FTLD-TDP-C cases, and investigate the relationship between FTLD-TDP-C pathologic inclusions and NL/G.
Methods. Twenty-six cortical and subcortical regions were immunostained with a phosphorylated TDP-43 antibody and evaluated for long DNs, short DNs, and NCIs in ten cases with FTLD-TDP-C (PPA-S N=7; bvFTD N=3). NL/G was assessed using hematoxylin-eosin stained sections. Specimens were obtained from the Northwestern Alzheimer Disease Center. TDP pathology and NL/G were graded using a semiquantitative 5-point scale. We calculated a "neuron-to-inclusion" score (TDP-C mean score - NL/G mean score) for each region per case to assess the relationship between the extent of FTLD-TDP-C pathology vs NL/G.
Results. Distribution heat maps showed that long DNs were most abundant in the left superior temporal and inferior parietal gyri, and inferior frontal gyri bilaterally. The amygdala, caudate, and putamen were heavily affected. Interestingly, NL/G was most severe in bilateral temporal poles, amygdala, and trans/entorhinal cortices, and not necessarily the cortical regions most heavily affected by TDP-43. At both group and individual levels, linear mixed models showed that regions with the *lowest* "neuron-to-inclusion" score (little pathology and high neuronal loss) differed significantly from those with high "neuron-to-inclusion" scores (abundant pathology and preserved neuronal densities) across all three TDP markers ($p<0.01$).
Conclusion. In cases with FTLD-TDP-C, there is extensive TDP-43 pathology and NL/G in both cortical and subcortical regions. There appears to be an inverse relationship between the extent of TDP-positive inclusions and NL/G, which may reflect a process whereby inclusions disappear as their associated neurons are lost. Future studies will aim to investigate the mechanistic basis of this phenomenon to better understand the putative substrates of neurodegeneration in clinical dementia syndromes.

Disclosures: **A. Kawles:** None. **Y. Nishihara:** None. **N. Gill:** None. **A. Feldman:** None. **H. Zhang:** None. **M.E. Flanagan:** None. **M.-. Mesulam:** None. **C. Geula:** None. **E.H. Bigio:** None. **Q. Mao:** None. **T. Gefen:** None.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG031867

Title: Loss of Valosin Containing Protein (VCP) activity enhances proteopathic seeding

Authors: *J. ZHU[1], D. DHAVALE[2], R. FRENCH[3], S. PITTMANS[2], J. HAINES[2], P. KOTZBAUER[1], Y. AYALA[4], A. DAVIS[1], C. C. WEIHL[1];
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Abstract: Protein inclusions such as tau, α-synuclein, and TDP-43 are considered a pathologic hallmark of many neurodegenerative diseases. These proteins are prone to misfold, aggregate, and template inclusion body formation. Accumulating evidence suggests that proteins in their oligomeric form can serve as a "seed", spread through an interconnected brain network, and induce new inclusions. This transmission correlates with disease progression. Insofar, those proteins are recognized as proteopathic seeds. We focuses on the mechanism of intracellular proteopathic seeding events. Previous studies have demonstrated that proteopathic seeds are endocytosed and damage the endolysosomal membrane. This damage facilitates seed escape from membrane-bound vesicles, subsequent templating the conversion of intracellular monomers forming new aggregates. We detected acceleration in seeding when treating cells with LLoMe—a drug that permeabilizes lysosome membranes— together with seeds. This indicates that endolysosome integrity is important for intracellular seeding event.Here, we report that the AAA ATPase, VCP, protects against proteopathic seeding by facilitating the clearance of damaged endolysosomes. VCP is a versatile protein required for protein homeostasis. We previously demonstrated that VCP is recruited to damaged endolysosome, and signals degradation by autophagy, termed as Endolysosomal damage response (ELDR). Moreover, disease mutations in VCP specifically impair ELDR. VCP disease mutations have been identified in multiple neurodegenerative diseases with proteopathic protein inclusions, including Frontotemporal dementia, ALS, and Parkinson's. This indicates that VCP may be essential for proteopathic seeding events. Using an α -synuclein FRET biosensor assay, we found both VCP inhibition and VCP disease mutations exacerbate α-synuclein seeding. A similar result was replicated in hippocampal neurons, in which both VCP knockdown and VCP mutant knock-in neurons show increasing α -synuclein aggregation following seeding. Screening different VCP cofactors, we identified that the ELDR-related cofactor, UBXD1 modifies the α-synuclein seeding similar to VCP. It suggests that VCP regulates α-synuclein seeding via ELDR. Additionally, mice carried a VCP disease mutation (n=3) exhibits significantly more α-synuclein aggregation than C57 control (n=7, p=0.015) 90 days after intrastriatal injection of α-synuclein pre-formed fibrils *in vivo*. Further, we observed the same protective effect of VCP on intracellular tau and TDP-43 seeding. Overall, our results support VCP as a gatekeeper for intracellular proteopathic seeding in general.

Disclosures: J. Zhu: None. **D. Dhavale:** None. **A. Davis:** None. **S. Pittmans:** None. **C.C. Weihl:** None. **R. French:** None. **Y. Ayala:** None. **J. Haines:** None. **P. Kotzbauer:** None.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: This work was funded by NEOMED research start-up funds to Dr. Christine Dengler-Crish.

Title: Wnt/b-catenin canonical pathway changes in brain and bone of an amyloid-dominant mouse model of Alzheimer's disease

Authors: *K. BRETLAND[1], L. LIN[1], K. BRETLAND[2,1], C. M. DENGLER-CRISH[1]; [1]Dept. of Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH; [2]Kent State Univ., Kent, OH

Abstract: Patients with Alzheimer's disease (AD) are at an increased risk for osteoporosis and bone fracture compared to neurotypical patients of the same age. This comorbidity negatively impacts quality of life in these patients and has been linked to increased mortality. As AD is the most common form of dementia and currently affects almost 6 million Americans, it is essential to identify mechanistic linkages between loss of bone integrity and neurodegeneration in this disease. The central and peripheral nervous systems regulate bone remodeling through complex circuitry and cell signaling pathways. One pathway of interest, canonical Wnt signaling, is shown to be disrupted in both osteoporosis as well as AD. Previously, our lab found evidence of disrupted Wnt signaling in the brain and bone of a mouse designed to model Alzheimer's-related tauopathy. In this project, we hypothesized that deficits in Wnt/β-catenin signaling would be associated with both bone loss and amyloid-β neuropathology in a rodent AD model that presents with low bone density, APP/PS1dE9 mice. Using radiographic bone densitometry, we tracked changes in bone density and body composition longitudinally across increasing pathological stages (2-13 months of age) in these mice. We then collected various brain (hippocampus, brainstem) and bone (femur, tibia) tissues for subsequent assays to measure protein and gene expression associated with the Wnt/β-catenin signaling pathway. Results were assessed with SPSS for each sex across pathological ages in comparison to sex and age-matched wildtype controls. Our preliminary results confirm evidence of a low bone density phenotype in APP/PS1 mice, as well as Wnt signaling impairments in brain and bone tissue in comparison to wildtype littermates. Interestingly, this seems to be a highly sex-dependent finding. These results could potentially support the idea of AD pathology being systemically driven. This may also provide a window for early intervention in AD, however, the sex differences must be examined further.

Disclosures: K. Bretland: None. **L. Lin:** None. **K. Bretland:** None. **C.M. Dengler-Crish:** None.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: American Heart Association Predoctoral Fellowship Award

Title: Inducing intergenerational resilience to vascular cognitive impairment and dementia by repetitive hypoxic conditioning

Authors: *K. D. BELMONTE[1], T. WILLS[1], J. M. GIDDAY[2];
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Abstract: BACKGROUND: Vascular cognitive impairment and dementia (VCID), secondary to stroke or chronic cerebral hypoperfusion, is the second leading cause of dementia following Alzheimer's disease. Although 25-30% of ischemic stroke survivors develop immediate or delayed VCID, there is no efficacious therapy. We have shown previously in mice that repetitive hypoxic preconditioning (RHC) induces a long-lasting resilience to acute stroke (PMID:21437933). More recently, we documented that untreated, first-generation adult progeny of mice exposed to RHC prior to mating are protected from retinal ischemic injury (PMID:32910134), consistent with accumulating evidence supporting the concept that long-lasting phenotypes induced by intermittent stressors may be inherited. Taken together, we hypothesized that RHC will induce resilience to VCID, and that such RHC-induced protection can also be inherited.
METHODS: Chronic cerebral hypoperfusion (CCH) was induced secondary to bilateral carotid artery stenosis with microcoils in both the parental F0 generation and their untreated F1 offspring of C57Bl/6J mice. Cohorts of F0 animals were directly exposed to either 8 wks of RHC or normoxia prior to CCH. F1-generation mice, derived from either RHC-treated animals prior to mating, or normoxic controls, never received direct RHC treatment but were properly age-matched to the F0 generation when CCH was established. Following 3 months of CCH, short-term recognition memory was assessed in vivo by novel object recognition (NOR) testing, and the following month, ex vivo measurements of hippocampal long-term potentiation (LTP) were recorded from the same animals as an electrophysiological metric of memory-associated synaptic plasticity.
RESULTS: CCH caused impairments in recognition memory in control mice from both generations. However, in F0 animals directly treated with RHC, as well as in their untreated adult F1 progeny, these CCH-induced memory impairments were prevented. Similarly, RHC treatment enhanced LTP formation in the CCH cohorts of mice directly treated with RHC, as well as in their untreated offspring, despite the latter having no direct exposure to RHC.
CONCLUSIONS: Our findings demonstrate that epigenetic-based therapeutics may hold promise for inducing resilience to VCID and raise the possibility that such a state of induced resilience may even be heritable.

Disclosures: **K.D. Belmonte:** None. **T. Wills:** None. **J.M. Gidday:** None.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer society
GENFI dataset

Title: Cerebellar lobule volumes and neuropsychiatric symptoms in genetic frontotemporal dementia

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Abstract: Introduction: Familial frontotemporal dementia (FTD) is a neurodegenerative disorder caused by three main autosomal dominant mutations. A paucity of studies have examined the impact of these mutations on the cerebellum as a cause of neuropsychiatric symptoms. Methods: 413 participants from the Genetic Frontotemporal dementia Initiative dataset were included in this study [167 non-carriers, 246 carriers]. We used the MAGeT Brain algorithm to estimate the volumes of the cerebellar lobules from standard T1-weighted images. Partial least square (PLS) analysis was used to identify a set of latent variables (LVs), that explain patterns of covariance between "brain" and "demographic/neuropsychiatric" data with the constraint that LVs explain as much of the covariance between the two matrices as possible. Here, our "brain" data included the volume of each cerebellar lobule and total brain volume (matrix size 413x13). Our "demographic/neuropsychiatric" data contained age, sex, years of education, estimated years of onset, genetic status, and 11 behavioral scores from the Cambridge Behavioural Inventory (CBI) for each subject (matrix size 413x16). Permutation testing and bootstrap resampling were performed (at $p<0.05$ threshold) to statistically test each LV and to assess the contribution of each "demographic/neuropsychiatric" data on these LVs, respectively. Results: Two LVs were significant in each hemisphere with highly similar results (Figure). LV1 demonstrated lower overall cerebellar volumes (except lobules I-II and X) to be associated with high CBI scores, being a mutation carrier, older, closer to onset, female sex and low education. LV2 exhibited higher lobule X volumes to be related to higher CBI scores in behavior, memory and everyday skills. Discussion: Within genetic FTD individuals, subjects with larger lobule X volume seemed to be at a higher risk for neuropsychiatric symptoms. This could be explained by a dysregulation of the cholinergic system which regulates plasticity, arousal and reward, since lobule X is known to be involved in this pathway.

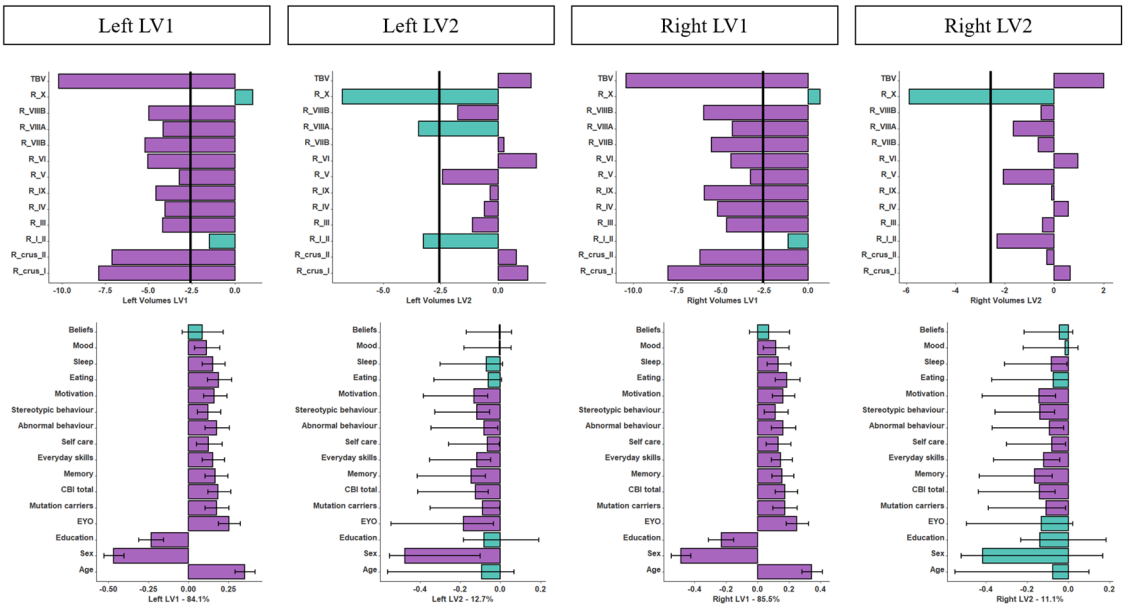

Figure : PLS between the left/right cerebellar subfield volumes and the demographic/neuropsychiatric symptoms information identified two significant LV on each hemisphere ($p<0.05$). Left LV1 explained 84.1%, left LV2 explained 12.7%, right LV1 explained 85.5% and right LV2 explained 11.1% of the covariance. **Upper row:** Bar plots describe the correlation of each subfield variable with the LV, purple color identifies variables significantly contributing to the LV, while turquoise color identifies nonsignificant variables. Black vertical line corresponds to a BSR threshold of 2.58. **Lower row:** Bar plots describe the correlation of each demographic/neuropsychiatric symptoms variable with the LV, with error bars denoting the 95% confidence interval, thus purple identifies variables significantly contributing to the LV.

Disclosures: A. Bussy: None. **J. Levy:** None. **M. Chakravarty:** None. **S. Ducharme:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Biogen, Ionis parmaceuticals and AZTherapies. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Speaker fees from Sunovion Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder of AFX Medical Inc. F. Consulting Fees (e.g., advisory boards); Consulting fees from Ionis Pharmaceuticals and Optina Diagnostics.

Digital Abstract Session

P107. Proteinopathy and Pathology Other than Abeta/Tau

Program #/Poster #: P107.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 NS084817
NIH Grant R01 DA044552
NIH Grant R01 DA044552-03S1
NIH Grant R01 DA033966
NIH Grant R01 NS060632

Title: Chronic exposure to methamphetamine reduces excitability of pyramidal neurons associated with decreased voltage-gated calcium channel activity inthemedial prefrontal cortex of rats.

Authors: *L. CHEN, V. T. NGUYEN, S. L. CASSODAY, A. DONNER, N. CHOUDHURY, L. AL-HARTHI, X.-T. HU;
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Abstract: Methamphetamine (Meth) is a highly addictive and widely abused psychostimulant. There is no FDA-approved medication for the treatment of Meth addiction. Elucidating the mechanism underlying Meth-induced neuropathology is critical for the development of efficacious pharmacotherapy. The medial prefrontal cortex (mPFC) is a key regulator of cognition and addiction; but it is profoundly altered by Meth and other psychostimulants. The mechanism by which Meth induce dysfunction of glutamatergic pyramidal neurons in the mPFC is not fully undersdood. To determine the impact of chronic Meth exposure on mPFC pyramidal neurons, we assessed their functional activity using Fischer F344 male rats at different ages. Adolescent rats (4-6 weeks of age; 4-6wk) received repetitive daily injection of Meth (5mg/ml/kg, s.c.) for 5 days, while adult rats (5-6 months of age, 5-6mo) were trained to self-administer (SA) Meth at the dose of 0.01mg/kg/infusion for one week, and then switched to the dose of 0.05mg/kg/infusion for another two weeks. Saline (SAL)-pretreated, or SAL-yoked F344 rats were used as control. Brain slices containing the mPFC were prepared for whole-cell patch-clamp recording after a withdrawal period of 1-4 days from Meth-SA or SAL. We found that the firing of mPFC pyramidal neurons was significantly decreased in rats after repeated Meth exposure and withdrawal compared to those pretreated with SAL in both adolescent and adult rats. We also performed whole-cell current-clamp recordings to assess voltage-sensitive Ca^{2+} influx via voltage-gated Ca^{2+} channels (VGCCs) that was indicated by evoked Ca^{2+} plateau potentials in mPFC pyramidal neurons. During this assessment, all neurons were evaluated under blockade of synaptic activities mediated by glutamate receptors and $GABA_A$ receptors, as well as functional activity of voltage-gated Na^+ and K^+ channels. We also found that mPFC pyramidal neurons from Meth-SA rats displayed a significant reduction of Ca^{2+} potentials in both the duration and area compared to those from SAL-yoked rats. Collectively, our findings demonstrate that chronic exposure to Meth significantly decreases the functional activity of mPFC pyramidal neurons in rat mPFC during an early stage of withdrawal. And the Meth-induced decrease of mPFC neuronal excitability is mediated by dysfunctional VGCCs with decreased functional activity, which is independent of dysfunctional NMDA receptors. Such alterations may contribute to the mechanism by which chronic Meth exposure disturbs cognition and causes addiction.

Disclosures: L. Chen: None. **V.T. Nguyen:** None. **S.L. Cassoday:** None. **A. Donner:** None. **N. Choudhury:** None. **L. Al-Harthi:** None. **X. Hu:** None.

Digital Abstract Session

P108. Biomarkers

Program #/Poster #: P108.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HMN grant 2018/42794

Title: Linking molecular biomarkers and behavior in preclinical models and patients with Alzheimer's disease

Authors: ***C. BJORKLI**[1], M. HEMLER[1], N. C. EBBESEN[1], R. NAIR[2], M. P. WITTER[2], C. G. KENTROS[2], A. KOBRO-FLATMOEN[2], A. SANDVIG[1], I. SANDVIG[1];
[1]Sandvig group Inst. for Neuromedicine, [2]Kavli Inst. for Systems Neurosci., NTNU, Trondheim, Norway

Abstract: **Aims**: Alzheimer's disease (AD) is a debilitating neurodegenerative disorder that causes severe deterioration of memory, cognition, behavior, and the ability to perform daily activities. The disease is characterized by the accumulation of two proteins in fibrillar form; Amyloid-β forms fibrils that accumulate as extracellular plaques while tau fibrils form intracellular tangles. Here we aim to translate findings from a commonly used AD mouse model to AD patients. **Methods**: Here we initiate and chronically inhibit neuropathology in lateral entorhinal cortex (LEC) layer two neurons in an AD mouse model. This is achieved by over-expressing P301L tau virally and chronically activating hM4Di DREADDs intracranially using the ligand dechloroclozapine. Biomarkers in cerebrospinal fluid (CSF) is measured longitudinally in the model using microdialysis, and we use this same system to intracranially administer drugs aimed at halting AD-related neuropathology. The models are additionally tested in a novel contextual memory task. **Results:** Preliminary findings indicate that viral injections of P301L tau into LEC layer two reveal direct projections between this region and the outer molecular layer of dentate gyrus and the rest of hippocampus. Additionally, phosphorylated tau co-localize with 'starter cells' and appear to spread from the injection site. Preliminary microdialysis results suggest that the concentrations of CSF amyloid-β and tau proteins mirror changes observed along the disease cascade in patients. The disease-modifying drugs appear to halt neuropathological development in this preclincial model. **Conclusions:** These findings will lead to a novel platform for translational AD research, linking the extensive research done in rodents to clinical applications.

Disclosures: **C. Bjorkli:** None. **R. Nair:** None. **M.P. Witter:** None. **C.G. Kentros:** None. **A. Kobro-Flatmoen:** None. **A. Sandvig:** None. **I. Sandvig:** None. **M. Hemler:** None. **N.C. Ebbesen:** None.

Digital Abstract Session

P108. Biomarkers

Program #/Poster #: P108.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: K01-AG061277
AARF-16–443681
P01-AG066597

Title: Prospective Motion Correction Improves Effect Size for Detection of Cortical Thickness in Neurodegenerative Disease

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Abstract: Magnetic resonance imaging (MRI) is critical for diagnostic and observational research of neurodegenerative diseases. However, subject motion can lead to reduced estimates of cortical thickness (CT, Reuter et al., 2015). Application of prospective motion correction using volumetric navigators (vNavs, Tisdall et al., 2012) has been demonstrated to reduce bias in gray matter estimates in healthy volunteers with artificial motion (Tisdall et al., 2016), but their application has not yet been studied in natural motion of neurodegenerative disease patients. In this study we investigated how prospective motion correction with vNavs influenced effect sizes for CT differences between participants clinically diagnosed with Alzheimer's Disease (AD, n=34), behavioral variant frontotemporal dementia (bvFTD, n=40), and healthy controls (n=26), recruited from the University of Pennsylvania Frontotemporal Degeneration Center. We hypothesized that group differences between AD and controls and bvFTD and controls would be larger when using vNavs than not correcting for motion. We collected two T1-weighted MRI sequences containing four echoes interspersed with vNavs on each individual, one applying motion correction, and one without. T1 images were processed using Advanced Normalization Tools (ANTs, Tustison et al., 2014). Using warps to a standardized template, a manually segmented brain atlas (Lausanne125, Daducci et al., 2012) was spatially normalized to each participant's native T1 space, and mean CT in each region of interest was calculated. CT estimates across 219 ROI were compared between patient and control groups in both MRI conditions, and effect sizes were calculated. CT comparisons in the motion corrected condition consistently demonstrated significant (false discovery rate corrected $p<0.05$) atrophy in anatomically relevant regions, while reducing spurious findings observed in the non-motion corrected condition. Effect sizes within the motion corrected condition were larger in the frontal pole and the inferior temporal lobes for both disease groups. Our results demonstrate that motion correction may allow for better discrimination between groups in biologically-plausible neuroanatomic regions.

Disclosures: **N.G. Kinney:** None. **A. Tranquille:** None. **P.R. Bharne:** None. **F.J. Nitchie:** None. **M.J. Martin:** None. **M. Grossman:** None. **S. Burke:** None. **C.A. Olm:** None. **P.A. Cook:** None. **J.C. Gee:** None. **D.W. Wakeman:** None. **M.D. Tisdall:** None. **C.T. McMillan:** None. **J.S. Phillips:** None.

Digital Abstract Session

P108. Biomarkers

Program #/Poster #: P108.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MV, LV, LuM and LDG are fellows of the Research Foundation Flanders (FWO).
Alzheimer's Research Foundation (SAO-FRA)
European Union Horizon 2020 Research and Innovation Program (2014-2020) (HERALD project, granted by the ATTRACT consortium)
The Centre for Eye Research Australia receives Operational Infrastructure Support from the Victorian Government.
XH and PvW acknowledge funding support from the H & L Hecht Trust and the Yulgilbar Alzheimer's Research Program.

Title: The App^{NL-G-F} mouse retina is a site for preclinical Alzheimer's disease diagnosis and research

Authors: *M. VANDENABEELE[1], L. VEYS[1], S. LEMMENS[2], X. HADOUX[3], G. GELDERS[1], L. MASIN[1], L. SERNEELS[4], J. THEUNIS[5], T. SAITO[6], T. C. SAIDO[6], M. JAYAPALA[7], P. DE BOEVER[5], B. DE STROOPER[4], I. STALMANS[2], P. VAN WIJNGAARDEN[3], L. MOONS[1], L. DE GROEF[1];
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Abstract: When a patient presents with Alzheimer's disease (AD) dementia, AD-related disease processes are already ongoing for 20 years. New methods to diagnose patients in this presymptomatic disease stage might hold the key to the development of successful AD therapies. In this light, the retina has emerged as a new target to diagnose and study AD, as it allows for the non-invasive characterization of central nervous system tissue, both in mice and patients. In this preclinical study, we aimed to gain more evidence for the early involvement of the retina in AD and for the potential of retinal examinations for follow-up of disease progression in AD mouse models. We report the results of a comprehensive phenotyping of the retina of the *App^{NL-G-F}* mouse, one of the first AD knock-in mouse models. Accumulation of amyloid beta (Aβ), one of the major hallmarks of AD, was assessed in the retina using ELISA, immunohistochemistry and *ex vivo* hyperspectral imaging. Furthermore, *in vivo* tests were applied to study the morphology and electrophysiology of the retina (optical coherence tomography and electroretinograms) and visual performance (optomotor test). We demonstrate that soluble Aβ accumulation is present in the retina of these mice early in life and progresses to Aβ plaque formation by midlife. This elevated Aβ burden, coincides with local microglia reactivity, astrogliosis, and abnormalities in retinal vein morphology. In addition, electroretinography recordings reveal subtle signs of neuronal dysfunction yet no clear neurodegeneration was observed and visual performance outcomes were unaffected in the *App^{NL-G-F}* mouse. Furthermore, we show that retinal hyperspectral imaging can be used to quantify retinal Aβ already early in life, underscoring its potential as a biomarker for AD diagnosis and monitoring. These findings suggest that the *App^{NL-G-F}* retina mimics the early, preclinical stages of AD, wherein Aβ accumulates but does not lead

to severe functional impairment yet. This model, combined with retinal imaging techniques, offers unique opportunities for drug discovery and fundamental research into preclinical AD.

Disclosures: M. Vandenabeele: None. **L. Veys:** None. **S. Lemmens:** None. **X. Hadoux:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); XH and PvW have filed an International Patent Application No PCT/AU2019/000003 relating to retinal hyperspectral imaging (HSI) and are co-founders of Enlighten Imaging PTY LTD, a start-up HSI company.. **G. Gelders:** None. **L. Masin:** None. **L. Serneels:** None. **J. Theunis:** None. **T. Saito:** None. **T.C. Saido:** None. **M. Jayapala:** None. **P. De Boever:** None. **B. De Strooper:** None. **I. Stalmans:** None. **P. van Wijngaarden:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); XH and PvW have filed an International Patent Application No PCT/AU2019/000003 relating to retinal hyperspectral imaging (HSI) and are co-founders of Enlighten Imaging PTY LTD, a start-up HSI company.. **L. Moons:** None. **L. De Groef:** None.

Digital Abstract Session

P108. Biomarkers

Program #/Poster #: P108.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA/NIH Grant R01 AG062566
NIA/NIH Grant P30 AG013854
NIH Grant R01 AG067781
NIH Grant R56 AG045571

Title: Integrity of Neuronal Size in the Entorhinal Cortex across the Cognitive Aging Spectrum

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Abstract: Integrity of Neuronal Size in the Entorhinal Cortex across the Cognitive Aging Spectrum

Authors:C. Nassif, A. Kawles, G. Minogue, Q. Mao, M.E. Flanagan, E.H. Bigio, M.-Marsel Mesulam, E.J. Rogalski, C. Geula, and T. Gefen
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Disclosures: None.

Abstract:

Background. With increasing age, there is a common and gradual decline in memory capacity. These common or “normal” declines in memory are associated with markers of Alzheimer’s

disease (AD) neuropathology, characterized by neurofibrillary tangles (NFTs) and amyloid plaques that typically appear in the entorhinal cortex (ERC). However, there is a rare group of individuals over age 80 designated as "Cognitive SuperAgers" who show exceptional memory at a level comparable to individuals 20-30 years their junior and appear to be immune to some of the deleterious effects of aging. In a recent study, we found that compared to same-aged cognitively-normal peers, SuperAgers showed significantly lower numbers of tangle-bearing neurons in the ERC. The goal of this study was to determine whether neuronal atrophy (i.e., size) in the memory-associated ERC differentiates SuperAgers from their cognitively-normal peers.
Methods. Postmortem specimens were collected from the following subject groups: SuperAgers (N=4; mean age, 89.9), cognitively-normal controls (N=6; mean age, 87.8), and individuals with amnestic Mild Cognitive Impairment (aMCI; N=5; mean age, 92.4), a prodromal stage of dementia of the AD type. ERC sections were obtained at a thickness of 40µm and stained with cresyl violet to visualize neurons. Neuronal size of cortical layers III and V were quantified using the ImageJ software (version 1.53a).
Results. One-way non-parametric ANOVAs determined no significant difference in neuronal size in the ERC between cognitively-normal controls (M=143.04µm^2; SD=77.49µm^2) and SuperAgers (M=143.48µm^2; SD=72.19µm^2); however, there was a significant difference in mean neuronal size between the aMCI group (M=130.11µm^2; SD = 44.51µm^2) and both the cognitively-normal control and the SuperAger groups (p<0.0001).
Conclusion. Despite exceptional episodic memory performance, neuronal atrophy in the ERC does not appear to be a strong discriminating factor between SuperAgers and their cognitively-normal peers. Instead, loss of neuronal integrity appears to be more closely associated with abnormal memory performance pathognomonic of aMCI, suggesting changes in neuronal size may emerge as a downstream consequence of accumulating AD neuropathology.

Disclosures: C. Nassif: None. **A. Kawles:** None. **G. Minogue:** None. **Q. Mao:** None. **M.E. Flanagan:** None. **E.H. Bigio:** None. **M. Mesulam:** None. **E.J. Rogalski:** None. **C. Geula:** None. **T. Gefen:** None.

Digital Abstract Session

P108. Biomarkers

Program #/Poster #: P108.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: U01 AG051406
U01 AG051412
AR-UK-PG2015-23
G1002252

Title: Support vector machine learning and diffusion-derived structural networks accurately predict amyloid quantity and longitudinal cognitive change in people with Down's syndrome who are aging

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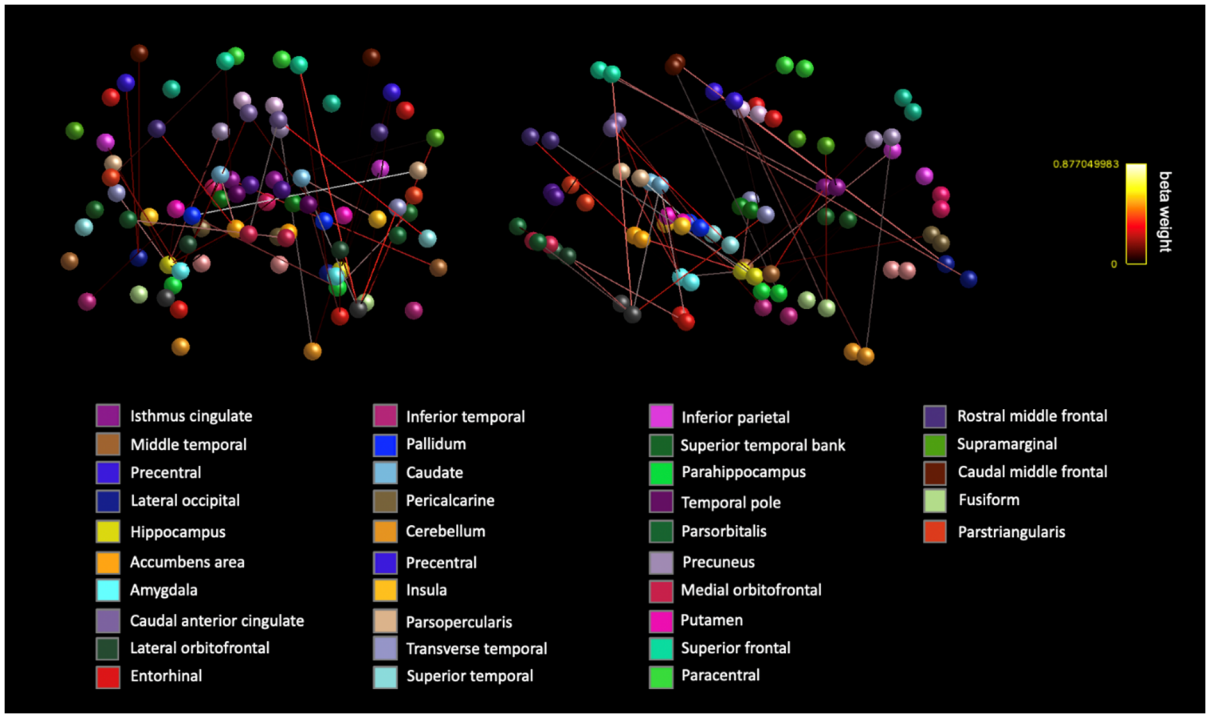
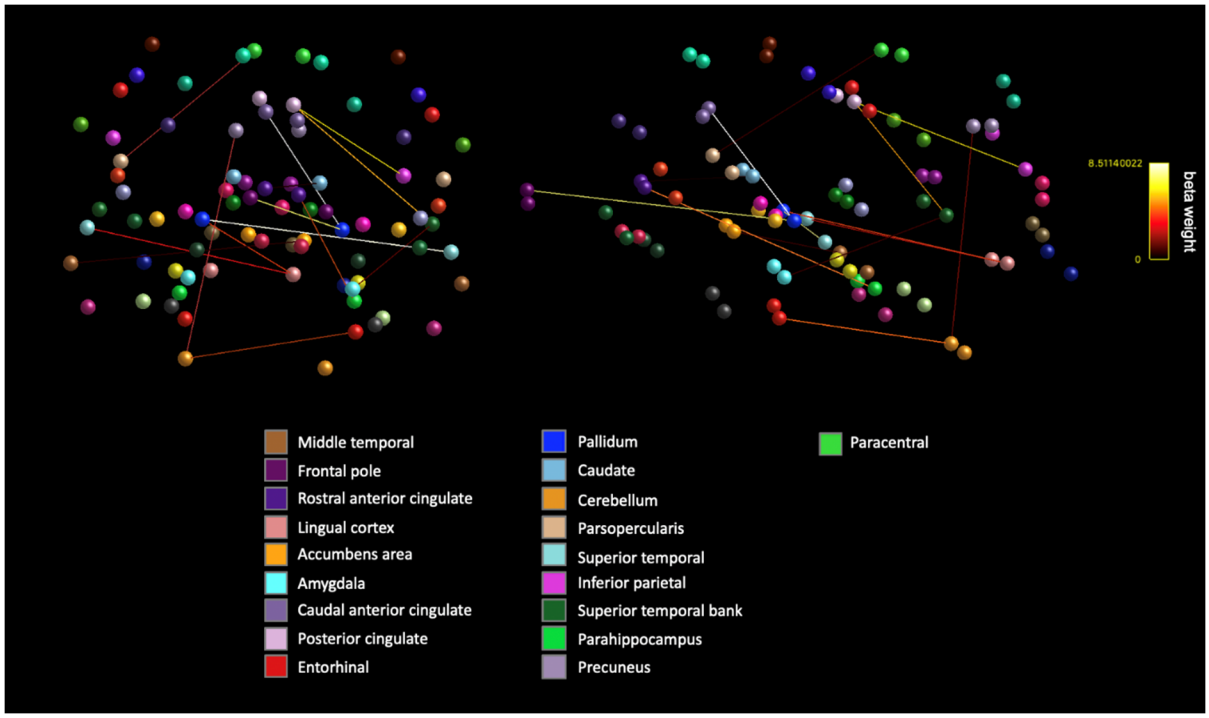
Abstract: Down's syndrome (DS) results from trisomy of chromosome 21, a genetic change which also confers a probable one hundred percent risk for the development of Alzheimer's disease (AD) neuropathology in later life. We aimed to assess the effectiveness of diffusion-weighted imaging and connectomic modelling for predicting brain amyloid plaque burden and longitudinal cognitive change using support vector regression. Ninety-five participants with DS successfully completed a full Pittsburgh Compound B (PiB) PET-MR protocol and memory assessment at two timepoints.

Linear regression support vector machines (SVMs) carried out on the selected features of the structural network predicted amyloid deposition via PiB binding with a root mean square error (RMSE) of 0.53 (SUVR) and an R^2 value of 0.19. The selected features of the structural network predicted longitudinal cognitive change with a RMSE of 8.8 (total score on cued recall) and an R^2 value of 0.26.

Here, we show that connection density of the structural network at baseline is a promising predictor of both current amyloid deposition and longitudinal cognitive change. Additionally, our findings indicate an important role for both network dysconnectivity and compensatory mechanisms during the trajectory of AD-development in people with DS. Taken together, these results demonstrate the integral role of the white matter during neuropathological progression and the utility of machine learning methodology for non-invasively evaluating AD prognosis.





Disclosures: **S.S. Brown:** None. **E. Mak:** None. **M. Grigorova:** None. **J. Beresford-Webb:** None. **M. Walpert:** None. **Y. Hong:** None. **T. Fryer:** None. **J. Coles:** None. **F. Aigbirhio:** None. **D. Tudorascu:** None. **A. Cohen:** None. **B.T. Christian:** None. **B. Handen:** None. **W. Klunk:** None. **D. Menon:** None. **P. Nestor:** None. **A. Holland:** None. **S. Zaman:** None.

Digital Abstract Session

P109. Dopamine and Non-Dopamine Pathways

Program #/Poster #: P109.01

Topic: C.03. Parkinson's Disease

Support: NIH-NINDS R01NS113746
NIH-OD P51OD011132
NIH-NINDS P50NS098685

Title: Three dimensional ultrastructural analysis of GABAergic terminals from the external globus pallidus in the subthalamic nucleus of rhesus monkeys

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Abstract: The subthalamic nucleus (STN) is part of the "indirect" pathway (striatum-external globus pallidus-STN) of the basal ganglia. Alterations of STN firing rates and patterns following nigrostriatal dopaminergic denervation are linked to the severity of parkinsonism and other diseases of basal ganglia origin (such as Huntington's disease or dystonia). Previous studies demonstrated that neuroplastic changes in the abundance, morphology and strength of GABAergic synapses from the external globus pallidus (GPe) to the STN contribute to parkinsonism. In 6-hydroxydopamine (OHDA)-treated mice with severe nigrostriatal degeneration, the increased strength of GPe-STN GABAergic synapses is associated with an increase in the number of synapses per GPe terminals (Fan et al., 2012, JNS 32:13718). This adaptation may contributes to abnormal firing patterns of STN neurons and the development of parkinsonian motor signs. Such knowledge does not exist for primate species. To enable enquires into disease-associated adaptive changes of the morphology of GPe terminals in the STN, we undertook a detailed quantitative 3D electron microscopic analysis of the morphometry of GPe terminals and their synapses in the STN in healthy monkeys. The single block facing/scanning electron microscopy (SBF/SEM) approach and the *Reconstruct* software were used to fully reconstruct 55 GABAergic pallidal terminals in the STN of 2 control rhesus monkeys. The pallidal terminals were identified either by anterograde labeling from the GPe or known ultrastructural features (large size, symmetric synapses, >3 mitochondria). We collected morphometric data for each terminal, including its volume, the flat area of synapses formed by the terminal, and the number and size of mitochondria within the terminal. Our data show that the volume of GPe terminals ranged from 1.13-26.41μm^3 (mean ± SEM= 4.02 ± 0.47). Each terminal formed large and complex fenestrated synapses with flat areas varying from 0.04-2.67μm^2 (mean ± SEM= 0.42 ± 0.05). The number of mitochondria per terminal ranged from 2-31 (mean ± SEM= 5.15 ± 0.55), while the volume of individual mitochondria varied between 0.06-0.34μm^3 (mean ± SEM=0.15 ± 0.008). Positive correlations were found between the volume of the terminal and the flat area of the synapses as well as the number and volume of mitochondria/terminal. Studies in MPTP-treated parkinsonian monkeys are in progress to compare the ultrastructural features of the GPe-STN terminals between the normal and parkinsonian states.

Disclosures: A. Martel: None. **R.M. Villalba:** None. **T. Wichmann:** None. **A. Galvan:** None. **Y. Smith:** None.

Digital Abstract Session

P109. Dopamine and Non-Dopamine Pathways

Program #/Poster #: P109.02

Topic: C.03. Parkinson's Disease

Support: NIA AG065682

Title: Identification of GPR139 as an attenuator of LID through striatal interneuron transcriptional profiling

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Abstract: Cholinergic interneurons (CINs) of the striatum have been recognized as a key mediator of L-dopa induced dyskinesia. We have previously shown that the Shh pathway attenuates L-Dopa induced dyskinesia (LID) in parkinsonian rodent and macaque models of Parkinson's disease (PD) in a mechanism that impinges on CIN activity (Malave et al., 2020, BioRX: https://doi.org/10.1101/2020.03.09.983759). The predominant source of Shh for CIN are dopaminergic neurons (DANs) located in the ventral midbrain and are the same population of neurons that degenerate in PD. The downstream transcriptional targets of Shh in CINs are not well defined. We utilized the immunoprecipitation technique, translating ribosome affinity purification (TRAP), to isolate mRNA from CINs for RNAseq in mice with and without Shh from DAN. CIN TRAP extractions were verified by qPCR and had enrichment of the CIN specific transcripts ChAT, GDNF, VAChT, M4, and M2 as well as depletion of the non-CIN transcripts M1 and Parv. The results from the RNAseq analysis identified the orphan G-coupled protein receptor GPR139 as a potential target for further investigation. First, we found that GPR139 is differentially expressed at the protein level in CIN in mice with and without Shh from DAN. GPR139 receptor agonism has been shown to block mu-opioid receptor activation by morphine and fentanyl. Interestingly, mu-opioid receptor antagonism has been shown previously to attenuate LID in animal models of PD. In a proof of principle study, we utilized the aphakia model of PD to ascertain if chronic L-dopa dosing paired with the GPR139 agonist JNJ-63533054 could attenuate the presentation and histological markers of LID. We find in preliminary results that GPR139 agonism reduces the appearance of three-paw dyskinesia in the aphakia model of PD. Histologically, the LID severity marker pERK is reduced with chronic GPR139 agonism in CIN. These results suggest that the transcriptional target of Shh, GPR139 attenuates expression of LID through a CIN resident mechanism. Further studies are needed to clarify the effects of GPR139 on CIN physiology and to determine the mechanism by which GPR139 impinges on LID formation and expression.

Disclosures: D.R. Zuelke: None. **A.H. Kottmann:** None. **K. Kennedy:** None. **P. Barkaus:** None.

Digital Abstract Session

P110. Rat and Mouse Toxin and Behavior Models

Program #/Poster #: P110.01

Topic: C.03. Parkinson's Disease

Support: Thomas Hartman Center for Parkinson's Research

Title: Estrogen sensitivity and sparing of Object-in-Place performance in 6-hydroxydopamine-lesioned female, male and gonadectomized male rats

Authors: *A. PATWARDHAN[1], J. PATEL[2], M. CONNER[1], M. KRITZER[1];
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Abstract: Early, pre-motor stages of Parkinson's disease (PD) are marked by treatment-resistant cognitive deficits whose underlying neurobiology is incompletely understood. However, the sex differences that characterize their type, prevalence and severity suggest key roles for sex hormones in modulating these non-motor signs (Jurado-Coronel et al., Front Neuroendocrinol, 2018). Recent work in our lab has shown striking protective effects of decreased androgen levels for deficits in spatial working and episodic memory that are induced in rats with partial bilateral neostriatal 6-OHDA lesions (Conner et al., Eur J Neurosci, 2020; Conner et al., Front in Neurol, 2020). Here we extend investigation to processes of associative memory for object identity and location using the Object in Place (OiP) task. In this task rats first explore an arena containing four distinct objects. After a delay, rats are reintroduced to the arena containing the same objects, albeit with two switched from their original locations. Driven by novelty preference, rats demonstrate associative memory by spending more time with displaced objects. We studied the effects of biological sex and sex hormones on OiP by comparing rats' discrimination index (DI) for displaced vs. stationary objects in male, female and gonadectomized male rats (GDX), and in GDX males given testosterone propionate (GDX-TP) or estrogen (GDX-E). We found robust DI that was greater in female vs. male rats, as well as striking deficits in DI in GDX rats that were partially attenuated by TP and fully rescued by E. We next evaluated impacts of sex and sex hormones in contexts of 6-OHDA lesions. Comparisons of sham and 6-OHDA-lesioned male, female, GDX, GDX-TP and GDX-E rats revealed no effects of sham surgery and profound 6-OHDA-induced DI deficits in male, GDX and, to a lesser extent, GDX-TP rats. However, in female and GDX-E rats, 6-OHDA lesions had no effect on DI. Importantly, the severity of DI deficits was unrelated to 6-OHDA lesion size and was not accompanied by obvious group differences in non-exploratory activities, e.g., grooming, rearing, ambulation. This strengthens conclusions that the memory functions tapped in OiP tasks are estrogen-sensitive and that elevated estrogen levels in the female and GDX-E groups protect these processes from harm in the 6-OHDA model. Together with our earlier data these findings also suggest that the distinct cognitive processes that are at risk in 6-OHDA rats include both estrogen- and androgen-sensitive domains that can be differentially attenuated/spared in sex- and hormone-specific ways. This in turn could help advance treatment for what are currently clinically intractable signs of PD.

Disclosures: **A. Patwardhan:** None. **M. Kritzer:** None. **M. Conner:** None. **J. Patel:** None.

Digital Abstract Session

P110. Rat and Mouse Toxin and Behavior Models

Program #/Poster #: P110.02

Topic: C.03. Parkinson's Disease

Support: SIP IPN Grant 20196737

COFAA felow

Title: Unilateral dopamine denervation in the globus pallidus of rat produces changes in anxiety-like behavior, memory and aggressiveness

Authors: K. BARÓN, J. CALZADA, E. CHUC-MEZA, *M. GARCIA-RAMIREZ;
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Abstract: External *Globus Pallidus* (GP) is a nucleus belonging to indirect pathway of basal ganglia and it is in a key position to influence processing of motor, associative and limbic information. GP receives dopaminergic innervation from Substantia Nigra pars compacta (SNc) In rodents, appetitive or aversive stimuli raise dopamine release in GP suggesting that dopaminergic innervation of GP is also involved in emotive process. Patients with globus pallidus infarctions shows acute cognitive changes, like inattention and amnesia. Besides in Parkinson`s disease patients disease it has also been observed anxiety and cognitive impairment, which are developed months or years before motor symptoms appear. The present work explores the effect of partial dopamine denervation in GP over anxiety, recognition memory, aggressiveness and motor activity in the rat. Unilateral dopaminergic lesion in GP was made with 6-OHDA (500 nL of 6-OHDA, 15 μg/μL) and 1 and 5 months after surgery, behavioral tests, were performed; Elevated plus maze (EPM), burying behavior (BB), novel object recognition test (NRT), Resident-intruder (RI) test and spontaneous motor activity (MA). Data were analyzed by one-way ANOVA followed by Student-Newman-Keuls post-hoc analysis. After a month of the surgery there were a significant decrease in time spent in open arms in EPM and increase burying time in BB, both indicative of anxiety. Recognition index was decreased in NRT revealing memory disfunction. Only 50% of lesioned rats increased aggression time in RI test. These results suggest that dopamine deficit in GP, could produce behavioral alterations resembling some of the neuropsychiatric symptoms observed in PD patients

Disclosures: K. Barón: None. **J. Calzada:** None. **E. Chuc-Meza:** None. **M. Garcia-Ramirez:** None.

Digital Abstract Session

P110. Rat and Mouse Toxin and Behavior Models

Program #/Poster #: P110.03

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1R43AG059509
University of Texas at Dallas

Title: Longitudinal Assessment of Skilled Motor Function and Levodopa Treatment Efficacy in DJ-1 Knockout Rats

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Abstract: DJ-1 knockout (DJ-1) rats have been shown to exhibit a moderate parkinsonian phenotype, with ca. 50% loss of midbrain dopaminergic neurons and deficits in some gross motor behaviors appearing between 6 and 8 m.o. In the current study, we provide additional characterization of skilled motor performance of DJ-1 rats from 2 to 12 months of age, and ask whether motor deficits in these rats are responsive to standard levodopa therapy. Male DJ-1 (n = 8) and wild-type Long Evans (WT, n = 8) rats were trained on an isometric pull task previously shown to be a sensitive assay of forelimb strength and coordination. The task requires subjects to reach 1.5 cm outside a training box and pull an isometric bar with sufficient force to receive reward. Rats performed two 30-minute training sessions daily, 5 days/week, beginning at 2 m.o. During Acquisition (2-10 m.o.), rats performed a standard version of the pull task in which pull force thresholds were low (100-200 g), and adapted on each trial to the average of the strongest 5 of the last 10 pulls. Our results demonstrate that DJ-1 rats exhibit significant deficits in pull force and percent correct performance on this task compared to WT rats. Impairment was detectable at ca. 8 m.o., as DJ-1 rats' performance stabilized, while WT performance continued to improve. At 10 m.o., the upper limit of the adaptive force threshold was increased to 300 g, increasing the difficulty of the task to determine whether the deficits exhibited by DJ-1 rats were due to an inability to execute the motor demands of the task. To our surprise, during this 5-week Challenge period, DJ-1 rats were able to increase their pull force, though performance remained significantly impaired compared to WT rats. Throughout the Acquisition and Challenge periods, DJ-1 rats exhibited no differences from WT in total distance traveled or number of rearing events in an open field, suggesting that gross motor performance was not significantly impaired. To evaluate whether the forelimb deficits we observed could be rescued by standard dopamine replacement therapy, we administered levodopa/benserazide (6 mg/kg, 10 mg/kg, respectively) daily for 3 weeks. During these Treatment sessions, drugs were systemically administered once per day, 30 minutes prior to the first training session. DJ-1 rats showed a small but significant increase in pull force over the 3-week Treatment period, while performance of WT rats was impaired by L-dopa administration. Combined, our results are consistent with a mild-to-moderate dopamine-mediated motor impairment in the DJ-1 KO rats that begins to present around ca. 8 m.o., but does not significantly worsen between 8 and 12 m.o.

Disclosures: C.A. Sanchez: None. **J. Brougher:** None. **D. Krishnan:** None. **C.A. Thorn:** None.

Digital Abstract Session

P110. Rat and Mouse Toxin and Behavior Models

Program #/Poster #: P110.04

Topic: C.03. Parkinson's Disease

Title: The string-pulling task as a novel and simple behavior to test for parkinsonian deficits in unilaterally 6-OHDA-lesioned rodents

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Abstract: The unilateral 6-hydroxydopamine (6-OHDA) toxin model of Parkinson's disease (PD) has been well characterized for decades. However, many of the standardized behavioral tasks established in this model are either experimenter-led movements, tests that assess the limbs independently, or drug-induced. Moreover, two of the behavioral tests that analyze both forelimb and paw function in the 6-OHDA model, the single-pellet reaching and staircase tasks, require extensive training. The string-pulling task, which has recently been described in rodents, assesses skilled motor coordination of both forelimbs and requires minimal equipment and training for both the experimenter and animal. In this pilot study we demonstrate the first use of the string-pulling task in a 6-OHDA rodent model of PD. Male Sprague-Dawley rats were placed in a box with a transparent window allowing for video recording. A cheerio-baited string was hung at the front of the box. The rats were trained according to established literature until they successfully retrieved the reward in 8 trials across 2 consecutive days. Once complete, rats (n=3/group) received either a unilateral 6-OHDA (2 sites; 10 μg/site) lesion or sham-surgery in the medial forebrain bundle. Rats were then tested for 8 consecutive trials at 1, 3, 7, 14, and 21 days post-lesion for string pulling performance. In this pilot, we analyzed the first 3 trials at the pre-lesion and 14-day post-lesion timepoints. All statistical analyses were performed using two-way ANOVA with post-hoc Sidak's multiple comparisons tests. While we found no between-group difference in the motivation of rats to initiate the string-pulling task, there was a significant effect of both timepoint and lesion in regard to completing the task. Specifically, 6-OHDA rats took longer to complete the task compared to pre-lesion ($p<0.05$) and sham-treated rats ($p<0.01$) at 14 days post-lesion. The 6-OHDA-lesioned rats also made significantly more errors, in the form of missing the string, compared to pre-lesion ($p<0.05$) and sham-treated rats ($p<0.01$) at 14 days post-lesion. Interestingly, there were no significant differences between the use of the contralateral limb at either timepoint, regardless of lesion. However, there was a significant increase in the use of the ipsilateral limb to pull the string at 14 days post-lesion compared to pre-lesion ($p<0.05$) and a strong trend compared to sham-treated rats ($p<0.058$). We are continuing to analyze the additional timepoints post-lesion and increase our sample size to establish the string-pulling task as a novel and simple behavioral task for use in the preclinical 6-OHDA PD model.

Disclosures: **M.J. Bartlett:** None. **C.J. Stopera:** None. **M.R. Sexauer:** None. **A. Vishwanath:** None. **G. Jordan:** None. **S.L. Cowen:** None. **T. Falk:** None.

Digital Abstract Session

P110. Rat and Mouse Toxin and Behavior Models

Program #/Poster #: P110.05

Topic: C.03. Parkinson's Disease

Support: This work was supported by CAPES, CNPq, and Fundação Araucária

Title: Fenofibrate promotes neuroprotection in a model of rotenone-induced Parkinson's disease

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Abstract: Parkinson's disease (PD) is a neurodegenerative andprogressive disease characterized by a degeneration of dopaminergic neurons in thesubstantia nigra pars compacta (SNpc) which causes a decreased in levels ofdopamine (DA) in the striatum. The etiology remains unknown but some likelycauses of PD include oxidative stress, mitochondrial dysfunction andneuroinflammation. Peroxisome proliferator-activated receptor (PPAR) agonistshave frequently been studied in models of PD and have shown protective effects inanimal models in which they were tested. In the current study, fenofibrate (100 mg/kg) (PPAR-alpha agonist) wasadministered orally for 15 days, 5 days prior to the injection of rotenone (2.5 mg / kg ip for 10 days).One, 7, 14 and 21 days after finishing the treatment with rotenone andfenofibrate the animals were subjected to the open field test. On the 21stday we performed the forced swimming test and on the 22nd a two-wayactive avoidance task. After 23 days, a subset of the animals had theirstriatum dissected to quantify DA and metabolites levels and the remaining wereperfused for quantification of tyrosine hydroxylase-immunoreactive neurons inthe SNpc. In addition, we evaluated the quantification of alpha-synuclein afterthe animals were treated for 5 days with fenofibrate treatment continuing forover 28 days with rotenone. After 29 days, the animals were perfused for immunohistochemistryanalysis of alpha synuclein in the SNpc and striatum. The results showed thatfenofibrate reduced depressive-like behavior induced by rotenone. Moreover, thePPAR-α agonist diminished the depletion of DA and protectedagainst dopaminergic neuronaldeath in the SNpc. Moreover, theadministration of fenofibrate attenuated the aggregation of α-synuclein in theSNpc and striatum in the rotenone-lesioned rats. The present study showed thatfenofibrate exerted neuroprotective effects, since rats treated before andafter the induction of parkinsonism demonstrated reduced behavioral,neurochemical and immunohistochemical changes.

Disclosures: D.C. Ramos: None. **J.K. Barbiero:** None. **S. Boschen:** None. **T. Bassani:** None. **C. Da Cunha:** None. **M.A.B.F. Vital:** None.

Digital Abstract Session

P111. LRRK2 Mechanisms, Targets, and Pathways

Program #/Poster #: P111.01

Topic: C.03. Parkinson’s Disease

Support: Funded by GlaxoSmithKline

Title: Role of LRRK2 in nigro-striatal degeneration in a 6-hydroxydopamine mouse model of hemiparkinsonism

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by degeneration of dopamine neurons in the substantia nigra pars compacta (SNpc). Mutations that increase kinase activity of Leucine-rich repeat kinase 2 (LRRK2), such as G2019S are a genetic cause of PD. Inhibition of LRRK2 is a current therapeutic strategy in development for PD. We investigated the interaction between LRRK2 activity and dopamine neurotoxicity in a mouse 6-hydroxydopamine (6-OHDA) injection model using LRRK2*G2019S^{GSK} knock-in mice, and pharmacological inhibition of LRRK2.
In experiment 1, we tested a range of 6-OHDA doses for inducing SNpc neurodegeneration, gait deficits, and altering locomotor response to amphetamine. In experiment 2, we compared 6-OHDA toxicity between LRRK2*G2019S^{GSK} homozygous knock in (KI) mice and wild type (WT) mice. In experiment 3, we treated 6-OHDA toxicity with administration of a LRRK2 inhibitor (MLi-2) in diet (~14 mg/kg/day). Sample size n=7-8 mice per group.
Mice were injected with 6-OHDA (2-8 µg in 1 µl PBS) in the right striatum. Mice were euthanized 4-5 weeks after injection. Brains were analyzed for nigrostriatal tyrosine hydroxylase loss and LRRK2 phosphorylation at s935.
8 µg 6-OHDA induced lasting gait impairment, reduced response to amphetamine, and TH loss in striatum and SNpc; however, the dose was not tolerated. Mice injected with 2 or 4 µg 6-OHDA showed TH loss in striatum and SNpc and developed temporary gait impairments. A dose of 6 µg 6-OHDA was chosen for subsequent experiments. Change from baseline in gait parameters "step cycle" and "stride length" was predictive of SNpc TH neuron loss. 6-OHDA produced similar TH loss in the striatum and SNpc in both WT and KI mice. 6-OHDA-WT mice showed slower gait and higher response to amphetamine compared to WT sham injection; however all KI mice sham- and 6-OHDA-injected behaved similarly to 6-OHDA-WT mice. pS935 LRRK2 was lower in KI mice compared to WT, with no effect of 6-OHDA. Daily treatment with MLi-2 inhibited pS935 LRRK2 by 41% in the brain and 61% in the lung. 6-OHDA+Veh mice displayed a gait impairment compared to Sham+Veh mice, and this impairment was not seen in 6-OHDA+MLi-2 mice. Nigrostiratal TH of 6-OHDA+Veh group was lower than that of Sham+Veh; however, TH stain in 6-OHDA+Veh did not differ from Sham+Veh.6-OHDA injection depletes dopamine in the SNpc and striatum and induces gait impairment. Mice carrying the G2019S LRRK2 mutation were not more sensitive to 6-OHDA toxicity than wild type mice. 6-OHDA-injected mice treated with MLi-2 had similar gait and TH staining to Sham-injected mice.

Disclosures: M.K. Schultz: A. Employment/Salary (full or part-time):; GlaxoSmithKline. **R.R. Osborn:** A. Employment/Salary (full or part-time):; GlaxoSmithKline. **G.A. Logan:** A. Employment/Salary (full or part-time):; GlaxoSmithKline. **S.L. Agrapides:** A. Employment/Salary (full or part-time):; GlaxoSmithKline. **J. Beavers:** A. Employment/Salary (full or part-time):; GlaxoSmithKline. **T.L. Gales:** A. Employment/Salary (full or part-time):;

GlaxoSmithKline. **C.E. Fishman:** A. Employment/Salary (full or part-time):; GlaxoSmithKline. **V. Sherina:** A. Employment/Salary (full or part-time):; GlaxoSmithKline. **C.A. Caswell:** A. Employment/Salary (full or part-time):; GlaxoSmithKline. **F.D. Tattersall:** A. Employment/Salary (full or part-time):; GlaxoSmithKline.

Digital Abstract Session

P111. LRRK2 Mechanisms, Targets, and Pathways

Program #/Poster #: P111.02

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS093383

Title: Gtp binding inhibitor, 68 attenuates LPS-induced B-cell singling and TNF alpha secretion

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Abstract: Mutations in the leucine-rich repeat kinase-2 (*LRRK2*) gene cause autosomal-dominant Parkinson's disease (PD) and contribute to sporadic PD. Common genetic variation in LRRK2 modifies susceptibility to immunological disorders including Crohn's disease and leprosy. Previous studies have reported that LRRK2 is expressed in B lymphocytes and macrophages, suggesting a role for LRRK2 in immunological functions. In this study, we characterized the LRRK2 protein expression and phosphorylation using human lymphoblasts. Lipopolysaccharide (LPS), a preinflammatory agent, treated human lymphoblasts resulted in increases of LRRK2 expression and kinase activities in a time dependent manner. Moreover, increase of LRRK2 protein expression by LPS appeared to be associated with activation of B cell signaling. Treatment with LRRK2 inhibitor, 68, reduced LPS-induced B-cell signaling and TNF-α secretion. These results suggested that LRRK2 is actively involved in preinflammatory responses in human lymphoblasts.

Disclosures: T. Li: None. **B. Ning:** None. **L. Kong:** None. **A. Sawa:** None. **C.A. Ross:** None. **W. Smith:** None.

Digital Abstract Session

P111. LRRK2 Mechanisms, Targets, and Pathways

Program #/Poster #: P111.03

Topic: C.03. Parkinson's Disease

Support: MetDePaDi RNAR1702
INSERM
CHU Lille

Université de Lille
Fondation de France R19199EK

Title: Lrrk2 and deficits of membrane trafficking in Parkinson Disease

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Abstract: Introduction: Parkinson's disease (PD) is the most common neurodegenerative motor disease. Mutations in the leucine rich repeat kinase 2 (*LRRK2*) gene are linked to autosomal dominant parkinsonism, and genomic variation at the *LRRK2* locus is associated with increased risk for sporadic PD. LRRK2 is a multi-phosphorylated protein and reduced phosphorylation is associated to disease. Dephosphorylation leads to alterations in LRRK2 interactions and subcellular localization. PD is characterized by impaired intracellular trafficking, however the link between LRRK2 phosphorylation and membrane trafficking is not fully understood. Interestingly, LRRK1, the closest homolog of LRRK2, interacts with VAMP7 (Vesicle Associated Membrane Protein 7) and blocks synaptic vesicle fusion. Furthermore, several vesicular Rab-GTPases are LRRK2 substrates including Rab8, Rab10, Rab29. Here we have studied how LRRK2 phospho-status affects its Rab substrates as well as the mutual regulation of LRRK2 phosphorylation sites.**Methods**: We used LRRK2 phospho-mimick and phosphor-dead mutants in a cluster of phosphorylation sites including S860-S910-S935-S955-S973-S976 to assess the phosphorylation changes on non-mutated sites induced by individual or combined phosphor-site mutations as well as the phosphorylation of the known LRRK2 substrates Rab8 and Rab10 *in cellulo*. In addition, phosphor-mutant LRRK2 was purified to assess *in vitro* kinase activity.**Results**: With analysis ongoing, our results demonstrate that the phospho-regulation of the tested phospho-sites is interdependent and that LRRK2's phospho-status affects the intensity of LRRK2's kinase activity on its Rab vesicular substrates *in cellulo*. This presents a direct link between LRRK2 phosphorylation and Rabs phosphorylation state. These results suggest that LRRK2 phosphorylation leads to the modification of its kinase activity. **Conclusions**: This study will contribute to improving our understanding of LRRK2's role in membrane perturbation in PD. We hypothesize that phosphorylation may alter LRRK2's subcellular localization thereby regulating its downstream effectors such as Rabs. The central role of LRRK2 phosphorylation and it localization is crucial to how vesicular trafficking might be defective in PD.

Disclosures: A. Marchand: None. **A. Sarchione:** None. **F. filippini:** None. **S. Nola:** None. **T. Galli:** None. **M. Chartier-Harlin:** None. **J. Taymans:** None.

Digital Abstract Session

P111. LRRK2 Mechanisms, Targets, and Pathways

Program #/Poster #: P111.04

Topic: C.03. Parkinson's Disease

Support: William N. & Bernice E. Bumpus Foundation Postdoctoral Fellowship
William N. & Bernice E. Bumpus Foundation Innovation Award
1R01NS119528

Title: Sustained activation of the DNA damage response and increased nuclear DNA damage in LRRK2 G2019S Parkinson's disease models

Authors: *C. P. GONZALEZ-HUNT, C. M. TOSTE, S. D. GOODSON, L. H. SANDERS; Neurol., Duke Univ. Med. Ctr., Durham, NC

Abstract: Recent studies from our laboratory demonstrated that LRRK2 G2019S-induced mitochondrial DNA (mtDNA) damage is LRRK2 kinase dependent and inhibition restores mtDNA integrity in Parkinson's disease (PD) models. However, whether or not there is also nuclear DNA damage in LRRK2 G2019S PD is unknown. We hypothesized that enhanced LRRK2 kinase activity alters signaling/repair pathways involved in genome maintenance, compromising global genome integrity. Utilizing CRISPR/Cas9-edited HEK293 cells carrying the G2019S mutation and isogenic controls, we measured nuclear DNA damage via comet assays and evaluated if the DNA damage response (DDR) was activated by western blotting. Our results show increases in nuclear DNA damage and activation of the DDR, represented by increases in phosphorylation of the kinase ataxia-telangiectasia mutated (ATM pS1981) and downstream substrates histone H2A.X (γH2A.X), CHK2 (pT68), and P53 (pS15). Next, we hypothesized that the increase in DNA damage and DDR activation would trigger cell cycle arrest; to test this we stained cells with propidium iodide and measured DNA content via flow cytometry. Our results show an increased proportion of cells in S phase, and a decreased proportion of cells in G0/G1. We also evaluated if inhibition of ATM or LRRK2 kinase activity could reverse H2A.X phosphorylation. As expected, ATM kinase inhibition was capable of reversing the γH2A.X increase we observe in LRRK2 G2019S HEK293 cells. Interestingly, LRRK2 kinase inhibition was also capable of reverting the γH2A.X increase back to baseline. In conclusion, our work shows that, in the context of the LRRK2 G2019S mutation, nuclear DNA damage is increased and the DDR is activated. We also found evidence of cell cycle arrest. Furthermore, our results show that inhibition of ATM or LRRK2 kinase activity can revert γH2A.X phosphorylation increases back to baseline, directly implicating mutant LRRK2 function in the DDR for the first time. We are currently working on further characterizing this novel signaling cascade in HEK293 lines, and evaluating DDR activation in a LRRK2 G2019S mouse model and induced pluripotent stem cell (iPSC)-derived neural progenitor cells. We are also investigating the potential impact of DDR dysregulation and DNA damage accumulation on PD pathogenesis. ATM function is critical for nervous system homeostasis, and its activation can lead to cycle arrest, DNA repair, or apoptosis. Dysregulation of the ATM signaling cascade could lead to cell death, and as such modulating ATM function has the potential to be a new strategy for therapeutic intervention in PD.

Disclosures: C.P. Gonzalez-Hunt: None. **C.M. Toste:** None. **S.D. Goodson:** None. **L.H. Sanders:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Escape Bio, Sanofi. C.

Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca.

Digital Abstract Session

P112. Parkinson's Disease: Alpha-Synuclein Pathology

Program #/Poster #: P112.01

Topic: C.03. Parkinson's Disease

Support: FRQS

Title: Longitudinal voxel-wise mapping of alpha-synuclein-induced brain pathology in a mouse model of Parkinson's Disease

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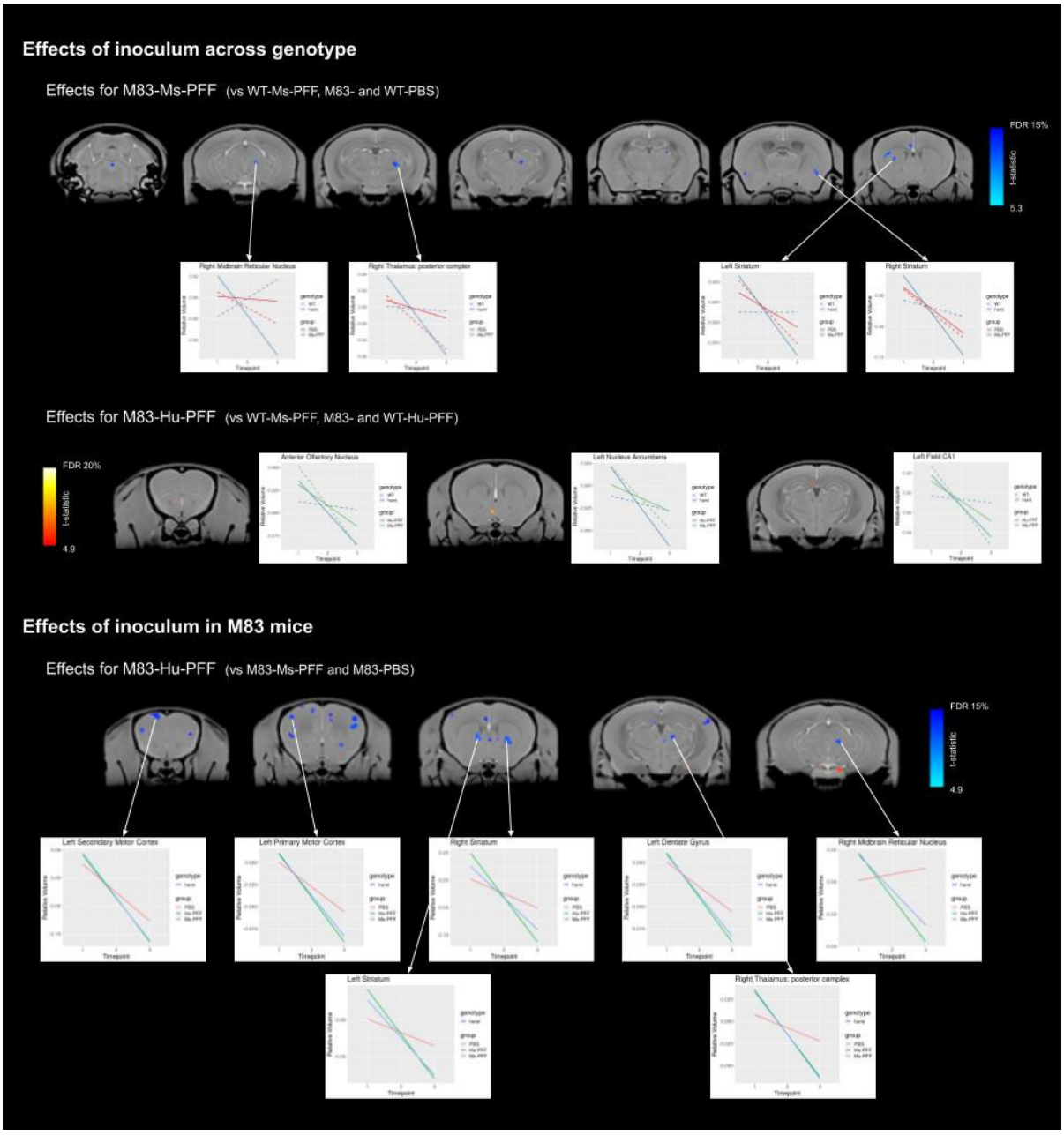
Abstract: The mechanisms underlying Parkinson's Disease (PD) pathology have not been elucidated. Recent evidence suggests aggregated misfolded alpha-synuclein (a primary component of Lewy bodies, [aSyn]), may propagate in a prion-like manner, mediating the spread of pathology and contributing to PD progression. Using an aSyn propagation mouse model of PD, with a known locus of pathology, we longitudinally examined aSyn-induced changes in anatomy using magnetic resonance imaging (MRI).
11-week old wild-type (WT) and hemizygous M83 $aSyn^{A53T}$ transgenic mice received an injection of either mouse [Ms-] or human [Hu-] preformed fibrils [PFF] of aSyn, or phosphate buffered saline (PBS; control group) in the right striatum (n~10 mice/group/sex). T1-weighted MRI images (100 μm^3 voxels) were acquired at -7, 30 and 90 days post-injection. Brain atrophy was assessed using deformation-based morphometry, to measure local differences between groups.
We observed significant changes in brain anatomy over time in M83-Ms-PFF compared to WT-Ms-PFF and M83- and WT-PBS groups; specifically in regions such as the bilateral striatum, and contralateral posterior nuclei of the thalamus (FDR 15%). There is an impact of the species of PFF, such that M83-Hu-PFF mice had steeper rates of decline in the contralateral anterior olfactory nucleus, contralateral nucleus accumbens and ipsilateral field CA1, compared to M83- and WT-Ms-PFF and WT-Hu-PFF mice (FDR 20%). When examining the effect of inoculum on M83 mice, significant voxel-wise changes were observed for the bilateral striatum, contralateral primary motor cortex, ipsilateral midbrain reticular nucleus and dentate gyrus (FDR 15%). No significant differences between the PFF groups compared to the PBS group were observed in WT mice.
The inoculation of aSyn PFF in the striatum gives rise to widespread patterns of PFF-induced brain atrophy, particularly involving regions that project to or receive input from the injection

site in both M83 and WT mice. However in certain regions, the presence of the mutation appears to further modulate the observed degeneration.



Disclosures: S. Tullo: None. **M. Chakravarty:** None. **D. Gallino:** None. **E. del Cid-Pellitero:** None. **E.A. Fon:** None.

Digital Abstract Session

P112. Parkinson's Disease: Alpha-Synuclein Pathology

Program #/Poster #: P112.02

Topic: C.03. Parkinson's Disease

Support: ANR MetDePaDi (RANR1702)
INSERM
CHU de Lille
Université de Lille
Fondation de France (R19199EK)

Title: Alpha-synuclein and deficits of membrane trafficking in Parkinson's Disease

Authors: *A. SARCHIONE[1], A. MARCHAND[1], F. FILIPPINI[2], T. GALLI[2], J. TAYMANS[1], M. CHARTIER HARLIN[1];
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Abstract: Introduction: Parkinson's Disease (PD) is a neurodegenerative disorder characterized by defects in membrane trafficking. SNCA, encoding α-synuclein (α-syn), is a major genetic determinants of PD pathogenesis involved in membrane trafficking. α-syn has recently emerged as regulator of SNARE (Soluble N-ethylmaleimide-sensitive-factor Attachment protein Receptor)-dependent vesicle fusion. Recently the vesicular SNARE protein VAMP4 emerged as novel PD-risk factor and VAMP7 as a mediator of ER-phagy secretion. Here we proposed to investigate the potential functional interactions between α-syn and VAMPs 4 and 7 as well as the aggregation profile of α-syn and its release in cell medium. **Methods**: The co-localization and interaction between α-syn and VAMPs was analyzed by immunocytochemistry and Proximity Ligation Assay. The released α-syn exocytosis was measured at different time points upon α-syn over-expression in cells. Exosomes isolation from cell culture media was performed in VAMP (2, 4, 7) KO. Pharmacological treatment with Bafilomycine A1 was performed in order to analyze the clearance of VAMPs upon lysosomal inhibition in cells. **Results**: We present preliminary results of PLA and co-localization testing the interaction between α-syn and VAMPs. Inhibition of lysosomal activity and effects on both VAMPs and α-syn levels were also tested. The α-syn release in cell medium is detectable up to 48h after transfection. We observed a reduced α-syn release in exosomes of PC12 KO for VAMP4 and VAMP7 compared to WT PC12 cells. **Conclusions**: α-syn and VAMPs are both involved in vesicular and membrane trafficking and our preliminary results may suggest an involvement of the same pathway in their clearance. In our perspectives, we will evaluate the effect of VAMPs on the α-syn aggregation and exocytosis in order to evaluate if the α-syn homeostasis could be modulated by SNARE proteins.

Disclosures: A. Sarchione: None. **A. Marchand:** None. **F. Filippini:** None. **T. Galli:** None. **J. Taymans:** None. **M. Chartier Harlin:** None.

Digital Abstract Session

P112. Parkinson's Disease: Alpha-Synuclein Pathology

Program #/Poster #: P112.03

Topic: C.03. Parkinson's Disease

Support: NINDS Grant NS092803

Title: Alterations in miR-219 expression associated with a-synuclein overexpression may contribute to impairments in oligodendrocyte maturation.

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Abstract: Introduction: Multiple System Atrophy (MSA) is a neurodegenerative disorder characterized by autonomic failure and parkinsonian features. The neuropathological hallmark of MSA is the aggregation of a-synuclein (asyn) in oligodendrocytes, conforming Glial Cytoplasmic Inclusions (GCI). GCI formation leads to impairments in oligodendrocyte differentiation. MiRNAs are small non-coding RNA molecules that regulate gene expression and multiple biological processes including development and cell differentiation. Our previous studies in *postmortem* MSA human striatum samples showed dysregulation in the expression of 59 miRNAs, with miR-219-2-3p showing the largest alterations in expression in comparison to healthy controls. MiR-219 regulates oligodendrocyte differentiation by inhibiting the expression of Lingo1, Etv5, and PDGFRa. MiR-219 is expressed by 2 loci (miR-219-1 and miR-219-2) and is processed into miR-219-5p, miR-219-1-3p and miR-219-2-3p.In this work we analyzed the expression of miR-219 in cellular and transgenic mouse models of MSA to determine the involvement of this miRNA in oligodendrocyte alterations. **Methods:** miRNAs were extracted from the *striatum* of MBP29 mice, which over-express asyn under the MBP promoter, and non-transgenic (NTG) littermates (n=6/group). We compared the expression of 600 miRNAs using the nCounter® Mouse v1.5 miRNA panel. The expression of miR-219 isoforms was validated by qPCR analysis. We applied the newly developed miRNAscope technology to determine cell-specific changes in miR-219.Adult rat hippocampal neuronal progenitor cells (ARH-NPC) were differentiated into oligodendrocytes and cells were transduced with lentivirus expressing human asyn (or Lv-control) prior to differentiation. **Results:** we identified 16 miRNAs with aberrant expression in MBP29 mice compared to NTG controls, including decreased levels of miR-219-5p and miR-219-2-3p. Neuropathological analysis showed alterations in the oligodendrocyte markers MBP and CNP in MBP29 mice. MiRNAscope assays demonstrated decreased expression of miR-219-2-3p localized in MBP positive cells.The levels of miR219 change during oligodendrocyte maturation in vitro, reaching the highest levels at day 14 of differentiation in control cells. In contrast, and similar to what we observed in MBP29 mice, ARH-NPC cells over-expressing asyn showed significant reductions in miR-219-5p and miR-219-2-3p expression; as well as alterations in CNP and PDGFRa markers at differentiation day 14. **Conclusions:** we propose that dysregulation of miR-219 in response to asyn accumulation may impair oligodendrocytes maturation contributing to MSA pathology.

Disclosures: **L.E. Cromberg:** None. **H. Romero:** None. **J. Mott:** None. **P. Desplats:** None.

Digital Abstract Session

P112. Parkinson's Disease: Alpha-Synuclein Pathology

Program #/Poster #: P112.04

Topic: D.07. Vision

Support: John Landman PhD Scholarship
Australian Government Research Training Program (RTP)
Australian Research Council Linkage LP160100126
Melbourne Neuroscience Institute Interdisciplinary Seed Fund

Title: Impaired retinal function & structure in A53T mice provide new insight into how Parkinson's disease manifests in the ageing eye

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Abstract: Purpose: Abnormal alpha-synuclein (α-syn) protein deposition within the central nervous system (CNS) has been recognised as one of Parkinson's disease's (PD) key hallmarks. As an embryological outpouching of the CNS, the retina provides an accessible means to study neurodegenerative disorders like PD. And as retinal assessments such as electroretinography (function) and optical coherence tomography (structure) are simple, non-invasive and inexpensive to conduct they may prove useful as biomarkers that can be used to streamline drug discovery from animal to human studies. For the first time, this study aims to quantify retinal biomarkers in a well-characterised murine PD model of α-syn overexpression.
Methods: Transgenic mice overexpressing human α-syn with A53T gene translocation and wildtype (WT) control littermates were assessed at 6 and 14 months of age (n=15-31/group). *In vivo* retinal function (electroretinography, ERG) and *in vivo* retinal structure (optical coherence tomography, OCT) were recorded. Dark-adapted and light-adapted ERG protocols were conducted to probe rod and cone pathways. ERG response parameters and OCT thickness profiles were analysed to assess changes to outer and inner retinal layers. Two-way ANOVA with Bonferroni correction for multiple comparisons (Prism, GraphPad) was used to compare differences between groups.
Results: Compared to WT controls, A53T mice exhibited significantly smaller and delayed ERG responses for both dark-adapted ERGs (genotype effect; photoreceptor and bipolar cell amplitude, $p<0.05$; bipolar cell timing, $p<0.0001$) and light-adapted ERGs (photoreceptor and bipolar cell amplitude, $p<0.001$; bipolar cell timing, $p<0.0001$). An interaction effect was also found in photoreceptor timing (dark- and light-adapted ERG, $p<0.05$). Light-adapted ERGs had a larger effect size than dark-adapted ERGs (Cohen's $d = -2.16$, effect size $r = -0.73$). OCT retinal thinning was also found in the outer retinal layers of A53T mice (outer plexiform layer, outer

nuclear layer, p<0.0001) but no changes were found in inner retinal layers (retinal nerve fibre layer, ganglion cell inner plexiform layer, inner nuclear layer, p = 0.06 to 0.37).
Conclusion: Accumulation of α-syn leads to outer retinal thinning and visual dysfunction in A53T transgenic mice at timepoints that model mid to advanced stages of PD. These retinal changes in function and structure provide a high throughput means to longitudinally study α-syn induced neurodegeneration and ultimately triage *in vivo* biomarker endpoints for PD drug discovery. Characterising retinal changes in PD models may also pave the way for a deeper understanding of vision loss in PD.

Disclosures: K.K.N. Tran: None. **V.H.Y. Wong:** None. **J.K.H. Lim:** None. **A. Shahanadeh:** None. **A. Hoang:** None. **D.I. Finkelstein:** None. **B.V. Bui:** None. **C.T.O. Nguyen:** None.

Digital Abstract Session

P113. Neuroprotective Mechanisms

Program #/Poster #: P113.01

Topic: C.03. Parkinson's Disease

Title: Using the proportionator to assess neuronal loss in an MPTP-treated mouse model of Parkinson's Disease

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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor and non-motor symptoms, including rigidity, tremor, and impaired walking and gait. The main pathological feature of PD is the cell death of striatofugal dopaminergic (DA) neurons located primarily in the substantia nigra pars compacta (SNc). The toxin-induced PD mouse model, in which 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration results in catecholamine depletion in the SNc, is commonly used to test novel therapeutics. This model reproduces many of the behavioral and physiological characteristics of the disease, including the selective vulnerability of striatofugal DA cells of the SNc, while DA cells of the neighboring ventral tegmental area (VTA) remain comparatively protected from the neurotoxin. However, the regions within which this neurodegeneration occurs varies somewhat across species, and even between strains. Some of this variability may be attributed to differences in expression of calbindin, a calcium-binding molecule, among DA cells of the ventral mesencephalic DA complex.
The present study undertook to demonstrate that restricting our sampling and analysis of neuronal loss to DA neurons that do not express calbindin, within a broader region of the ventral mesencephalic DA complex than just the SNc is a more sensitive assay of MPTP-induced neurodegeneration than counting all of the DA neurons within the same region.
This study uses advanced stereological analysis to accurately evaluate neuronal changes in the MPTP mouse model. The tyrosine kinase inhibitor, Nilotonib, was used as an agent of rescue.

Immunofluorescent (IF) methods were used to generate a duplex stain for tyrosine hydroxylase (TH) and calbindin. The proportionator method of area sampling was used to unbiasedly sample from this subpopulation of neurons in mounted sections that were captured from the ventral mesencephalic DA complex using a systematic uniform random sampling (SURS) method. The increased sensitivity and specificity of molecular phenotyping combined with the proportionator technique enables us to capture drug effects more efficiently compared to traditional approaches.

Disclosures: **M.A. Staup:** None. **R. Pussinen:** None. **T. Huhtala:** None. **J. Baily:** None.

Digital Abstract Session

P113. Neuroprotective Mechanisms

Program #/Poster #: P113.02

Topic: C.03. Parkinson's Disease

Support: R01NS095799

Title: Tip60 HAT activity prevents locomotor defects and short term memory loss in multiple neurodegenerative conditions

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Abstract: Epigenetic mechanisms, such as histone acetylation, regulate dynamic gene expression that is critical for several neural functions including learning and memory. Consequently, loss of neural histone acetylation has been implicated in multiple neurodegenerative conditions, yet the mechanisms underlying such alterations remain unclear. Our lab has previously shown that imbalance in Tip60 histone acetyltransferase (HAT) and histone deacetylase 2 (HDAC2) in a well characterized *Drosophila* AD model results in epigenetic repression of critical synaptic plasticity genes and functional cognitive deficits. Interestingly, these phenotypes are restored by increasing Tip60 HAT levels in the AD brain, supporting a neuroprotective role for Tip60 in AD linked neurodegeneration. Here we show that similar to AD, disruption of Tip60 HAT/HDAC2 balance and repression of synaptic plasticity genes is a shared early event in Parkinson's Disease (PD), Huntington's Disease (HD) and Amyotrophic Lateral Sclerosis (ALS). Further, chromatin immunoprecipitation (ChIP) studies reveal that repressed neuroplasticity genes show reduced Tip60 enrichment and reduced histone acetylation at all gene loci examined with certain genes also showing inappropriate HDAC2 enrichment. Functional neuronal consequences of each of these disease conditions are reminiscent of human pathology and include locomotion, synapse morphology, and short term memory deficits. Since Tip60 overexpression is neuroprotective in AD linked neurodegeneration, we wanted to further explore if Tip60 plays a general neuroprotective role in multiple neurodegenerative conditions. Remarkably, increasing Tip60 HAT levels specifically in the learning and memory center of the *Drosophila* brain protects against locomotion and short-

term memory function deficits. Together, our results support a model by which Tip60 protects against neurological impairments in different neurodegenerative diseases *via* similar modes of action, giving hope for a unified therapeutic approach.

Disclosures: **A. Bhatnagar:** None. **M. Beaver:** None. **P. Panikker:** None. **H. Zhang:** None. **F. Elefant:** None. **R. Snook:** None.

Digital Abstract Session

P113. Neuroprotective Mechanisms

Program #/Poster #: P113.03

Topic: C.03. Parkinson's Disease

Support: NIH-R56NS109608
ABRC ADHS18-198846

Title: Behavioral analysis in the progressive unilateral 6-OHDA-lesion rat model indicates a neuroprotective effect of sub-anesthetic ketamine-treatment

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Abstract: Low-dose, sub-anesthetic ketamine has been shown to be neuroprotective in rodent models of cerebral ischemia and traumatic brain injury. Additionally, a recent publication from our lab demonstrated that treatment with sub-anesthetic ketamine (10-hr infusion; 5 x 20 mg/kg; *i.p.*; 2-hrs apart) can reduce levodopa (L-DOPA)-induced dyskinesia (LID) in a rat model of 6-hydroxydopamine (6-OHDA), while also providing acute anti-parkinsonian activity. Specifically, we have shown that increased brain-derived neurotrophic factor (BDNF) release underlies the long-term anti-dyskinetic activity of 10-hr sub-anesthetic ketamine treatment in LID rats. There is also evidence in the literature for ketamine having anti-inflammatory effects via activation of both microglia and astrocytes. Therefore, we hypothesized that sub-anesthetic ketamine may exhibit neuroprotective effects in the progressive 6-OHDA rat model of Parkinson's disease (PD). To test this, male Sprague-Dawley rats were administered either ketamine or vehicle via a treatment protocol (6-hr treatment; 3 x 20 mg/kg; *i.p.*; 2-hrs apart) that began 6-hrs prior to a unilateral intrastriatal (2 sites; 13.75 µg/site) lesion. Animals were then treated daily with the 6-hr treatment protocol for 7 days post-lesion. Baseline behavioral activity was established prior to the lesion and animals were retested at 14 and 28-days post-lesion. We have completed the analysis of the amphetamine-induced rotation test, which provides an estimate of the extent of the parkinsonian lesion, at the 28-days post-lesion timepoint. Rats were injected with D-amphetamine (5 mg/kg; *i.p.*) and rotational asymmetry was assessed by counting the net ipsilateral rotations (mean ± SEM) over 90-minutes. Ketamine (211 ± 116) treated

animals showed a significantly decreased number of net ipsilateral rotations compared to the vehicle (666 ± 187) treated group (Two-tailed t-test; *p < 0.05; n=15), suggesting a neuroprotective effect of ketamine. Currently we are performing unbiased stereology on dopaminergic neurons in the substantia nigra to further evaluate ketamine's neuroprotective effects. To investigate anti-inflammatory activity, we are staining for the microglia and astrocyte markers IBA1 and GFAP, and conducting a morphometric analysis to identify changes in activation of these cell types post-ketamine. In addition, video analysis of other behavioral tests, including the cylinder, vibrissae-elicited forelimb placing, and the forelimb adjusting steps tests, are ongoing to further analyze the effects of ketamine treatment in the progressive unilateral 6-OHDA model of PD.
Funding: NIH-R56NS109608; ABRC ADHS18-198846.

Disclosures: C.J. Stopera: None. **M.J. Bartlett:** None. **K. Bernard:** None. **M.R. Sexauer:** None. **A. Esqueda:** None. **S.J. Sherman:** None. **H. Morrison:** None. **T. Falk:** None.

Digital Abstract Session

P113. Neuroprotective Mechanisms

Program #/Poster #: P113.04

Topic: C.03. Parkinson's Disease

Title: Motor learning of novel choreography investigated with mobile eeg

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Abstract: We are investigating dance-related learning in an ecologically valid setting using Mobile Brain/Body Imaging (MoBI) to better understand potential therapeutic applications for neurorehabilitation. Previous studies from members of our group have shown that dance-based learning over 8-months produced BOLD signal changes in SMA using fMRI (Bar & DeSouza 2016) and resting state alpha power increases in frontal cortex post-dance in people with Parkinson's disease (PwPD) compared to controls (Levkov et al 2014). PwPD who dance weekly over 3-yrs show a slowdown in disease progression (DeSouza & Bearss, 2018). Our current project uses mobile neuroimaging (MoBI) to assess the impact of motor learning while subjects (n=17) learn a 30-sec choreography (Barnstaple et al 2020) with the aim of examining brain and motor dynamics involved. The choreography was specifically designed to include elements common to many dance forms without referencing a specific dance style, image, or affect, and synthesized music unfamiliar to all participants was the training stimulus. Recordings used motion capture data synced with continuous recording of wireless mobile EEG (Brain Products ActiCap; 128 electrodes) in a dedicated 150 m^2 lab space. Movements through space were recorded using 10 HTC Vive trackers running on Steam VR. 30-sec trials included (i) watching VIDEO (4 times), (ii) LIVE performances of the choreography (6 times), (iii) moving with the

teacher RB (LEARN; 3 to 20 times until 80% criteria was reached rated by experimenter LJ), (iv) imagining performing from a first-person perspective (IMAGINE; 6 times), and (v) finally performing in space (PERFORM; 6 times). Music was rated for affective valence pre/post motor learning. All participants reached our target of 80% or higher accuracy in reproducing the movement sequence within 20 LEARN trials, with expertise in dance resulting in 10.13 trials to reach criteria. Subjects also reported the degree to which they felt as though they were "dancing" over 6 PERFORM trials; scores consistently increased, corresponding to a subjective sense of self-expression in the knowledge of the dance.

Disclosures: J.F.X. DeSouza: None. **R.E. Barnstaple:** None. **K. Gramann:** None. **L. Jaufmann:** None. **J. Protzak:** None. **S. Jeung:** None.

Digital Abstract Session

P113. Neuroprotective Mechanisms

Program #/Poster #: P113.05

Topic: C.03. Parkinson's Disease

Support: NIH R01 1R01NS102735-01A1
Farmer Family Foundation Parkinson's Research Initiative

Title: Systemic MC1R activation modulates immune response and protects the nigrostriatal dopaminergic system in MPTP+LPS model of PD

Authors: *P. SRIVASTAVA, W. KAI, T. STIMPSON, M. SCHWARZSCHILD, X. CHEN; Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Objective: We previously identified a role of Melanocortin receptor 1 (MC1R) in dopaminergic neuron survival. MC1R is present on immune cells and can regulate the immune system. The present study explores effects of systemically-administered NDP-MSH, an MC1R agonist and existing drug that does not cross BBB, in modifying the immune response and protecting nigrostriatal dopaminergic system in the MPTP+LPS mouse model of PD. **Methods:** Male C57BL/6 mice were treated i.p. with MPTP (17 mg/kg) and LPS (1 mg/kg) from day 1 to day 4 and NDP-MSH (400 μg/kg) or vehicle from day 1 to day 12 following which the mice were sacrificed. Immune cells in the blood, striatal dopamine, and nigral TH+ neurons and glia, and expression of occludin, a marker for BBB integrity were accessed. **Result:** NDP-MSH treatment significantly attenuated striatal dopamine depletion (by 37%, $p<0.001$) and nigral TH+ neuron loss (by 33%, $p<0.01$) induced by MPTP+LPS. NDP-MSH reduced microglia activation and IBA1+ cells in the nigral region. No significant difference in occludin expression was observed in NDP-MSH treated mice vs controls in the striatum. NDP-MSH treatment in MPTP+LPS mice significantly reduced blood $CD4^+$ T cells ($p=0.006$) and B cells ($p=0.04$). NDP-MSH did not show any protective effect in MC1R mutant mice following MPTP+LPS. **Conclusion:** A peripherally acting MC1R agonist confers protection on dopaminergic nigrostriatal neurons and reduces hyperactivate states of microglia. Whether the neuroprotection

by NDP-MSH results, at least in part, from its immune-modulating effects remains to be determined.

Disclosures: **P. Srivastava:** None. **W. Kai:** None. **T. Stimpson:** None. **M. Schwarzschild:** None. **X. Chen:** None.

Digital Abstract Session

P113. Neuroprotective Mechanisms

Program #/Poster #: P113.06

Topic: A.01. Neurogenesis and Gliogenesis

Support: K99MH125329

Title: Sars-cov-2 infects neural cell types in developing human cortex

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Abstract: The novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causes the life-threatening illness, COVID-19, and is responsible for the current global pandemic and over one million deaths world-wide. Although SARS-CoV-2 infection most notably impairs respiratory and cardiac function, the capacity to infect other cell types and organ systems is currently being elucidated. Strikingly, many patients suffering with or having recovered from COVID-19, can present with a range of neurological symptoms including seizures, encephalopathy, stroke, headaches, dizziness, loss of smell and taste and a general inability to focus. In order to investigate the biological origin of COVID-19 related neurological insults, we sought to test whether neural tissue could be directly infected by SARS-Cov2. We utilized adult cortical surgical samples, primary developing human cortical tissue, and cortical organoid models to evaluate the capacity for coronavirus infection and replication in the human brain. In cortical tissue cultures treated with SARS-Cov-2, we observed significant infection in vascular cells innervating the brain, as well as in some neural cell types. In developing tissue, we observed modest infection in radial glial cells, the stem cells of the developing cortex, and there was minimal infection in dividing cells. Importantly, the neuronal domain of the developing cortex, the cortical plate, was devoid of infected neurons. However, we identified a population of glial precursors that had consistent SARS-CoV-2 infection and robust viral replication. In contrast, organoids of comparative stages did not demonstrate infection of any cell types present, perhaps due to lack of glial diversity or maturation in these models. Despite minimal expression of the SAR-COV-2 receptor, ACE2, in cell types present in primary cortical tissue, glial cells were preferentially infected. Possibly an alternative entry factor may meditate infection in these cells. Our study provides evidence of direct infection of specific human neural cell types with implications for the vulnerability of the developing brain and the potential for neural infection after SARS-CoV-2 exposure in postnatal life.

Disclosures: **M. Andrews:** None. **T. Mukhtar:** None. **A. Kriegstein:** None.

Digital Abstract Session

P114. Parkinson's Disease: Neural Dynamics

Program #/Poster #: P114.01

Topic: C.03. Parkinson's Disease

Support: Doris Duke Clinical Scientist Development Award (#2014101)
NIH COBRE NIGMS P20 GM103645
Neurosurgery Research and Education Foundation (NREF)
Lifespan Norman Prince Neurosciences Institute
Brown University Carney Institute for Brain Science

Title: Neural dynamics of parkinsonian tremor onset and maintenance

Authors: *P. M. LAURO[1], S. LEE[1], W. F. ASAAD[2];
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Abstract: Parkinsonian tremor, a common symptom of Parkinson's disease (PD), is characterized by oscillatory (4-6 Hz) motor output in distal limbs. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) alleviates tremor in patients, suggesting the STN is involved in tremor. To precisely understand the STN's role in tremor dynamics, we obtained microelectrode STN recordings and electrocorticography (ECoG) recordings from sensorimotor cortex from 10 patients with PD undergoing DBS implantation surgery (9M, 1F; 65.2 ± 7.4 years). To capture tremor dynamics, patients performed a motor performance task where they followed a moving onscreen target with a joystick-controlled cursor. Tremor was captured from the 3-8 Hz bandpassed joystick trace, and was scaled to that of age-matched healthy control subjects (3M, 11F; 62.4 ± 10.0 years). Based on population-based tremor thresholds, behavioral and neural data from patients were split into 4 second epochs to measure separate components of tremor: absence, onset, and sustained tremor.

Tremor onset epochs were characterized by increased tremor frequency power (~ 5 Hz) and decreased high-beta frequency power (20-30 Hz) in STN, motor cortex, and somatosensory cortex relative to tremor absence epochs. At the same time, alpha-low beta frequency power (10-20 Hz) increased in premotor and parietal cortices. Each of these spectral power changes remained during tremor maintenance episodes.

While both low- and high-beta frequencies exhibited phase-locking across motor and somatosensory cortices during tremor absence epochs, this phase-locking decreased during onset and sustained tremor epochs. Simultaneously, premotor and parietal cortices exhibit increased phase-locking at beta frequencies during onset and sustained tremor epochs. The STN exhibited increased tremor frequency phase-locking and beta frequency phase-locking with premotor and parietal cortices, with tremor frequencies dominant during onset epochs and beta frequencies during sustained tremor epochs.

Taken together, these results suggest that changes in tremor are reflected by dynamic changes in cortical connectivity. While motor-somatosensory cortices may be coupled during volitional movement, premotor-parietal cortices become more coupled during Parkinsonian tremor.

Preliminary evidence suggests that the STN preferentially phase-locks with premotor and parietal cortices, acting as a subcortical node for this transition.

Disclosures: P.M. Lauro: None. **W.F. Asaad:** None. **S. Lee:** None.

Digital Abstract Session

P114. Parkinson's Disease: Neural Dynamics

Program #/Poster #: P114.02

Topic: C.03. Parkinson's Disease

Support: National Institute of Neurological Disorders and Stroke (RO1NS064040)

Title: Compensatory Cortical Oscillatory Activity During Movement Activation and Inhibition in Parkinson's Disease

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Abstract: Background: Response activation and inhibition are functions fundamental to executive control that are disrupted in Parkinson disease (PD). Impairments in movement activation and inhibition likely underlie the common motor signs observed in PD. Brain network reorganization observed after dopamine depletion in the nigrostriatal pathway may be associated with preservation of similar task performance in early to moderate PD. **Methods:** We examined oscillatory power amplitude, peak latency and frequency in cortical networks subserving motor activation and inhibition using magnetoencephalography (MEG) to identify abnormalities associated with PD. We used a whole-head biomagnetometer (CTF MEG, Coquitlam, Canada) with 275 first-order axial gradiometers and 27 reference sensors. MEG data was reconstructed in source space using a structural MRI (1.5 T GE Signa scanner) collected with a multiplanar rapidly acquired gradient echo imaging sequence and repetition time, 7.87 s; echo time, 2.69 ms; flip angle, 8 degrees; slices, 200; field of view, 256 mm; resolution, 1x1x1.2 mm). Participants (N=18 PD, 18 age-matched controls) performed a cue/target task requiring initiation of an un-cued movement or inhibition of a cued movement, with 120 trials during a 70-minute scan. Data were stimulus-locked (target onset=0 ms) followed by 25 ms time windows until 1112.5 ms post-target onset. Data were passed through a filter and partitioned into time windows at 100, 150, 200, and 300 ms to capture spectral peaks in the MEG signal. Twenty-five ms steps were estimated in alpha (8-12 Hz), beta (12-30 Hz) and gamma (30-55 Hz). Behavioral and latency data were evaluated using repeated measures ANOVA with t-test post-hoc analysis and an alpha threshold of 0.05. **Results:** We found decreased power and delayed peak latency in the beta band in medial frontal gyrus and in alpha band in pre-supplementary motor area at 387.5 ms during activation of an un-cued movement in PD patients, but reaction time was not statistically different from controls ($F(1,70)=2.25$, $p=0.14$). **Conclusions:** Abnormal alpha activity and relatively normal response activation reaction times in the PD group could be related to

compensation for failing dopaminergic neurons (up to 80% of substantia nigra neurons by symptom onset). This interpretation is supported by fMRI/genomics studies reporting asymptomatic Parkin or LRRK2 mutation carriers who had altered brain network activity and connectivity compared to healthy non-carriers with similar performance of internal movement selection. Disentangling compensatory changes from pathological disinhibition will advance our understanding of the pathophysiology of PD.

Disclosures: **N. Glassy:** None. **L. Hinkley:** None. **E. Disbrow:** None.

Digital Abstract Session

P114. Parkinson's Disease: Neural Dynamics

Program #/Poster #: P114.03

Topic: C.03. Parkinson's Disease

Support: NIH UH3 NS103468

Title: Internodal analysis reveals multiple beta oscillations within the basal ganglia in patients with Parkinson's disease.

Authors: ***S. L. SCHMIDT**[1], J. J. PETERS[1], K. PALOPOLI-TROJANI[1], K. T. MITCHELL[2], W. M. GRILL[1], D. A. TURNER[3];
[1]Duke Univ., Durham, NC; [3]Neurosurg., [2]Duke Univ. Med. Ctr., Durham, NC

Abstract: Deep brain stimulation (DBS) is the primary surgical intervention for treatment of tremor and/or fluctuations of levodopa-responsive motor symptoms in Parkinson's disease (PD). Leads are implanted into either the subthalamic nucleus (STN) or globus pallidus (GP: GPi/e). The 16-channel RC+S (Medtronic, PLC), allows connection of four standard DBS leads, and we tested combined STN + GPi DBS in a cohort of 3 participants with PD. The RC+S allows simultaneous sensing of local field potentials (LFP) of four leads. Beta oscillatory activity (13-30 Hz) has been suggested as a biomarker which correlates with severity of rigidity and bradykinesia. Beta spectral power is enhanced in the untreated state and reduced with levodopa or DBS. Conversely, STN - GPi coherence in the narrowband gamma range (~ 60 - 90 Hz) has been found to increase after administration of levodopa or therapeutic DBS, particularly in patients with dyskinesia. Given the communication through coherence hypothesis and the information block model of DBS, we probed internodal interactions between the STN and GP. We recorded LFP from 3 awake participants in a series of experiments initiated ≥ 3 mo after implantation of DBS leads. With DBS off, we observed prominent beta oscillations throughout the motor circuit and significant interactions between nodes as measured by magnitude-squared coherence (MSCoh) and phase-locking value (PLV). Indeed, multiple frequency-distinct beta-oscillations appeared across the nodes. The MSCoh profiles varied by participant but were relatively consistent within each participant over time. In general, a band of coherence occurred within the low beta band (< ~20 Hz) between most regions. Conversely, MSCoh in the high beta band (> ~20 Hz) was broader in frequency range but observed only between the STN and GP of

the same hemisphere. MSCoh in both the low and high beta bands was decreased substantially during DBS. The lag time determined by PLV confirmed that the coherence was not simply volume conduction between regions. We analyzed the MSCoh between nuclei in narrowband gamma at multiple points during the participants' levodopa cycles. We did not observe dyskinesia, a narrowband gamma spectral power peak, or gamma coherence in any participant at any point, even though the participants demonstrated dyskinesia pre-operatively. Together these results suggest that inter-nodal analyses including MScoh and PLV in beta frequency oscillatory activity may be a useful tool for assessing the presence of Parkinsonian symptoms and as control signals for adaptive DBS. Clinical studies were approved by the IRB of Duke University Health System and participants provided informed consent.

Disclosures: **S.L. Schmidt:** None. **J.J. Peters:** None. **K. Palopoli-Trojani:** None. **K.T. Mitchell:** None. **W.M. Grill:** A. Employment/Salary (full or part-time):; Deep Brain Innovations LLC, NDI Healthcare Fund. **D.A. Turner:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic PLC.

Digital Abstract Session

P114. Parkinson's Disease: Neural Dynamics

Program #/Poster #: P114.04

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS grant R01-NS100908
OD P51-OD011132

Title: Temporal variability in the firing of external globus pallidus neurons in normal and parkinsonian monkeys

Authors: ***A. GALVAN**, X. HU, K. HEFFERNAN, A. DEVERGNAS, T. WICHMANN; Emory Univ., Atlanta, GA

Abstract: Based on extracellular recordings obtained *in vivo* in monkeys, most neurons in the external segment of the globus pallidus (GPe) show high frequency firing interspersed by pauses while a smaller proportion of neurons fire at low frequency and generate bursts. Traditionally, these two patterns of firing have been considered to arise from distinct subpopulations of GPe neurons, but it is possible that the heterogeneity of firing patterns originates, instead, from temporal variations in the firing behavior of individual neurons. In rodents, GPe neurons show significant temporal variations in firing rates (Diester et al, 2013). In monkeys that have been treated with the neurotoxin MPTP to induce parkinsonism, GPe neurons show a reduction in the firing rate and an increase in the proportion of firing in bursts. Most of the available studies, however, are based on seconds or, at most, a few minutes of recording of neuronal activity. To examine the temporal stability of GPe neuronal firing in normal and parkinsonian monkeys, we obtained long duration (7-60 min) recordings of well-isolated GPe neurons. Four young adult monkeys (one male, three females) received chronic recording chambers aimed at the GPe.

Extracellular electrophysiologic recordings were then conducted using tungsten electrodes and standard recording procedures, while the monkeys were sitting on a primate chair. In two of the monkeys, recordings were conducted before and after induction of stable parkinsonism with MPTP. All recordings were conducted while the animals were awake. The data was collected to computer disk and spikes sorted. Timings of spikes were converted to inter-spike intervals (ISIs) for further analysis. The data were binned in one-minute bins, and for each bin we obtained several firing parameters (firing rate, ISI-coefficient of variability (ISI-CV), bursting parameters). In most of the recorded GPe neurons, substantial changes in firing parameters were seen across the recorded time in both normal and MPTP-treated monkeys. However, the temporal variability in firing rates, as well as in other parameters of firing, was larger in normal than in parkinsonian conditions. For example, in the normal monkeys, GPe neurons firing rates varied from 80 to 35 spikes/sec, while GPe cells in the parkinsonian state the range was from 54 to 28 spikes/sec. Our results suggest that the firing of individual GPe neurons shows very high temporal variability, this could explain the heterogeneous firing patterns that have been ascribed to GPe neurons in *in vivo* monkey recordings. Our results also indicate that the temporal variability in firing of GPe neurons is reduced in the parkinsonian state.

Disclosures: **A. Galvan:** None. **X. Hu:** None. **K. Heffernan:** None. **A. Devergnas:** None. **T. Wichmann:** None.

Digital Abstract Session

P115. Parkinson's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P115.01

Topic: C.03. Parkinson’s Disease

Support: NIH NS045962

Title: Phosphodiesterase 10A inhibitor for Parkinson's Disease therapy

Authors: ***B. KOCHOIAN**[1,3], C. G. SINON[1,3], K. PENDERGRAST[1,3], S. M. PAPA[2,3]; [2]Neurol., [1]Emory Univ., Atlanta, GA; [3]Yerkes Natl. Primate Res. Ctr., Atlanta, GA

Abstract: BACKGROUND: L-Dopa improves mobility in Parkinson’s disease (PD), but long-term therapy is associated with efficacy decline and abnormal involuntary movements, called L-Dopa-induced dyskinesias (LID). Previous work has revealed decreased striatal expression levels of the cyclic nucleotides, cAMP and/or cGMP, second messengers that play a major role in dopaminergic signaling cascades of striatal projection neurons (SPNs). Restoring cyclic nucleotide levels with inhibition of phosphodiesterases (PDEs), enzymes that catabolize cAMP and cGMP, may help regulate DA signaling in SPNs. PDE10A is highly expressed in the striatum and has substrate affinity for both cAMP and cGMP. To understand the impact of PDE10A function on striatal SPN responses to DA, we assessed the effect of PDE10A inhibition directly on the SPN activity in the primate model of PD. METHODS: An MPTP-treated macaque with advanced parkinsonism and reproducible LID was used for striatal single-cell

recordings with local microinjection of a selective PDE10A inhibitor (PDE10A-I). Our approach was to analyze firing changes of SPNs with continuous recordings from the OFF state, to 1 min and 5 min after local PDE-I injection, and to 20 min following a systemic L-Dopa administration, ON state, and to the point of peak-dose dyskinesias. RESULTS: SPNs displayed a variable response in firing to local injection of PDE10A-I. Among SPNs that increased firing rate following PDE10A-I injection, there was a synergistic effect of L-Dopa sustaining the frequency increase. At the point of dyskinetic response, however, we observed an inversion of the initial firing rate changes induced by L-Dopa (ON state onset). This unstable frequency increases induced by L-Dopa are consistent with the expression of LID and expressed the D1 receptor (D1R) expressing SPN response to dopaminergic stimulation. DISCUSSION: Our previous work has demonstrated that systemic administration of PDE10A-I decreases dyskinesias. Our present analysis of the SPN response to PDE10A inhibition revealed synergistic action of PDE10A-I with L-Dopa on positive modulation of D1R-like SPNs. However, persistence of inversion of firing rate changes in the ON state suggests that PDE10A-I does not stabilize DA responses of D1R-like SPNs, and that opposite effects can be expected on D2R-like SPNs. Our results support further investigation into the mechanisms underlying the anti-dyskinetic effect of PDE10A-I.

Disclosures: B. Kochoian: None. **C.G. Sinon:** None. **K. Pendergrast:** None. **S.M. Papa:** None.

Digital Abstract Session

P115. Parkinson's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P115.02

Topic: C.03. Parkinson’s Disease

Support: NIH R37 NS040894

Title: Temporally non-regular patterns to improve the efficacy of deep brain stimulation (DBS) for Parkinson's disease (PD)

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Abstract: DBS is an effective treatment for movement disorders such as PD for individuals who are refractory to pharmacological intervention alone. The dependency of DBS efficacy on stimulation frequency is well established, with only high frequencies (greater than ~100 Hz) providing symptom relief and low frequencies potentially exacerbating symptoms. We previously demonstrated that the effects of DBS are also dependent on the temporal pattern of stimulation. This novel parameter space provides opportunity to improve the efficacy of DBS by suppressing oscillatory activity in the basal ganglia. Here, we tested in intraoperative experiments the effects of two temporally non-regular patterns of stimulation on both oscillatory

activity in the basal ganglia and motor symptoms in participants with PD. The Institutional Review Boards at Duke University and Emory University approved the study protocol. Individuals undergoing DBS implantation or implantable pulse generator replacement surgeries were recruited and gave written informed consent. We simultaneously delivered DBS and recorded oscillatory activity from the subthalamic nucleus (STN) in each participant (n = 17). Motor symptoms were also recorded before and during DBS according to each participant's dominant symptom (bradykinesia or tremor). Bradykinesia (n = 11) was evaluated using an alternating finger tapping task, and tremor power (n = 6) was measured with a hand-mounted accelerometer. Five stimulation conditions were tested: *Off*, *Low* (regular 10 Hz), *High* (regular 185 Hz), and two temporally non-regular stimulation patterns with geometric mean of 185 Hz (named *Absence* and *Presence*). The absence and presence stimulation patterns were both periodic and characterized by the absence or presence of short bursts of pulses, respectively. Analysis consisted of paired signed rank tests between *Off* and *On*. In the tremor cohort, *High* ($p = 0.031$) and *Presence* ($p = 0.031$) reduced tremor power compared to *Off*, while *Absence* and *Low* did not. Reduction in tremor was not correlated with a reduction in beta power in this cohort. In the bradykinesia cohort, *High* ($p = 0.0078$) and *Absence* ($p = 0.014$) improved the performance on the alternating finger tapping task compared to *Off*, while *Presence* and *Low* did not. Additionally, *High* ($p = 0.0078$), *Absence* ($p = 0.0098$) and *Presence* ($p = 0.039$) reduced beta power compared to *Off*, while *Low* did not. Temporally non-regular DBS patterns were as effective as traditional high frequency stimulation in treating the motor symptoms of PD and suppressing pathological beta activity.

Disclosures: K. Palopoli-Trojani: None. **S.L. Schmidt:** None. **J.J. Peters:** None. **D.A. Turner:** None. **W.M. Grill:** A. Employment/Salary (full or part-time):; Deep Brain Innovations LLC, NDI Healthcare Fund.

Digital Abstract Session

P115. Parkinson's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P115.03

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS R56 NS109608
Arizona Biomedical Research Commission (ABRC) grant ADHS18-198846

Title: Ketamine disrupts 80-Hz gamma oscillations in parkinsonian rats with L-DOPA-induced dyskinesia

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease, and symptoms include debilitating motor and cognitive deficits. While levodopa (L-DOPA) is the leading treatment for PD, long-term administration leads to L-DOPA-Induced Dyskinesias (LID), which are uncontrollable involuntary movements. A common neurophysiological feature of LID is the emergence of 80-Hz oscillations in the motor cortex (M1) following administration of L-DOPA. Ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, can be effective in treating depression, chronic pain, and post-traumatic stress disorder. Sub-anesthetic infusion of ketamine has recently been shown to reduce LID in preclinical studies and in human case studies. In M1, ketamine administration produces narrowband low-gamma oscillations in control animals that are notably distinct from 80-Hz gamma in LID. Given ketamine's capacity to reduce LID and generate 'competing' low-gamma oscillations, we hypothesized that ketamine would reduce the 80-Hz signature in LID. We induced LID in 6-hydroxydopamine-lesioned (6-OHDA) rats by treating animals regularly with L-DOPA (12 mg/kg for ≥10 days). During each experimental session, control and LID-expressing rats were injected with saline or L-DOPA (L-DOPA: 12 mg/kg, i.p.; subjects: n=1 control, n=1 6-OHDA, n=8 total sessions). L-DOPA or saline injections were followed 1-hour later by a ketamine injection (20 mg/kg, i.p.). Single-unit and local-field responses were measured from each hemisphere through a dual-bundle 16-tetrode hyperdrive (AP: 1.5, ML: ±2.2mm). Preliminary data indicated that LID-associated 80-Hz oscillations and dyskinetic movements were suppressed within 5 minutes following ketamine injection, with the 80-Hz oscillations being replaced by robust low-gamma (~50 Hz). Action potentials of neurons in M1 were also phase-locked to changes in 80-Hz power but not 80-Hz phase during LID. These preliminary data support the hypothesis that ketamine reduces LID through the disruption of 80-Hz gamma in M1.

Disclosures: S.L. Cowen: None. **A. Vishwanath:** None. **M.J. Bartlett:** None. **G. Jordan:** None. **V. Boudreau:** None. **S.J. Sherman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pending patent application for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease. **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pending patent application for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease..

Digital Abstract Session

P115. Parkinson's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P115.04

Topic: C.03. Parkinson's Disease

Support: The Grainger Foundation

Title: Transient non-motor symptoms during optimization of stimulation parameters in Parkinson's disease patients reveals the integrative role of subthalamic nucleus: A Tractography-based analysis

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Abstract: Deep brain stimulation (DBS) is a treatment option for people suffering from severe Parkinson's disease (PD). A popular clinical target associated with relief of Parkinsonian symptoms is the subthalamic nucleus (STN), which is responsible for integrating signals associated with control of voluntary movement and control of emotions. While the characteristic result of DBS is a decrease in motor symptoms (e.g., tremor), undesired psychiatric side effects can also occur during treatment. Currently, there is a lack of understanding of the mechanisms by which DBS evokes motor-symptom relief and their implications in emotion and cognition during stimulation. Tractography-based modeling of patient-specific pathways activated by STN DBS can offer insight into the underlying circuitry responsible for the cognitive and psychiatric effects of stimulation.

This study examines a cohort of 15 PD patients who underwent bilateral STN-DBS (Medtronic quadripolar electrode 3387) and experienced transient non-motor side effects during optimization of stimulation parameters. These transient non-motor symptoms (TNMS) presented at specific combinations of contact location and stimulus amplitude (i.e., voltage), and manifested as confusion, crying, a sense of euphoria, feeling anxious, or feeling relaxed.

The aim of this study is to determine if there is a correlation between these TNMS, the stimulation parameters, and engagement of specific neural pathways. Pathway activation modeling, which is used to determine engagement of neural pathways, involves modeling of white matter pathways in the brain as well as the electric field produced by specific stimulation parameters used both during their TNMS events and the relief of their motor symptoms. Electric field models are combined with multi-compartment cable models of axons along the reconstructed white matter pathway models to simulate axonal activation in response to the presence of specific electric fields. The findings of axonal activation modeling are then correlated with the TNMS observed. We expect to find similar pathway activation in individuals experiencing similar TNMS symptoms. We also expect to observe differences between DBS parameters that resulted in therapeutic benefits and DBS parameters that evoked TNMS. The knowledge gained from this study will further our understanding of which pathways are associated with psychiatric side effects related to STN DBS and will represent the first step toward improved targeting for improved therapeutic efficacy of STN DBS for movement and psychiatric disorders.

Disclosures: S.G. Hillan: None. **B. Howell:** None. **G. Goss:** None. **M. McDonnell:** None. **A. Twohy:** None. **A. Makhlouf:** None. **O. Abulseoud:** None. **M.A. Frye:** None. **J.L. Lujan:** None.

Digital Abstract Session

P115. Parkinson's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P115.05

Topic: C.03. Parkinson’s Disease

Title: Differential dopaminergic axonal degeneration manifest into tremor-like and rigidity-like Parkinsonian symptoms: A computational investigation

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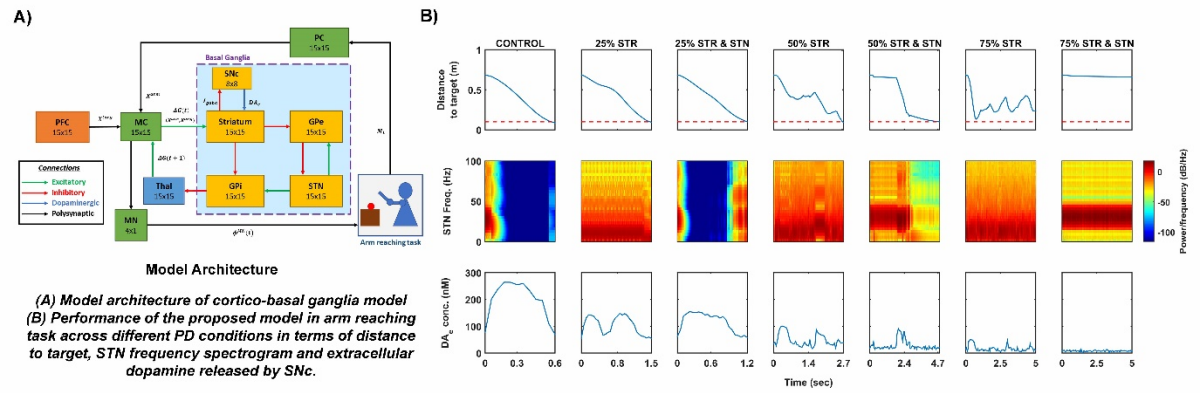
Abstract: A major underlying cause of Parkinson's disease (PD) is the idiopathic loss of dopaminergic neurons in Substantia Nigra pars compacta (SNc). Dopamine (DA) deficiency due to the loss of SNc neurons manifests in cardinal symptoms of PD, including tremor, rigidity, bradykinesia, and postural imbalance. To investigate the PD condition in detail and manage its symptoms, it is important to have an integrated multiscale computational model that can replicate the symptoms at the behavioral level by evoking the key cellular and molecular underlying mechanisms that contribute to the pathology. A realistic simulation of various symptoms of PD can go a long way toward achieving this goal. In line with this approach, we developed a multiscale integrated model of cortico-basal ganglia motor circuitry for arm reaching task incorporating a detailed biophysical model of SNc dopaminergic neuron. Computational models of the basal ganglia tend to take a dichotomous approach, describing exclusively at a detailed biophysical level or at abstract information processing level using concepts from Reinforcement Learning (RL), without working out the relationship between the two levels. In our model, we replace the abstract representations of reward with the realistic variable of extracellular DA released by a network of SNc cells and incorporate it with the RL-based behavioral model, which simulates the reaching task. The phasic DA release from the SNc subsystem is analogous to the reward prediction error in RL terminology. In this model, we introduce the pathological PD condition by modulating the number of surviving SNc neurons, which affects the amount of DA released extracellularly. Differential degeneration of dopaminergic axonal projections to two regions of the basal ganglia viz., striatum and subthalamic nucleus exhibits a differential manifestation of symptoms of PD such as tremor on one hand and bradykinesia and rigidity on the other. The model explores the cellular and anatomical basis to the two key PD patient categories viz., tremor-dominant and rigidity-dominant.



(A) Model architecture of cortico-basal ganglia model (B) Performance of the proposed model in arm reaching task across different PD conditions in terms of distance to target, STN frequency spectrogram and extracellular dopamine released by SNc.

Disclosures: S.S. Nair: None. **V.R. Muddapu:** None. **V. Chakravarthy:** None.

Digital Abstract Session

P116. Therapeutic Strategies

Program #/Poster #: P116.01

Topic: C.03. Parkinson's Disease

Title: 3D Human Midbrain-like Organoid (hMLO) Model Using Patient-Derived Induced Pluripotent Stem Cells (iPSCs) For The Exploration Of Parkinson's Disease (PD) Pathology

Authors: *L. A. STRUZYNA, N. G. HATCHER, L. YAO, M. J. MARINO, M. E. HOFMANN, M. L. WATT;
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Abstract: PD is the second most common neurodegenerative disease, affecting 2% of people over the age of 65. Despite significant research efforts, the vast majority of neuroprotective treatment strategies for PD fail in clinical trials - in part due to inadequate disease models. Recent studies have suggested organoid models recapitulate complex functions that make them superior to conventional 2D cultures for the interrogation of human disease. Therefore, we have generated hMLOs from human PD patient iPSCs in an attempt to establish a more physiologically-relevant model in which to assess PD pathology. In a protocol adapted from Jo et al. (2016), human PD patient-derived iPSCs were aggregated into embryoid bodies, after which they were exposed to a series of growth factors and small molecules in order to differentiate them towards a midbrain neuronal lineage. The embryoid bodies were embedded in matrigel and placed on an orbital shaker, after which they matured into midbrain organoids over months *in vitro*. The organoids were characterized using a variety of histological, cell biological, and biochemical techniques. As anticipated, the hMLOs demonstrated an organized 3D cytoarchitecture with ventricle-like structures that contain discrete cell layers. Young organoids (<25 days *in vitro*) exhibited midbrain progenitor markers, while mature organoids revealed the presence of the dopamine transporter and tyrosine hydroxylase-positive dopaminergic neurons that produced neuromelanin. Patch-clamp electrophysiology studies with hMLO slices demonstrated a neuronal phenotype with the firing of repetitive action potentials. Furthermore,

LC-MS analyses demonstrated the ability to evaluate glycosphingolipid content. Ongoing work is evaluating organelle function, alpha synuclein aggregation, and gene expression within the organoids. Human midbrain-like organoids have the potential to provide an improved *in vitro* discovery platform in which to examine mechanisms of PD pathology and interrogate neuroprotective treatments for PD. However, such analyses require a deep understanding of these complex models. Toward that end, we are extending the work of previous groups in order to provide novel insight into lipid content, organelle function, and alpha synuclein accumulation within these PD patient iPSC-derived hMLOs. This extended panel of characterization metrics will hopefully enable refined detection and interrogation of PD pathophysiologically-relevant perturbations, aiding in our understanding of the mechanisms involved in PD onset and identification of therapeutics.

Disclosures: L.A. Struzyna: None. **N.G. Hatcher:** None. **L. Yao:** None. **M.J. Marino:** None. **M.E. Hofmann:** None. **M.L. Watt:** None.

Digital Abstract Session

P116. Therapeutic Strategies

Program #/Poster #: P116.02

Topic: C.03. Parkinson's Disease

Support: NIA AG065682
G-RISE T32GM136499

Title: Activation of D2 receptor on cholinergic interneurons is critical for the induction of L-Dopa induced dyskinesia

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Abstract: Dopamine neuron (DAN) loss is a hallmark sign of Parkinson's Disease (PD). L-Dopa therapy, the gold-standard treatment for PD, attenuates bradykinesia and akinesia in patients. Unfortunately, prolonged L-Dopa use eventually produces a debilitating side-effect called L-Dopa induced dyskinesia (LID) in ~90% of medicated PD patients. The mechanistic underpinnings of LID remain obscure and are of interest for the purpose of improving PD therapies. Cholinergic interneurons of the striatum (CIN) are projection targets of DAN and undergo pathophysiological changes in response to DAN degeneration. These changes to CIN physiology have been suggested key for LID formation, a contention supported by studies demonstrating that manipulations of CIN activity in animal models of LID can impact LID expression. While a link between aberrant CIN function and LID is increasingly clear, the nature of this relationship remains disputed. Namely, both increased and decreased CIN activity have been implicated in LID. Therefore, to reconcile these results, the changes to CIN physiology

following DAN loss and subsequent L-Dopa treatment, as well as the mechanisms responsible for those changes, warrant closer examination.
DAN are known to signal CIN via numerous signaling factors, all of which are lost as DAN degenerate in PD. L-Dopa therapy restores dopamine (DA) signaling but fails to restore the additional signaling factors released by DAN to CIN. We recently found that release of the signaling peptide sonic hedgehog (Shh) from DAN to CIN is crucial in the prevention of aberrant CIN physiology and LID induction. Additionally, loss of Shh signaling on CIN reduces levels of neuronal activity marker p-rpS6 among dorsolateral CIN. These observations suggest that hypo-active CIN physiology may be a key driver of LID in the striatum. CIN express both inhibitory D2 receptors and facilitatory D5 receptors. Here, we further probe the degree to which CIN hypo-activity, specifically through activation of D2 following L-Dopa administration, may be responsible for LID induction. We hypothesize that reducing inhibition from D2 signaling on CIN can help maintain normal CIN physiology during L-Dopa therapy and confer LID resistance. To test this, we utilized CIN specific ablation of the D2 receptor in 6-OHDA animals treated with L-Dopa. Behavioral scoring of abnormal involuntary movements was used to quantify LID in D2 knockout and heterozygous mice. Additionally, we report differences in CIN p-rpS6 levels as well as the LID cytochemical marker p-ERK. Our initial results suggest that ablation of D2 signaling from CIN prevents the induction of LID behavior and associated changes in CIN physiology.

Disclosures: S. Uribe-Cano: None. **K. Liu:** None. **L. Malave:** None. **A.H. Kottmann:** None.

Digital Abstract Session

P116. Therapeutic Strategies

Program #/Poster #: P116.03

Topic: C.03. Parkinson's Disease

Support: Research Grant from The Army Research Office (ARO) # W911NF-16-1-0311
Merit Award from the Veterans Administration (VA) Biomedical Laboratory Research and Development 1I01BX003303-01

Title: Four Salvage NAD^+ Biosynthetic Pathway Enzymes Moonlight as Molecular Chaperones to Protect Against Proteotoxic Stress

Authors: *M. PINKERTON;
Univ. of Miami.

Abstract: Four Salvage NAD^+ Biosynthetic Pathway Enzymes Moonlight as Molecular Chaperones to Protect Against Proteotoxic Stress
Authors: Meredith Pinkerton1, Andrea Ruetenik1, Viktoriia Bazylianska1, Eva Nyvltova1, and Antoni Barrientos1,2*
Neurodegenerative proteinopathies involve the misfolding and aggregation of disease-specific proteins in the brain. Searches for neuronal maintenance factors have identified the NAD+

salvage biosynthetic pathway enzyme NMNAT (nicotinamide mononucleotide (NMN) adenylyltransferase) as an effective suppressor of proteotoxicity. Although there has been some controversy regarding whether the neuroprotective effect of NMNAT is mediated by increased NAD+ levels or a non-catalytic chaperone role of NMNAT, it is now accepted that both may contribute. Screens in yeast models of HD and PD in our lab have allowed us to identify not only the NMNAT homologs Nma1/2 but three additional enzymes of the NAD+ salvage pathway that achieve similar protection against extended polyglutamine- and a-synuclein-induced proteotoxic stress: Npt1, Pnc1, and Qns1. Under proteotoxic stress, the four proteins are recruited to promote the clearance of misfolded and oligomerized proteins. We have shown that the suppression mechanism by NAD+ proteins is independent of their catalytic activity. In vitro and in vivo studies have indicated that the four proteins have the ability to act as molecular chaperones. Our data suggest that they perform holdase and foldase chaperone activities to contribute to the reduction of misfolded toxic proteins, while promoting their refolding. The proteotoxicity suppression mechanism requires the presence of the HSP90 chaperones HSP82/HSC82. In the case of Nma1, structure-function relationship studies have shown that the C-terminus of the protein is essential for its chaperone activity and presumably for interaction with HSP90. Our data illustrates the existence of an evolutionarily conserved strategy of repurposing housekeeping enzymes under stress conditions, such as age-associated neurodegenerative proteotoxicities.

Disclosures:

Digital Abstract Session

P116. Therapeutic Strategies

Program #/Poster #: P116.04

Topic: C.03. Parkinson's Disease

Support: JSPS KAKENHI Grant Number 20K17932
JSPS KAKENHI Grant Number 17K10899
JSPS KAKENHI Grant Number 16KK0182
JSPS KAKENHI Grant Number 26461272
JSPS KAKENHI Grant Number 17K07096
NCI/DTP Open Chemical Repository for providing the compounds used in our assays and the makers of the DOCK program Suite (UCSF)

Title: Phospho-serine 276 of nuclear factor kappa b is involved in the genesis of l-dopa-induced dyskinesia

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Abstract: Nuclear factor kappa B (p65/RelA) is ubiquitously distributed in the body and regulates various genes involved in inflammation, stress, and immune responses, and cell

survival and proliferation. In this study, we observed that phosphorylation of the serine 276 residue of p65 (p65-pS276) was regulated by dopamine signaling in mouse striatum. Differential dopaminergic modulation across naïve and 6-hydroxydopamine-induced parkinsonian mice resulted in an increase or decrease in the expression of p65-pS276 in the dorsal striatum. The expression pattern of p65-pS276 induced by dopamine D1/D2 synergy was strikingly similar to that of c-fos, and over 80% of c-fos mRNA-positive cells induced by high-dose apomorphine were also p65-pS276 positive. Administration of a small-molecular p65-pS276 inhibitor prevented striatal c-fos expression and motor stereotypy induced by dopamine D1/D2 synergy. Moreover, continuous administration of this agent in a mice model of LDOPA-induced dyskinesia decreased ΔfosB expression in the dorsal striatum and dyskinetic movements as well. These results indicated that p65-pS276 could be a molecular target for the treatment of motor stereotypy and L-DOPA-induced dyskinesia.

Disclosures: R. Morigaki: None. **M. Ogawa:** None. **S. Okita:** None. **T. Matsuda:** None. **Y. Takagi:** None. **S. Goto:** None.

Digital Abstract Session

P116. Therapeutic Strategies

Program #/Poster #: P116.05

Topic: C.03. Parkinson's Disease

Support: Vinnova Grant 2019-01458

Title: Mesdopetam suppresses sensitization and AIMs in the rodent unilateral 6-OHDA lesion model of Parkinson's disease

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Abstract: Introduction
Mesdopetam is a dopamine D3 antagonist, displaying an agonist-like binding mode at the orthosteric receptor site but devoid of intrinsic activity. It is currently in Phase II clinical development for the treatment of L-DOPA induced dyskinesias (LIDs) in advanced Parkinson's disease. It has been shown to reduce bad ON-time (time with troublesome dyskinesias) with 4-5 hours, in a well-tolerated dose range. In the 6-OHDA lesioned hemiparkinsonian rat model, mesdopetam has previously been reported to dose-dependently reduce established LIDs. In this study, the effect of co-administering mesdopetam with L-DOPA in hemilesioned rats was explored.

Methods
Female rats with unilateral 6-OHDA lesions in the MFB (post-mortem verified by DA

transporter autoradiography) were used. Two weeks after surgery, proper lesioning was confirmed by an acute apomorphine challenge rotation test. Then chronic treatment was started with either: Saline (N=6); L-DOPA/benserazide (10/7.5mg/kg, i.p.; N=6); mesdopetam (3 or 10mg/kg, s.c; N=6); amantadine (40mg/kg, i.p.; N=7); L-DOPA/benserazide combined with mesdopetam (N=6); L-DOPA/benserazide combined with amantadine (N=7). Behavioural assessments were performed on Day 1, 7, and 14 of the chronic treatment regime. Contralateral rotations were measured by RotoRat® over 2.5 hours post-drug administration. Rat abnormal involuntary movements (AIMs) were scored from 5-min video recordings captured at 90 min post-injection. The experiments were approved by the local ethical committee at Karolinska Institute and conducted in accordance with the European Communities Council Directive (86/609/EEC).

Results

Rats treated with L-DOPA alone displayed increasing rotational behaviour over the 14 Days of repeated dosing, along with increased AIMs scores. Co-treatment with mesdopetam or amantadine suppressed AIMs to a similar degree. In contrast, only co-treatment with mesdopetam countered the L-DOPA-induced increase in contralateral turns over the 2-week period of repeated dosing.

Conclusions

Co-treatment with mesdopetam, at 3 or 10 mg/kg, suppressed L-DOPA-induced AIMs over 14 days. Furthermore, the sensitization (reflected by increased rotational behaviour) in rats receiving repeated doses of L-DOPA was clearly suppressed, especially at the 3 mg/kg dose. This effect was not seen with the comparator amantadine, which reduced AIMs but did not affect the L-DOPA sensitization. It is concluded that mesdopetam, in addition to its previously described acute antidyskinetic effects, may prevent the development of dyskinesias by blocking L-DOPA sensitization.

Disclosures: **P. Svenningsson:** None. **Y. Yang:** None. **S. Hjorth:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; IRL AB, Sweden. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IRL AB, Sweden. **S. Waters:** A. Employment/Salary (full or part-time):; IRL AB, Sweden. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IRL AB, Sweden. **N. Waters:** A. Employment/Salary (full or part-time):; IRL AB, Sweden. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IRL AB, Sweden. **P. Svensson:** A. Employment/Salary (full or part-time):; IRL AB, Sweden. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IRL AB, Sweden. **J. Tedroff:** A. Employment/Salary (full or part-time):; IRL AB, Sweden. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IRL AB, Sweden.

Digital Abstract Session

P116. Therapeutic Strategies

Program #/Poster #: P116.06

Topic: C.03. Parkinson's Disease

Support: DOD Grant W81XWH-17-1-0699
NIH Grant P30 ES006096
Kerman Family Fund
Selma Schottenstein Harris Lab for Research in Parkinson's
Parkinson's Disease Support Network - Ohio, Kentucky & Indiana

Title: Intranasal carnosine mitigates alpha-synuclein pathology and motor dysfunction in the Thy1-aSyn mouse model of Parkinson's disease

Authors: J. M. BROWN[1], L. S. BAKER[1], ***K. B. SEROOGY**[2], M. GENTER[1];
[1]Envrn. Hlth., [2]Neurol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Parkinson's disease (PD) is a common and debilitating neurodegenerative disorder. Early symptoms of PD include motor dysfunction and impaired olfaction (hyposmia). Toxic aggregation of alpha-synuclein (aSyn) is a hallmark of PD neuropathology and includes aSyn accumulation in the olfactory bulb (OB) and substantia nigra pars compacta (SNpc). Thy1-aSyn transgenic mice exhibit many features of PD, including olfactory and sensorimotor impairment, making these mice a useful model for studying drug intervention in PD. Carnosine is an endogenous dipeptide with antioxidant and anti-aggregating properties. Previously, we showed that treatment with 2.0 mg/d intranasal (IN) carnosine for two months improved motor behavior in Thy1-aSyn mice. Here, we tested whether an elevated dose of the dipeptide would provide greater benefit for mitigation of motor and olfactory deficits in the transgenic mice. In addition, neuronal expression of aSyn in the OB mitral cell layer (MCL) and of aSyn and tyrosine hydroxylase (TH) in the SNpc were assessed in both the Thy1-aSyn and wild-type mice after IN carnosine treatment. After baseline behavioral testing, two-month-old male wild-type and Thy1-aSyn mice were randomly assigned to treatment groups (n=25-46/group) and received daily IN vehicle, 2.0 or 4.0 mg/d carnosine treatment for eight weeks. Mice were then reassessed for motor and olfactory function in the challenging beam traversal (CBT), spontaneous activity and buried pellet tests. Animals were then sacrificed and the brains processed for aSyn and TH immunohistochemistry, and labeled cells were counted in the SNpc and OB MCL using design-based stereology. Intranasal carnosine dose-dependently mitigated progressive gait deficits in Thy1-aSyn mice in the CBT. There were no effects of IN carnosine treatment on olfactory function. Thy1-aSyn mice treated with IN carnosine exhibited fewer aSyn+ somata in the SNpc vs. vehicle-treated transgenic mice. Carnosine treatment did not affect the number of aSyn+ somata in the MCL, nor the number of TH+ cells in the SNpc of either genotype. Overall, these results demonstrate that IN administration of carnosine decreases somatic aSyn accumulation in the SNpc, which may underlie its mitigation of motor deficits in Thy1-aSyn mice.

Disclosures: **J.M. Brown:** None. **K.B. Seroogy:** None. **M. Genter:** None. **L.S. Baker:** None.

Digital Abstract Session

P116. Therapeutic Strategies

Program #/Poster #: P116.07

Topic: C.03. Parkinson's Disease

Support: Conacyt Grant 257597

Title: Levodopa and celecoxib interact synergistically to alleviate allodynia in hemiparkinsonian rats

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Abstract: Pain is a common non-motor symptom of Parkinson's disease patients, with a prevalence between 29-82%. Consequently, it´s vital to find pharmacological treatments for the management of PD-associated pain symptoms, in order to improve patients' quality of life. This study tested the degree of interaction between levodopa and celecoxib combination in allodynia induced by unilateral 6-OHDA injection into the SNpc in rats. Allodynia was evaluated using von Frey filament with up-down method. For acute treatment, at 20 days' post-lesion with 6-OHDA. The animals were administrated with levodopa (3, 6, 10, 25 mg / kg, i.p., n = 6), or CXB (2.5, 5, 10, 20 mg / kg, ip, n = 6) allodynia was evaluated for 8 h. For sub-acute treatment (10 days), the same group of animals used for acute treatment received levodopa (3, 6, 10, 25 mg / Kg, ip, per day, every 24 h, n = 6), celecoxib (2.5 , 5, 10, 20 mg / Kg, ip, per day, every 24 h, n = 6), tactile allodynia was evaluated. Isobolographic analysis was employed to define the nature of the drug interaction using a dose ratio (0.5:0.5). The ED_{50} calculated from Levodopa or celecoxib was considered as a theoretical dose for each drug. The experimental ED_{50} of the L-DOPA + CXB combination was calculated by the following scheme: 1) L-DOPA ED_{50} + CXB ED_{50}; 2) ½ (L-DOPA ED_{50} + CXB ED_{50}); 3) ¼ (L-DOPA ED_{50} + CXB ED_{50}); 4) 1/8 (L-DOPA ED_{50} + CXB ED_{50}). For acute or sub-acute (10 days) treatment, the animals received an intraperitoneal injection with the dosing scheme before indicated and allodynia was evaluated. Acute and sub-acute (10 days) treatment with a single dose of levodopa (3-25 mg / Kg, i.p.) or celecoxib (2.5-20 mg/kg, i.p.) induced dose-dependent antiallodynic effect in hemiparkinsonian rats. For acute treatment, the theoretical ED_{50} of the combination was 13.5 mg/kg, this value was higher than the experimental ED_{50} value 3.9 mg/kg. Moreover, for sub-acute treatment (10 days), the theoretical ED_{50} to the combination was 11.2 mg/kg. while the experimental ED_{50} was 3.1 mg/kg. These data suggest that levodopa and celecoxib combination produces an antiallodynic synergistic interaction at the systemic level. Therefore, the combination of levodopa and CXB may use as an analgesic strategy in Parkinson's patients

Disclosures: **B. Godínez-Chaparro:** None. **C. Rodriguez-Ramos:** None. **M. Benítez-Díaz Mirón:** None. **G. Garza-Mouriño:** None.

Digital Abstract Session

P117. Therapeutic Strategies: Preclinical Animal Models ? Other Therapeutics

Program #/Poster #: P117.01

Topic: C.03. Parkinson's Disease

Support: NIH R37 NS040894

Title: The Cortical Evoked Potential as a Biomarker for Deep Brain Stimulation Efficacy

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Abstract: Deep brain stimulation (DBS) applied at a fixed amplitude and frequency is effective at alleviating the motor symptoms of Parkinson's disease (PD), but efficacy may be improved by using customized temporal patterns of stimulation or closed-loop control. A necessary element in the development of these techniques is an electrophysiological biomarker that can serve as a proxy for DBS-mediated changes in symptoms. Many such biomarkers have been proposed, with nearly all quantifying some type of spectral feature in the cortico-basal ganglia-thalamic network. However, these spectral biomarkers have high inter-patient variability, and the mechanisms by which DBS alters them is uncertain. We assessed the utility of an alternative, non-spectral biomarker: the cortical evoked potential (cEP) following DBS in the subthalamic nucleus (STN), in the 6-OHDA lesioned rat model of parkinsonism. We hypothesized that changes in the cEP magnitude and latency between effective and ineffective DBS settings would have a stronger correlation with symptom reduction than conventional, spectral biomarkers. For the conventional biomarkers, we quantified the M1 ECoG beta band power, beta band bursting, and phase-amplitude coupling (PAC). We obtained within-animal correlations of each biomarker with symptom reduction by applying stimulation frequencies that spanned a range of efficacies (13 - 200 Hz) and yielded a spectrum of symptom changes for each animal that were correlated with each biomarker. To quantify symptom reduction, we used methamphetamine-induced circling (N=9) and an adjusting steps task (N=6). High stimulation frequencies decreased the cEP magnitude, increased the cEP latency, and reduced the cortical beta band power, beta band bursting, and PAC. However, the cEP features exhibited a stronger correlation with symptom reduction across stimulation frequencies than the spectral biomarkers, for both behavioral tasks. The cEP magnitude and latency had r2 values of 0.73 ± 0.05 (mean ± SEM) and 0.74 ± 0.05 for the adjusting steps task and 0.64 ± 0.04 and 0.63 ± 0.07 for the circling task, respectively. Alternatively, the highest average r2 values for the spectral biomarkers were 0.46 ± 0.07 for the adjusting steps task (beta band power) and 0.54 ± 0.11 for the circling task (beta band bursting). The cEP features exhibited stronger correlations with symptom reduction from STN DBS than conventional cortical spectral biomarkers. This strong correlation may be indicative of a mechanistic link between the observed changes in cEP magnitude and latency at high stimulation

frequencies and the cortical mechanism by which STN DBS alleviates parkinsonian motor symptoms.

Disclosures: **I.R. Cassar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **A. Czeiszperger:** None. **K. Hancock:** None. **W.M. Grill:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder.

Digital Abstract Session

P117. Therapeutic Strategies: Preclinical Animal Models ? Other Therapeutics

Program #/Poster #: P117.02

Topic: C.03. Parkinson's Disease

Support: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP 2018/18695-9)
Hospital Sírio Libanês

Title: Understanding how subthalamic stimulation influences striatal dopamine, glutamate, and GABA release in a model of Parkinson's disease

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Abstract: Introduction: The underlying mechanisms of deep brain stimulation (DBS) of the subthalamic nucleus (STN), a well-established treatment for Parkinson's disease (PD), remains largely unknown. STN-DBS induces anti-inflammatory mechanisms[1], and considering the importance of glial modulating for synapse regulation, here we aimed to understand the striatal release of dopamine (DA), glutamate (GLU), and GABA before, during, and after the first and fifth session of STN-DBS in a rat model of PD. **Methods:** Male Wistar rats were injected with 6-OHDA neurotoxin and implanted with guidance cannula in the left striatum, and implanted or not with stainless steel electrodes in the left STN. After 7 days, the nigrostriatal lesion was assessed by apomorphine-induced rotation. On the 8th day, 6-OHDA+DBS ON animals were stimulated 2 h/day/5 days (130 Hz, 60 μs, 0.1 mA). Striatal microdialysis samples were collected before, during, and after the 1st and 5th session of DBS in the 6-OHDA+DBS ON and 8 and 12 days after the surgeries in the 6-OHDA and 6-OHDA+DBS OFF animals. Statistical analyses: 2-way-ANOVA and Bonferroni post-hoc test. **Results:** Rat model of PD was validated by increase on rotational behavior, related with inhibition of striatal DA fibers[2]. On the 12th day, 6-OHDA

animals showed reduction of 20% of DA ($p < 0.0001$) and increase of 40% of GLU release in the striatum. Six-OHDA+DBS OFF animals showed a DA reduction of 40% ($p < 0.0001$) and GLU inhibition of 30% ($p < 0.05$), comparing the 8th and 12th day after neurotoxin. No change in the striatal GABA release was observed. In 6-OHDA+DBS ON rats, the 1st stimulation induced a transitional increase of DA and GLU initially; but as the stimulation continues, DA decreases consistently. Before the last stimulation, striatal DA release was 20% decreased when compared to 8th day and decreased consistently in 40% during and after the last stimulation. Striatal GLU levels of 6-OHDA+DBS ON maintain below 6-OHDA levels, and above 6-OHDA+DBS OFF, while GABAergic release accompanies the transition and stability of GLU. **Conclusion:** Our findings may suggest that STN ablation induced by the electrode implantation elicits an initial but not sustainable protection. The increase of striatal GLU release may suggest excitotoxicity that contributes to the extensive inflammation in PD. Additionally, DBS inhibits the striatal DA release probably by regulating the motor pathways downstream circuitry from the striatum and induces an optimal balance between GLU and GABA release which may contribute to the improvement of symptoms. Hence, by decreasing inflammation, STN-DBS regulates the synaptic plasticity, thus improving the neuronal circuitry impairment.

Disclosures: **A. Campos:** None. **G. Mauricio:** None. **K. Vieira:** None. **M.C. Carvalho:** None. **A.V.V. Auada:** None. **I. Lebrun:** None. **R.C.R. Martinez:** None. **E.T. Fonoff:** None. **C. Hamani:** None. **R.L. Pagano:** None.

Digital Abstract Session

P117. Therapeutic Strategies: Preclinical Animal Models ? Other Therapeutics

Program #/Poster #: P117.03

Topic: C.03. Parkinson's Disease

Title: Aerobic exercise increases CD68+ M2 microglia and TH+ levels in rotenone-induced parkinsonian rats quantified rapidly in fresh-frozen tissue using a computer-based infrared immunohistochemistry imaging method

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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by reduced tyrosine hydroxylase (TH) dopamine neurons and progressive motor impairment. Using a 14-day systemic (IP) rotenone injection in rats, we show that M1 microglial markers (CD11b+IBA-1+) are increased, while therapeutic treadmill exercise increases microglial M2 markers (CD11b+CD68+) coincident with high TH levels and increased rotarod latency-to-fall. Rotenone-treated (RO) Sprague Dawley rats exhibited a 57.1% lower latency-to-fall than vehicle-injected controls (CTRL) (56.3±4.9 v 131.3±18.2 s, n=5, $p<0.0001$), while the RO-with-exercise group (RE) exhibited performance similar to controls (127.5±11.1 s), concurrent with

TH levels (CTRL v RO: $p<0.0001$). M2 markers were significantly lower in RO compared with CTRL (8.4±1.1 v 17.0±1.7 counts, $p<0.0001$), while RE M2 levels were higher than RO (12.2±1.4, $p=0.0013$). Levels of pAkt observed by Western blot in RE sample suggest trophic factor modulation.
Furthermore, we present a rapid, robust, fresh-frozen approach to IHC, without committing the tissue to IHC via fixation and cryopreservation while maintaining long-term storage. We used a sensitive LiCor-based infrared quantification of TH in midbrain sections that can be directly compared across studies. In fresh-frozen tissue that was stored for up to 1 year prior to IHC reaction, this method was highly sensitive to rotenone treatment in 3-month-old Sprague-Dawley rats, correlating with a significant loss decrease in latency-to-fall by approximately 2.5 fold. Using the in situ method presented here, the measured RO midbrain showed 31% lower TH signal than CTRL ($p<0.01$, $n=5$). TH infrared counts versus rotarod latency-to-fall indicates a positive slope and significant correlation of $R^2=0.68$ ($p<0.05$, $n=10$). This rapid, instrument-based quantification method by in situ infrared detection may have minimal bias and good power to quantify TH levels in brain tissue. This approach also allows for the identification of multiple targets by IHC with the simultaneous performance of downstream molecular analysis in the same tissue, allowing for reduced animal use per study.

Disclosures: J.W. Sonne: None. **C. Frosti:** None. **C.M. Seavey:** None. **C. Lemieux:** None. **A. Bunea:** None. **J.S. Groshong:** None.

Digital Abstract Session

P117. Therapeutic Strategies: Preclinical Animal Models ? Other Therapeutics

Program #/Poster #: P117.04

Topic: C.03. Parkinson's Disease

Support: MJFF008162
NeuraCell

Title: Engineered intrabodies regulate levels of pathogenic alpha-synuclein in Parkinson's Disease models

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Abstract: Preventing the intracellular pathological aggregation of the protein alpha-synuclein (α-syn) may be an effective strategy in modifying the progression of synucleinopathies, such as Parkinson's Disease (PD). We have generated nanobody constructs with affinity for aggregation-critical α-syn target epitopes that can be delivered intracellularly (intrabody) as genes via viral vectors. Upon fusion with a negatively-charged proteasomal targeting motif (PEST) that enhances nanobody solubility, anti-α-syn nanobodies function by 1) interfering with target

aggregation regions and 2) mediating α-syn clearance via proteasomal degradation. Here, we report the development of a family of our lead, humanized intrabody construct, VH14-hPEST, for the therapeutic targeting of intracellular α-syn. Using point-mutations at conserved residues critical for PEST interaction with the 19S proteasome cap, we engineered several variants of PEST that modify the efficiency of proteasomal clearance activity. We have generated a family of VH14-hPEST constructs that can reduce total α-syn levels to 60% (VhP), 20-30% (VhP-mid), or <10% (VhP-Low) of control levels. We also generated the VhP-OFF construct that retains α-syn engagement but abolishes intrabody proteasomal interaction. VhP and VhP-Low intrabodies significantly decreased the level of endogenous α-syn expression compared to VhP-OFF when delivered to 3D forebrain organoids generated with iPSCs derived from PD patients with triplications of the α-syn gene (SNCA) ($p=0.0002$), validating engagement in human cells. We aimed to evaluate whether intrabody therapy would protect against neurodegeneration in a progressive rat model of PD. To achieve the broadest CNS distribution of AAV-intrabody therapy, we compared the transduction efficiencies of the engineered viral vector, AAV.PHP.eB (PHP), and its parent serotype, AAV9, when delivered into the cisterna magna of adult rats. PHP achieved vastly superior biodistribution throughout the neuraxis, warranting its use for in vivo experiments. We induced synucleinopathy using injections of α-syn pre-formed fibrils into the striatum of aged rats and delivered intrathecal PHP-VhP and PHP-VhP-Low one month post-lesion. Animals treated with both VhP and VhP-low significantly reduced the number of pathological aggregates in the substantia nigra compared to control-treated animals, confirming effective target engagement in vivo ($p=0.0003$). These data demonstrate the utility of intrabody-mediated regulation of protein levels and highlight the use of this strategy for potential clinical application across numerous neurodegenerative proteinopathies.

Disclosures: **D. Chatterjee:** None. **L. D'Brant:** None. **A. Messer:** None. **D. Butler:** None. **J.H. Kordower:** None.

Digital Abstract Session

P117. Therapeutic Strategies: Preclinical Animal Models ? Other Therapeutics

Program #/Poster #: P117.05

Topic: C.03. Parkinson's Disease

Support: COFAA Fellowships Chuc-Meza & Garcia-Ramirez
SIP-IPN 20195050
Actipulse Neuroscience® equipment loan

Title: Low intensity repetitive transcranial magnetic stimulation in a rodent model of Parkinson`s

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Abstract: Repetitive transcranial magnetic stimulation (TMSr) is a cortical stimulation technique that takes advantage of electromagnetic induction to cause neuronal depolarizations. It has been used in the therapy of various psychiatric and neurological conditions, as depression and Parkinson's, respectively. The instruments and protocols reported make use of magnetic field intensities of the order of 2 to 3 Teslas and stimulation frequencies of 4 to 25 Hz. Although these parameters have shown positive effects in the aforementioned conditions, they also have collateral effects such as seizure induction epileptic, hypomania, headaches, neck pain and paresthesia. An alternative is offered by the low intensity TMSr (0.2 to .5 mT and frequencies from 8 to 560 Hz). To evaluate the effect of such a technique on an experimental Parkinson's model, a daily stimulation protocol was tested for 2 months in hemilesioned rats with 6-hydroxydopamine in the medial forebrain bundle. The results demonstrated a significant reduction in the number of turns induced by amphetamine, as well as a reduction in anxiogenesis evaluated with the defensive burial test and a lower number of dead neurons. There were no effects on motor activity or induction of seizures either during or in the treatment-free intervals. Thus, these results would support the use of this type of technique, especially to reduce the non-motor symptoms observed in Parkinson's.

Disclosures: E. Chuc-Meza: None. **A. Nazario-Pallares:** None. **E. Guarneros-Bañuelos:** None. **M. Garcia-ramirez:** None.

Digital Abstract Session

P117. Therapeutic Strategies: Preclinical Animal Models ? Other Therapeutics

Program #/Poster #: P117.06

Topic: C.03. Parkinson’s Disease

Support: NIH K99 AA025991 (AGS)
NIH ZIA AA000407 (DML)

Title: Ketone ester-enriched diet protects against progressive motor dysfunction in MitoPark mice

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Abstract: Parkinson’s disease (PD) is a progressive, neurodegenerative disease characterized by motor and non-motor dysfunctions. Its pathology involves a progressive loss of midbrain dopamine neurons (DANs) and converging lines of evidence suggest a central role for mitochondrial dysfunction, and subsequent increases in oxidative stress, in PD etiology. The MitoPark (MP) mouse is a model of PD in which the mitochondrial *tfam* gene is knocked out of DANs. This model recapitulates several aspects of PD, such as the gradual development of motor deficits, responsiveness to L-DOPA, and protein inclusions. Thus, in contrast to other PD models with rapid loss of DANs, the gradual onset of motor dysfunction and DAN degeneration in MP

mice enables the study of potential therapeutic interventions on the preservation of motor function and neurodegeneration. One such strategy involves decreasing the metabolic load on mitochondria by inducing a state of ketosis and thereby providing an alternative energy source for neurons which could decrease neuronal oxidative stress and enhance monoamine synthesis (by increasing tetrahydrobiopterin levels, a critical cofactor for monoamine synthesis). To test this hypothesis, we administered a β-Hydroxybutyrate ketone ester-enriched (KEE) diet or standard rodent chow to control and MP mice beginning at four weeks. At six weeks, we began weekly testing of spontaneous and challenged locomotor activity with open field and rotarod, respectively. We found that MP mice fed standard rodent chow had impaired spontaneous and challenged motor function beginning at 14 weeks, relative to control mice fed either the standard diet or KEE diet. In contrast, MP mice fed the KEE diet did not develop significant motor deficits in either task up to our endpoint of 20 weeks. We also examined ex vivo striatal dopamine release with fast-scan cyclic voltammetry in all groups. Similar to rotarod and open field tests, we found that standard chow-fed MP mice had significantly reduced dopamine release relative to control groups at 20 weeks, and that this reduction was partially ameliorated in MP mice fed the KEE diet. Finally, we assessed the loss of midbrain DANs and striatal dopamine axons via tyrosine hydroxylase immunoreactivity and found that DAN numbers and striatal dopamine axon immunoreactivity were decreased in MP mice relative to WT mice, regardless of which diet they were fed. The most parsimonious explanation of our behavioral, voltammetric, and histological results is that the KEE diet enhances monoamine synthesis in remaining DANs in MP mice. We therefore believe that a ketone ester dietary supplement may be a promising adjunct therapeutic treatment for PD.

Disclosures: **A.G. Salinas:** None. **J.A. Nadel:** None. **V. Mahajan:** None. **M.T. King:** None. **R.L. Veech:** None. **D.M. Lovinger:** None.

Digital Abstract Session

P117. Therapeutic Strategies: Preclinical Animal Models ? Other Therapeutics

Program #/Poster #: P117.07

Topic: C.03. Parkinson’s Disease

Title: Evaluation of Anti-Parkinson’s activity of herbomineral (Abhrak bhasma) on MPTP induced Parkinson’s disease in mice model

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Abstract: Background: The study was designed to validate the claims in Ayurveda regarding the efficacy of Ayurvedic drugs in neurodegenerative disorders. It was decided to conduct an efficacy study of a herbomineral drug (*Abhrak bhasma*) on Parkinson’s disease induced in C57BL/6 mice. Parkinson’s disease has symptom descriptions in Ayurveda and modern medicine. The latter offers only the symptomatic therapy by replacing dopamine, the neurotransmitter involved, but does not slowdown or reverse the loss of dopaminergic neurons.

Method: 8 weeks old male C57BL/6 mice were used for the study. Parkinsons disease was induced by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The dose selected for inducing Parkinsons disease was 20 mg/kg, i.p., twice a day at an interval of 12 hours for four consecutive days. The positive control used was l-Dopa. The test drug used was a herbomineral (*Abhrak bhasma*). The groups in the study were- Healthy normal group, Disease group, Positive control group and Test drug group. The model was for a period of 15 days and MPTP was injected between 07th day to 11th day. L-Dopa and test dug were fed on all 15 days. The disease group was fed with saline. Behaviour tests- Rotarod test, Photoactometer, Paw stride length and Open field test was carried on the 16th day. After carrying out the tests, the mice were sacrificed and striatum was dissected out. One set of striatum were used for Neurotransmitter (Dopamine) and its metabolites (Homovanillic acid and 3,4-Dihydroxyphenylacetic acid) and the other set were used for Immunohistochemistry of Tyrosine hydroxylase neurons. **Result:** Bahaviour studies showed that the test group had a significant improvement in Rotarod test, Open field test, Paw stride length and Open field test. Neurotrasmitter (Dopamine) and its metabolites (Homovanillic acid and 3,4-Dihydroxyphenylacetic acid) levels were improved in the test drug when compared to the disease group. Immunohistochemistry of Tyrosine hydroxylase neurons in Striatum showed a significant protection of neurons in the group fed with the test drug. **Conclusion:** The drug (*Abhrak bhasma*) was found to be effective in reducing the inflammation in the brain of the mice indicating its anti-inflammatory properties. There was a significant improvement in the neurotransmitter levels and immunohistochemistry suggesting that the drug plays a protective role against the MPTP agent.

Disclosures: A. Shankar: None.

Digital Abstract Session

P117. Therapeutic Strategies: Preclinical Animal Models ? Other Therapeutics

Program #/Poster #: P117.08

Topic: C.03. Parkinson's Disease

Support: NIH-R01NS091238
T32AG061897-2

Title: Evaluation of a neuroprotective PACAP glycopeptide as systemically delivered CNS active drug to treat Parkinson's disease

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Abstract: Pituitary adenylate cyclase-activating peptide (PACAP), is an endogenous neuropeptide hormone that has been extensively studied as a protective therapeutic in a variety of

neurodegenerative settings due to its potential for initiating cellular processes that promote survival and inhibit potentially harmful processes such as inflammation. This multi-faceted response to PACAP is due to interactions with PAC1, VPAC1 and VPAC2 receptors. However, despite PACAP's trophic and anti-inflammatory properties, it has not realized its translational potential due to its poor pharmacokinetic profile (non-linear PK/PD), and limited blood-brain barrier (BBB) permeability. We have designed and synthesized a glycosylated PACAP analog ($PACAP_{1\text{-}27}$S-Lac) that is able to stimulate cAMP production *in vitro* using individual CHO cell lines expressing either PAC1, VPAC1, and VPAC2 receptors. Glycosylation stabilizes a peptide normally susceptible to peptidase activity and enhances BBB penetration by favoring the alpha helical conformation of the peptide to increase the probability of membrane interaction, which along with negative membrane curvature promoted by the presence of the sugar residue facilitates transcytosis at endothelial tissues at the BBB. Using *in vivo* microdialysis in rats coupled with LC-MS^3 we show $PACAP_{1\text{-}27}$S-Lac (n=3, 15 mg/kg, *i.v.*) is able to penetrate the BBB. $PACAP_{1\text{-}27}$S-Lac is detected in the striatum at ~376 nM at 60 min, compared to native PACAP with estimated ~41 nM at 60 min. To test for protective ability of the $PACAP_{1\text{-}27}$S-Lac, we began a study using a mild progressive unilateral 6-hydroxydopamine (6-OHDA; 2 x 27.5 µg) rat PD model (n=16/group). In the treatment group rats were injected (*i.p.*) with 15 mg/kg of $PACAP_{1\text{-}27}$S-Lac 6-hrs prior, 48-hrs and 72-hrs post 6-OHDA injection. Analysis of a subset of animals at 4 weeks post-6-OHDA lesion shows that PACAP-treated rats exhibit a trend towards reduced ipsilateral turning behavior (n = 6/group, 27.5 mean turns/100 min in vehicle-treated, compared to -75.53 in PACAP-treated, p=0.0678) in response to the indirect dopamine agonist amphetamine, consistent with reductions in overall parkinsonian lesion size. Unbiased stereology of dopaminergic neurons in the substantia nigra is underway (n=8/group) to evaluate the extent of neuroprotection. Preliminary analysis of a subset of these rats indicates a lesion size of 20% in the PACAP-treated compared to 30% in the vehicle-treated groups. Further video analysis, including the cylinder, vibrissae-elicited forelimb placing, and forelimb adjusting steps tests, will be done to determine the exact extent of neuroprotective activity, if any, that PACAP has.

Disclosures: K. Bernard: None. **M.J. Bartlett:** None. **C. Liu:** None. **G. Molnar:** None. **C. Apostol:** None. **L. Szabò:** None. **S.J. Sherman:** None. **L. Madhavan:** None. **J.M. Streicher:** None. **R. Polt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Glycosylated PACAP/VIP Analogues with Enhanced CNS Penetration for Treatment of Neurodegenerative Diseases. **M.L. Heien:** None. **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Glycosylated PACAP/VIP Analogues with Enhanced CNS Penetration for Treatment of Neurodegenerative Diseases.

Digital Abstract Session

P117. Therapeutic Strategies: Preclinical Animal Models ? Other Therapeutics

Program #/Poster #: P117.09

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS045962

Title: Striatal GluN2B gene silencing improves motor responses to L-Dopa in hemiparkinsonian rats

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Abstract: Introduction: Dopamine (DA) denervation of the striatum in Parkinson's disease (PD) results in profound alterations in striatal projection neuron (SPN) activity. Studies of SPN dysregulation demonstrate a role for glutamate signaling in SPN hyperactivity following DA depletion. DA substitution therapies are associated with long-term efficacy decline and side effects including L-DOPA-induced dyskinesias (LID). Antagonism of NMDAR signaling has shown anti-LID effects, but pharmacological approaches lack specificity for the striatum. We undertook a gene therapy approach to silence the expression of GluN2B subunit gene in SPNs to explore a potential new treatment for PD.
Methods: Rats received nigrostriatal 6-OHDA lesions and injections of rAAV-GluN2B shRNA (n=8) or scrambled shRNA control virus (n=6) in the ipsilateral striatum. Motor deficits were assessed at baseline and 30-minutes after L-DOPA injection. Rats were then given daily injections of L-DOPA/benserazide (8/8 mg/kg i.p.) for 15 days. Abnormal involuntary movement (AIMs) scoring and rotation tests were performed on days 1, 4, 8, 12, & 15.
Results: Rats with 6-OHDA lesion demonstrated a decrease in forehand and backhand adjusting steps, and increased latency to initiate stepping with the contralateral forepaw. On day 16 of chronic L-DOPA treatment, adjusting step test and initiation step test performance was equivalent in both forepaws after LDOPA injection. Additionally, rats that received rAAV-GluN2B shRNA injection in striatum performed more overall steps with the contralateral paw after LDOPA injection than those receiving scrambled shRNA injection. Silencing of GluN2B gene expression in the striatum resulted in an increase in total rotations and significantly reduced AIMs in response to L-Dopa from day 8 to the end of chronic treatment.
Discussion: Our results demonstrate that striatal NMDAR/GluN2B gene silencing in hemiparkinsonian rats effectively reduced AIMs while increasing the antiparkinsonian action of L-Dopa. Therefore, these rodent tests of GluN2B gene therapy showed efficacy for improving motor responses to L-Dopa over long-term treatment, and thereby support further investigation into the potential clinical application of this gene therapy for PD.

Disclosures: C. Sinon: None. **B. Kochoian:** None. **S.M. Papa:** None. **J. Dooyema:** None. **K.I. Pendergrast:** None. **Y. Li:** None. **R. Halverson:** None.

Digital Abstract Session

P118. Therapeutic Strategies: Neuromodulation and Exercise

Program #/Poster #: P118.01

Topic: C.03. Parkinson's Disease

Support: NIH Grant T32NS007480-20
NIH Grant K23NS097576

Title: Directional basal ganglia evoked potentials for a guided objective deep brain stimulation programming

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Abstract: Deep brain stimulation (DBS) devices require postoperative programming to realize the therapeutic benefit, determining which parameters (e.g. contact combination) provide the best symptomatic benefit and the fewest side effects. This process relies on the skills of the clinician and the patient exam, requiring a time consuming trial and error approach, which has been further exacerbated by the recent introduction of higher contact-density electrodes known as segmented DBS leads. In this study, we provide a proof of concept platform that could be used to objectively guide programming and placement of leads by using DBS local evoked potentials (DLEP) in an intraoperative setup. We have explored DLEP in the subthalamic nucleus (STN) and ventral intermediate nucleus (VIM), using low-frequency (10 Hz) and therapeutic high-frequency (130 Hz) stimulation, and their relationship to the electrode location. We enrolled 14 patients affected by either Parkinson's disease (PD) or essential tremor (ET), implanted with segmented DBS leads. We have developed post processing tools to reliably recover EP activity from commercially available clinical amplifiers (e.g. Neuro Omega), facilitating the deployment in the current surgical process. DLEP activity was recovered after each stimulation pulse by subtracting the modeled DBS artifact. DLEP is a strongly stereotyped oscillatory activity (typical amplitude range of 50-550 μV) with a high signal-to-noise ratio (SNR), which allows single-trial DLEP detection without relying on multi-epoch averages. Our initial results show that DLEP had a consistent difference between STN and VIM targets (absent in the latter). We demonstrate that our post-processing pipeline is sufficient to reliably recover DLEP from noisy recordings which follow a brief saturation recovery of the amplifier. In addition, the recorded DLEP could evaluate the accuracy of STN targeting, as contacts nearer to the motor STN (based on imaging and microelectrode recordings) typically manifested higher DLEPs amplitudes while leads off target showed no DLEPs. Consistently with previous work, we found that DLEP in the STN had a better SNR compared to spontaneous beta band activity. Furthermore, we believe that DLEP can address a few pitfalls of STN beta based lead localization, such as its relative time-domain instability (as the underlying STN beta bursts are not strongly stereotyped) and the difficulty of defining a priori pathological beta amplitude thresholds, center frequency, and bandwidth. In conclusion, we show how this method can be implemented in a data-driven approach to help guide the placement and the initial programming of DBS segmented leads.

Disclosures: **E. Opri:** None. **F. Isbaine:** None. **N. Au Yong:** None. **J.T. Willie:** None. **R.E. Gross:** F. Consulting Fees (e.g., advisory boards); Medtronic, Inc.. **S. Miocinovic:** None.

Digital Abstract Session

P118. Therapeutic Strategies: Neuromodulation and Exercise

Program #/Poster #: P118.02

Topic: C.03. Parkinson's Disease

Title: TPS (transcranial pulse stimulation) significantly reduces Parkinson's disease symptoms

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Abstract: Background Shockwaves are used in medicine since 1980. First application was for extracorporeal kidney stone disintegration. Meanwhile, low intensity shock waves proofed to be efficient for the treatment of non-unions, tendon and muscular pain, wound healing, heart insufficiency, erectile dysfunction, aesthetics and finally also neurological indications like Alzheimer's disease. The working principle is the mechanical stimulation of biological processes called mechanotransduction resulting in increased cell metabolism, release of nitric oxide (eNO) and of numerous growth factors like VEGF, BMP, TGF-β, GABA, BDNF and GDNF. There is also an anti-inflammatory effect, stimulation of stem cells and of the innate immune system. **Materials and Methods** Transcranial Pulse Stimulation (TPS), former under the term TESWT (Transcranial Extracorporeal Shockwave Therapy) uses shockwave pulses for mechanical stimulation of the brain tissue. Due to the application through the scull, the intensity is significantly reduced: pressure amplitude down to 35% and energy intensity accordingly to approximately 15%. The average power density is less than 0.1W/cm^2. There is no heating effect, no microlesions. Four patients with Parkinson's disease have been treated with TPS in a feasibility study. The treatment was performed with the Neurolith (Storz Medical AG, CE marked) and consisted of 6 approximately 30 minutes sessions within two weeks. 6000 pulses/session and energy flux density of 0.2mJ/mm^2 at 5Hz were applied during each session. Additionally, a maintenance treatment (one session) was applied once per month. **Results** UPDRS, motoric part decreased by more than 50% within the treatment period. UPDRS, all 3 items was reduce by 44%. This positive outcome was maintained by monthly treatment over the time period of over 3 years. There are no negative side effects. Placebo controlled, randomized trial is ongoing. **Conclusions** Parkinson's disease treatment with TPS is safe and effective. Nevertheless, a regular maintenance treatment is necessary in order to maintain the positive outcome of the initial treatment. Further clinical trials with larger number of patients are necessary. The broad scope of shockwave stimulated biological effects in the brain seems to be an interesting and promising extension of the traditional treatment. The Neurolith is fitted with IR based documentation and navigation system using patient's MRI or CT brain images allowing further optimization of the Parkinson's disease treatment.

Disclosures: P. Novak: A. Employment/Salary (full or part-time):; Science&Technology Dir., Storz Medical AG. **H. Lohse-Busch:** A. Employment/Salary (full or part-time):; Rheintal Klinik, Bad Krotzingen. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Storz Medical AG, Tägerwilen.

Digital Abstract Session

P118. Therapeutic Strategies: Neuromodulation and Exercise

Program #/Poster #: P118.03

Topic: C.03. Parkinson's Disease

Support: Davis Phinney Foundation
TeCK Fund
Kent State University EHHS Mid-Career Seed Award

Title: Optimization of high-cadence cycling parameters to improve motor function in people with Parkinson's disease

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Abstract: Previous research has shown that dynamic high-cadence cycling can improve motor symptoms of Parkinson's disease (PD) such as tremor and bradykinesia. However, these improvements were not homogenous, with some participants experiencing greater benefits than others. To gain insight into how individual characteristics, bike settings and cycling performance affects functional changes, data from a previous study where individuals with PD rode the dynamic cycle with different resistance settings was used to build several preliminary predictive models. PURPOSE: To examine which variables contribute to greater improvement in symptom scores after one dynamic bike session. We hypothesized that participants who had higher body mass index (BMI), increased age, more severe symptoms, and higher PD medication dosages were less likely to contribute effort during the study. METHODS: Thirteen participants completed three sessions of dynamic biking under three different resistance settings. UPDRS-III was assessed before and after each session and bike settings (cadence, power) were recorded every second. Entropy of cadence was calculated using Matlab and data were analyzed using repeated measures ANOVA and multiple linear regression. RESULTS: There were no significant differences in cadence, entropy of cadence, average power, effort defined as >65% of positive power, or change in UPDRS-III score between the three bike settings. However, there was a high correlation (r^2=-0.62, p=0.01) between effort and BMI, which remained significant (r^2=-0.85, p<0.005) for participants who completed all three sessions within 10 days but not for those who took longer to complete the sessions (r^2=-0.59, p=0.1). There was also a significant difference in the change in UPDRS-III scores between upper and lower body regions (p<0.03), where high effort mitigated positive changes. These findings were used to create a preliminary model explaining variance in effort (R^2 adjusted=0.62, p=0.04). CONCLUSION: Participants characteristics such as pre-UPDRS, age, BMI, and daily medication play a role in the amount of exerted effort during bouts of dynamic cycling. This study suggests that effort during dynamic cycling is significantly affected by intrinsic participant characteristics. Future studies will test and improve on the preliminary model, and will examine techniques to increase effort during a cycling session.

Disclosures: **P. Gates:** None. **R. Melczak:** None. **A.L. Ridgel:** None.

Digital Abstract Session

P119. Molecular Markers, Imaging and Wearable Sensors: Advancing Diagnostics and Treatment

Program #/Poster #: P119.01

Topic: C.03. Parkinson's Disease

Support: R35 NS116883-01

Title: Platform for online neuropsychological testing (PONT)

Authors: ***W. SABAN**[1], R. IVRY[2];
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Abstract: A major challenge for neuropsychological research arises from the fact that we are dealing with a precious and limited resource: The patients. Not only is it frequently difficult to identify and recruit individuals appropriate for the question at hand, but their ability to participate in research projects can be limited. As a result, neuropsychological studies typically include small sample sizes (e.g., n=8) and it can take quite a bit of time to complete a single study (e.g., 2 years), let alone a package of studies that might make for a comprehensive story with appropriate controls. As a step towards addressing these issues, we have developed a platform for online neuropsychological testing (PONT). PONT is an online neuropsychological testing protocol that includes five primary steps: 1) Contacting support group leaders to help advertise the project; 2) Having interested individuals initiate contact with us given IRB contact rules; 3) Conducting interactive, remote neuropsychological assessments; 4) Automated administration of the experimental tasks; 5) Obtaining feedback and providing payment. The platform uses a Gorilla interface and is designed to handle a range of experimental protocols (e.g., surveys, reaction time tasks) that can be run on different devices (computer, phone, tablet). To date, we have developed PONT as a tool to accelerate our research program on subcortical contributions to action and cognition, reaching out to Parkinson and ataxia support groups. By contacting approximately 600 support group coordinators, we have recruited around 150 individuals with spinocerebellar ataxia (SCA) and 135 individuals with Parkinson's disease (PD) over the past nine months. We have also completed four experiments testing motor (sequence learning) and cognitive (arithmetic) abilities, with an average of 20 SCA, 20 PD, and 25 matched controls in each experiment. The results demonstrate the feasibility and efficiency of conducting online experiments on people with different neurological disorders. Participants like PONT because they can complete the experiments at home and at a time that fits into their personal schedule. We have encountered some limitations with this approach; for example, it is difficult to handle problems that come up when a participant finds a task too difficult or doesn't understand the instructions. Nonetheless, PONT has great potential to significantly increase the sample size in neuropsychological studies, make data collection much more efficient, and increase the impact

of neuropsychological research. And, of course, this type of research can thrive during the pandemic.

Disclosures: W. Saban: None. **R. Ivry:** None.

Digital Abstract Session

P119. Molecular Markers, Imaging and Wearable Sensors: Advancing Diagnostics and Treatment

Program #/Poster #: P119.02

Topic: C.03. Parkinson's Disease

Title: Deep Learning for Differentiating Parameter Configurations of Deep Brain Stimulation for Treating Parkinson's Disease Incorporating Conformal Wearable and Wireless Inertial Sensors as an Evolution for Network Centric Therapy

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Abstract: The application of deep learning offers an advanced opportunity for evolving Network Centric Therapy for the treatment of movement disorders, such as Parkinson's disease. Network Centric Therapy consists of the application of wearable and wireless inertial sensor systems with Internet connectivity to Cloud computing resources. In particular, deep learning is uniquely equipped to differentiate between various parameter configurations for deep brain stimulation. Utilizing a conformal wearable and wireless inertial sensor system with a profile on the order of a bandage, the tremor response to an assortment of deep brain stimulation parameter configuration settings is objectively quantified for a subject with Parkinson's disease. The acquired inertial signal data enables deep learning to achieve considerable classification accuracy with respect to the assortment of deep brain stimulation parameter configuration settings. The results implicate the utility of advanced concept synergy of conformal wearable and wireless inertial sensor systems, Cloud computing, and deep learning for attaining considerable classification accuracy that further realize the potential of Network Centric Therapy. These achievements further evolve the objective of optimizing deep brain stimulation parameter configurations in a real-time and closed-loop context.

Disclosures: R.C. LeMoyne: None. **T.J. Mastroianni:** None.

Digital Abstract Session

P119. Molecular Markers, Imaging and Wearable Sensors: Advancing Diagnostics and Treatment

Program #/Poster #: P119.03

Topic: C.03. Parkinson's Disease

Support: PI11/02109
PIM2010ERN-0033

Title: Functional correlates of response inhibition in impulse control disorders in Parkinson's disease

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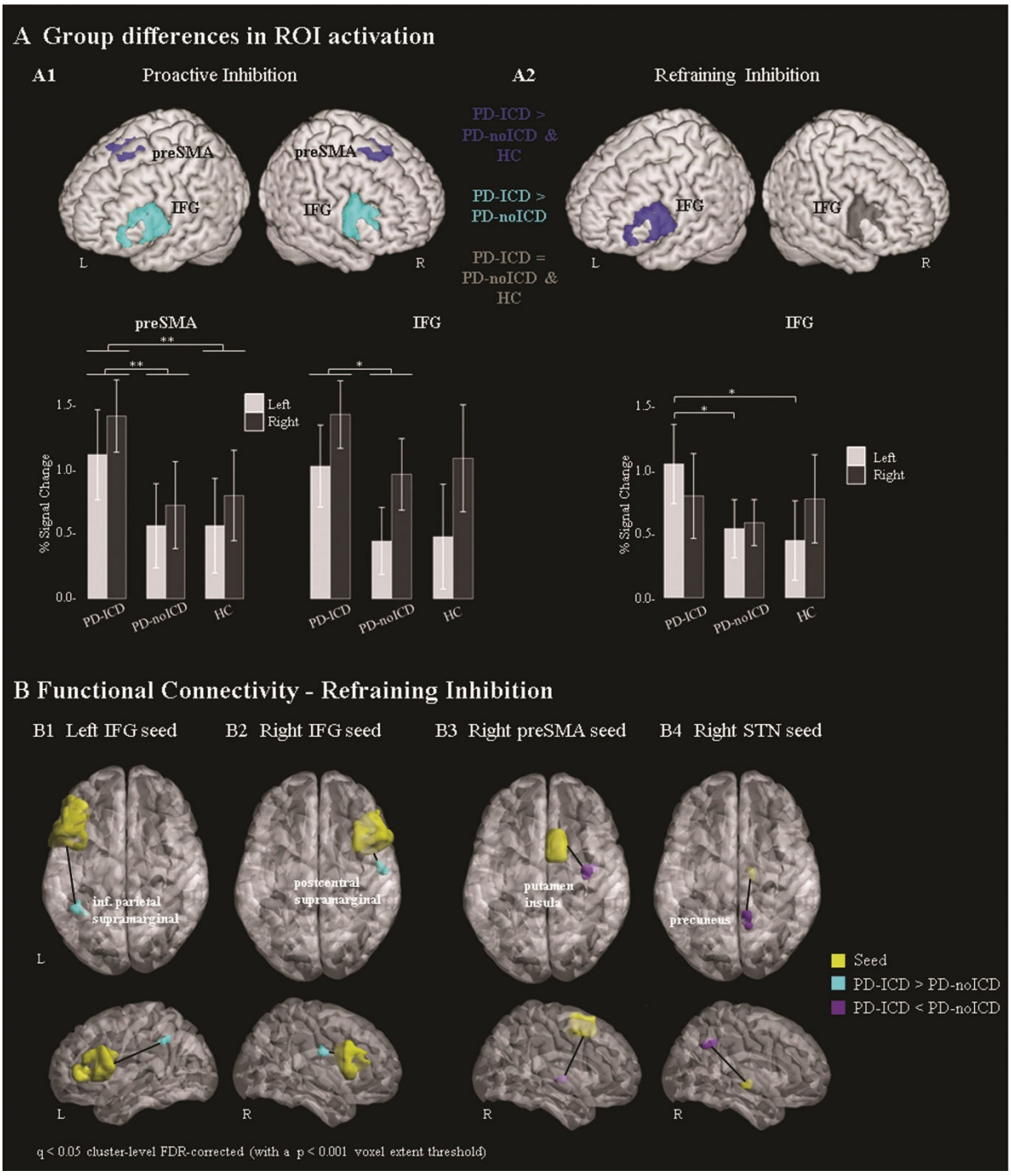
Abstract: Background: Impulse control disorder is prevalent in Parkinson's disease (PD) and has a strong negative impact on the quality of life of those affected. Although impulsivity has classically been associated with response inhibition deficits, previous evidence from PD patients with impulse control disorder has not revealed behavioral dysfunction in response inhibition. Here, our objective was to investigate the neural underpinnings of two aspects of inhibitory control: *proactive inhibition,* inhibition that has been prepared beforehand, and *restrained inhibition*, inhibition of an impulse to inhibit.**Methods**: Eighteen PD patients with impulse control disorders, 17 PD patients without the complication, and 15 healthy controls performed a version of the conditional Stop Signal Task during functional magnetic resonance imaging. Whole-brain contrasts, regions of interest, and whole-brain functional connectivity analyses were conducted.**Results**: PD patients with impulse control disorder exhibited bilateral hyperactivation of two areas of the stopping network - inferior frontal gyrus (IFG) and presupplementary motor area - while performing proactive inhibition. When engaged in restrained inhibition, they showed hyperactivation of the left IFG, an area linked to action monitoring. Additionally, restrained inhibition showed changes in the functional coactivation of inhibitory regions with left inferior parietal cortex and the right supramarginal gyrus.**Conclusions**: PD patients with impulse control disorder completed the inhibition task correctly, while showing altered engagement of inhibitory and attentional areas. During proactive inhibition they showed bilateral hyperactivation of two inhibitory regions, while during restrained inhibition they showed additional involvement of attentional areas responsible for alerting and orienting, suggesting PD patients with impulse control disorder deploy additional mechanisms to maintain an adequate inhibitory performance.



Disclosures: T. Esteban-Peñalba: None. **P.M. Paz-Alonso:** None. **I. Navalpotro-Gómez:** None. **M.C. Rodríguez-Oroz:** None.

Digital Abstract Session

P119. Molecular Markers, Imaging and Wearable Sensors: Advancing Diagnostics and Treatment

Program #/Poster #: P119.04

Topic: C.03. Parkinson's Disease

Title: AMP PD: Collaborating in the discovery of biomarkers to accelerate the development of therapies for Parkinson's disease

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Abstract: Aims: The Accelerating Medicines Partnership in Parkinson's Disease (AMP PD; https://amp-pd.org/) is a public-private partnership between the National Institutes of Health (NIH), the U.S. Food and Drug Administration (FDA), with industry (Celgene, GSK, Pfizer, Sanofi and Verily), non-profit (the Michael J. Fox Foundation; MJFF) organizations and managed by the Foundation of the National Institutes of Health (FNIH). AMP PD aims to identify and validate diagnostic, prognostic, and progression biomarkers to improve clinical trial design and identify pathways for therapeutic development.

Method: The AMP PD research plan encompasses a deep molecular characterization and longitudinal clinical profiling of PD patient data and biosamples with the goal of identifying and validating diagnostic, prognostic and/or disease progression biomarkers for Parkinson's disease (PD). The program includes open sharing of harmonized molecular (e.g. whole genome sequencing and transcriptomics) and clinical data to enable dissection of new targets, disease subtypes, and identification of predictive markers for disease progression and disease prognosis. AMP PD utilizes well-characterized cohorts with existing biosamples and clinical data that are collected under comparable protocols using common data elements. The core cohorts include the MJFF and NINDS BioFIND Study, the Harvard Biomarkers Study (HBS), the NINDS Parkinson's Disease Biomarkers Program (PDBP), and the MJFF Parkinson's Progression Marker Initiative (PPMI). Data from new cohorts, including the International Lewy Body Dementia Genetics Consortium Genome Sequencing in Lewy body dementia case-control cohort, the MJFF LRRK2 Cohort Consortium (LCC), and the Study of Isradipine as a Disease Modifying Agent in Subjects With Early Parkinson Disease, Phase 3 (STEADY-PD3), will be in the AMP PD platform by end of 2020.

Results: The AMP PD Knowledge Platform provides a controlled access mechanism to analyze data for PD biomarker discovery. The clinical diagnosis of participants in Release 1.0 includes 2,005 idiopathic PD patients, 963 healthy controls, 64 prodromal subjects, 62 clinically diagnosed PD subjects without evidence of dopamine deficit, and 705 participants of genetically enriched cohorts with GBA or LRRK2 variants in which 304 were affected (details to be made available on bioRxiv).

Conclusions: The AMP PD cloud-based infrastructure fosters the sharing of tools and data, supports nimble data analysis, decreases storage and compute costs, and facilitates collaboration with the over-arching goal of enabling the discovery of biomarkers for clinical trial design and therapeutic development.

Disclosures: C.R. Swanson-Fischer: None. **E. Appelmans:** None. **S. Biswas:** None. **M. Bookman:** None. **D. Alonso:** None. **C. Wonders:** None. **J.M. Ward:** None. **D. Babcock:** None. **A. Singleton:** None. **H. Iwaki:** None. **.. and AMP PD Consortium:** None.

Digital Abstract Session

P119. Molecular Markers, Imaging and Wearable Sensors: Advancing Diagnostics and Treatment

Program #/Poster #: P119.05

Topic: C.03. Parkinson's Disease

Title: Decreased Dorsal Attention Network grey matter volume associated with persistent working memory impairment in early stage Parkinson's Disease

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Abstract: Background: On average, 1 in 4 individuals diagnosed with Parkinson's disease (PD) will also have mild cognitive impairment (PD-MCI). PD-MCI is a risk factor to dementia. Identifying early biomarkers is essential to advancing treatments to prevent progression of dementia. Cross-sectional studies have identified decrease grey matter volume relative to impairment in various cognitive domains affected in PD. However, performance on cognitive tests can fluctuate overtime in PD patients, questioning whether cognitive impairment is a stable trait of the individual. Identifying grey matter differences in PD patients with persistent, non-fluctuating cognitive impairment may be a better approach to identifying biomarkers of progressive cognitive decline.
Methods: To further explore the relationship between grey matter volume and persistent attention/working memory (WM) impairment, we analyzed anatomical images and cognitive data from the Parkinson's Progressive Marker Initiative (PPMI; www.ppmi-info.org/). PPMI provides longitudinal data collected from early, drug naïve PD patients. This allows for the

characterization of individuals who show persistent or fluctuating cognitive changes over several years. WM was measured using the Letter Number Sequencing (LNS) test. Participants with persistent WM impairment (n= 10) were defined by an LNS standardized test score of 8 or less for 4 consecutive years since baseline imaging. Persistent unimpaired WM (n=10) was defined by an LNS standardized test score of 10 or more for 4 consecutive years since baseline imaging. The two groups were matched on sex, age, years of education, and disease duration. Grey matter volume was measured using voxel-based morphometry (VBM) through SPM12 and CAT12. Region of interest (ROI) analysis, focusing on the dorsal attention network (DAN; Schaeffer et al., 2018 100-parcel atlas), was conducted.
Results: Compared to PD patients with persistent unimpaired WM, those with persistent impaired WM had decreased grey matter density/volume in the bilateral visuomotor cortex (p=0.005), bilateral intraparietal sulcus (p=0.04), and right superior parietal lobule (p=0.021).
Significance: Our results demonstrated a loss of grey matter volume in key regions of the DAN related to persistent impairment in WM performance. The DAN is a critical cognitive network that supports encoding and maintenance of external information. This promising preliminary study warrants further investigation into brain regions that could potentially support targets for cognitive rehabilitation therapies.

Disclosures: R. Alsakaji: None. **S. Kletzel:** None. **S. Livengood:** None. **N. Chaudhry:** None. **K. Colletta:** None. **S. Jones:** None. **O. Ibik:** None.

Digital Abstract Session

P119. Molecular Markers, Imaging and Wearable Sensors: Advancing Diagnostics and Treatment

Program #/Poster #: P119.06

Topic: C.03. Parkinson's Disease

Support: NIH Fellowship TL1R001431
NIH Fellowship F31NS116938-01

Title: Discoidin Domain Receptor 1 alters brain angiogenesis and autophagy: a microRNA sequencing analysis

Authors: *A. J. FOWLER[1], J. AHN[2], F. PAGAN[3], Y. TORRES-YAGHI[3], C. MOUSSA[2]; [1]Georgetown Univ. Med. Ctr., Washington, DC; [2]Georgetown Univ., Washington, DC; [3]MedStar Georgetown Univ. Hosp., Washington, DC

Abstract: We used an adaptive-design clinical trial to evaluate the effects of DDR1 inhibition via Nilotinib on microRNAs in the cerebrospinal fluid Parkinson's patients. Nilotinib is a tyrosine kinase inhibitor that potently and preferentially inhibits (IC_{50} 1nM) Discoidin domain receptors (DDRs) and is FDA-approved for the treatment of chronic myelogenous leukemia (CML) as an inhibitor of c-Abelson (IC_{50} >20nM). We conducted a phase 2 study that enrolled 75 moderately severe Parkinson's patients, who were randomized into a single dose of 100mg,

200mg, 300mg and 400mg nilotinib versus placebo (n=15 per group). These participants were re-randomized into multiple dose of nilotinib, 150mg and 300mg, versus placebo for 12 months (n=25 per group). Following 3 months wash-out, participants were randomized into an open label treatment of nilotinib, 150mg and 300mg for 12 months (n=30-33 per group). Lumbar punctures were performed after a single dose (baseline) and multiple dose at 12 months and the CSF was analyzed using next generation miRNA sequencing. Approximately 2500 miRNA were detected in the CSF. A cross-sectional analysis between treatment groups after a single dose show no differences in miRNA expression. The multiple dose study showed that nilotinib was measured in the CSF in a dose-dependent manner up to (C_{max}) 1.8 nM and 3.4 nM in nilotinib, 150mg and 300mg, respectively, achieving a pharmacologically adequate CSF concentration that would inhibit DDRs. Analysis of longitudinal expression changes between 12-months and baseline detected approximately 140 significantly differentially expressed miRNAs. Gene ontology analysis of the miRNAs in the Nilotinib treated participants show strong regulation of inflammation, blood brain barrier (angiogenesis) and protein clearance via ubiquitination and autophagy. Several miRNA highly correlate with the sum of Unified Parkinson's Disease Rating Scales (UPDRS) II and III at 12 months and can predict clinical outcomes progression at 27 months. These data are in agreement with pre-clinical evidence and suggest that miRNA sequencing in human CSF provides an alternative approach to determine biomarkers based on regulation of pathways that are relevant to neuropathology. Furthermore, this study suggests that data from smaller Phase 2 studies can be used to predict biomarker and clinical outcomes in larger Phase 3 trials.

Disclosures: **A.J. Fowler:** None. **J. Ahn:** None. **F. Pagan:** F. Consulting Fees (e.g., advisory boards); KeiferRX LLC. **Y. Torres-Yaghi:** None. **C. Moussa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Georgetown University. F. Consulting Fees (e.g., advisory boards); eiferRX LLC.

Digital Abstract Session

P120. Cellular and Molecular Mechanisms in Neurodegenerative Disease

Program #/Poster #: P120.01

Topic: C.06. Neuromuscular Diseases

Support: ABRC Grant #ADHS18-198843

Title: Linking TBI secondary injuries to FTLD- and ALS-like neurodegeneration

Authors: *G. R. BJORKLUND, J. C. WONG, S. E. STABENFELDT;
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Abstract: In the United States, approximately 2.8 million traumatic brain injury (TBI) related emergencies were reported annually. These injuries resulted in approximately 2.5 million emergency room visits, 282,000 hospitalizations, and 56,000 deaths placing a tremendous burden on the public health system. A traumatic brain injury (TBI) initiates primary and secondary

damage that may lead to a significantly increased risk for the development of neurodegenerative disorders such as frontotemporal lobar dementia (FTLD) and motor neuron diseases like amyotrophic lateral sclerosis (ALS). Currently there are no standard treatments or cures for TBIs or associated neurodegenerative disorders other than symptomatic treatment and supportive therapies. In this study, our aim is to determine the impact of a unilateral TBI located within the motor cortex on neuronal degeneration spatially distant (forebrain and cervical spinal cord) from the site of injury. Adult male C57BL/6J mice with no genetic predisposition to either ALS or FTLD were subjected to a unilateral controlled cortical impact (CCI) in the primary motor cortex. Tissue sections from the forebrain, hindbrain, and cervical spinal cord were then examined for clinically relevant biomarkers of FTLD and ALS via immunohistochemistry. Specifically, neuronal cells were examined to determine the extent to which TAR DNA Binding Protein 43 (TDP-43) exhibited translocation from the nucleus accompanied by cytosolic accumulation with or without abnormal levels of ubiquitination. These cellular pathologies of TDP-43 are strongly associated with neurodegenerative diseases in humans that include both ALS and FTLD. Time points for the analysis include 7, 14, and 28 days post injury (DPI). Current preliminary results indicate the widespread loss of neuronal nuclear TDP-43 accompanied by an apparent cytosolic aggregation primarily on the ipsilateral side of the injury in both the forebrain and cervical spinal cord. This observation is accompanied by heavy patterns of ubiquitination and is most prominent at 7 DPI. By 28 DPI, a number of neurons appear to have recovered nuclear TDP-43 although cytosolic aggregations of TDP-43 were persistent as compared to uninjured control samples. As TDP-43 aggregation is considered a cornerstone of ALS and is present in the majority of FTLD cases, these early results have shown a possible link between TBIs and the development of FTLD- and ALS-like neurodegenerative diseases.

Disclosures: G.R. Bjorklund: None. **J.C. Wong:** None. **S.E. Stabenfeldt:** None.

Digital Abstract Session

P120. Cellular and Molecular Mechanisms in Neurodegenerative Disease

Program #/Poster #: P120.02

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NINDS NS085770
NINDS NS075075
NIA AG056258
NIDCD DC008552
NIA AG13854
NACC AG016976

Title: Basal forebrain cholinergic system resilience to tauopathy in corticobasal degeneration

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Abstract: The basal forebrain cholinergic neurons (BFCN) provide the only source of cortical cholinergic innervation and are involved in memory and attention. The BFCN display neurofibrillary tangle (NFT) pathology early in the pathologic cascade of AD. Cholinergic system degeneration in AD is associated with extensive loss of cortical cholinergic axons. There is also a correlation between the NFT in the BFCN and memory scores in aging, MCI and dementia. These features serve as the foundation for cholinergic therapy in AD. The aim of the present study was to investigate the vulnerability of the cholinergic basal forebrain to another tauopathy known as corticobasal degeneration (CBD), which is characterized by accumulation of 4-repeat tau. In a cohort of five autopsied participants, ranging in age at death between 54-71 years (80% male), we quantified BFCN, AT8 phosphorylated tau pathology, and number of cholinergic axons in five cortical regions. BFCN were quantified using hemotoxylin stained histological sections, hyperphosphorylated tau via AT8 immunoreactivity, and cholinergic axons were assessed using acetylcholinesterase histochemical reactivity. Density of cortical cholinergic axons in CBD patricipants was compared with published data from a cohort of cognitively normal and AD participants (Geula et al., Cerebral Cortex, 6:165-177, 1996). The percentage of cholinergic basal forebrain neurons with CBD-related tauopathy ranged from 30 to 71% and was qualitatively ranked as moderate in 4 and severe in 1. Numbers of BFCN remained relatively constant across particpants and showed no correlation with CBD-related AT8 immunoreactivity ($p>0.05$). In fact, we observed the second greatest number of BFCN in the participant with the highest AT8 immunoreactivity in BFCN. The density of cholinergic axons in CBD was not significantly different from normal controls ($p>0.05$). In contrast, the density of these axons was significantly reduced in AD participants when compared with controls ($p<0.005$) or CBD participants ($p<0.03$). The results of the present study demonstrate that different tauopathies have dramatically different effects on the cellular integrity of BCFN. The paired helical filaments of the AD-related tauopathy contain a mixture of 3-repeat and 4-repeat tau and have a profound degenerative effect on the BFCN. In contrast, the CBD-related tauopathy, comprised of 4-repeat tau species, has no such effect. These findings stand to provide new insights into the neurotoxicity of tauopathies and also suggest that, unlike AD, cholinergic therapy is not indicated in CBD.

Disclosures: S.R. Dunlop: None. **Y. Nishihira:** None. **I. Ayala:** A. Employment/Salary (full or part-time):; Northwestern University. **E. Bigio:** None. **T. Gefen:** None. **M.E. Flanagan:** None. **M. Mesulam:** None. **C. Geula:** None.

Digital Abstract Session

P120. Cellular and Molecular Mechanisms in Neurodegenerative Disease

Program #/Poster #: P120.03

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: UNAM-DGAPA-PAPIIT IA210620

Title: An in-silico study to identify new biomarkers and therapeutic targets for Progressive Supranuclear Palsy disease

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Abstract: *IIntroduction:* Progressive supranuclear palsy (PSP) is a sporadic, uncommon neurological disorder. It is considered the second most frequent form of atypical parkinsonism. It is characterized by the presence of neurofibrillary tangles in the brainstem, basal ganglia, and cerebellum. Currently, there has not be identified an apparent trigger of this disease, many MAPT mutations related to the inclusion of exon 10 and environmental factors have been reported as significant risk factors, but the molecular mechanisms involved in this disease remains elusive. Approximately 40% of the diagnosed patients are misled with related neurodegenerative diseases, and no specific biomarkers has been discovered. A wide range of bioinformatics tools, and available databases could be used to identify possible diagnostic biomarkers; these tools could help us study dysregulated cellular and molecular processes that could lead us to find new therapeutic targets and possible drug targets. *Methods:* In the present work we used online available transcriptomic profile data of confirmed PSP patients: a GEO2R microarray datasets from blood tissue (GSE34287, GSE6613, and GSE99039); and a microarray dataset from medial temporal lobe (MTL)(E-MEXP-2280). Raw data were processed and normalized in R system packages; differential expression analysis was performed with limma package, differentially expressed genes (DEGs) were selected, and further functional enrichment analysis and KEGGp analysis were conducted with topGO and limma packages. *Results*: In blood data sets enrichment analysis showed an increased dysregulation in gene ontologies and KEGGp related to mRNA processing, metabolic process, apoptosis, inflammatory response, and synaptic process. Besides, in MTL we observed an increased dysregulation expression of genes related to mRNA processing, metabolic process, ROS regulation and synaptic process. *Conclusions*: Our results correlate with the dysregulation of genes related to neuropathophysiological processes, like mitochondrial metabolic genes and apoptosis-related genes. Furthermore, we observe the marked dysregulation of genes involved in mRNA processing in both blood and brain tissue, this agrees with previous reports were RNA splicing and RNA binding proteins could be playing an essential physiopathological role in the progression of some tauopathies. We propose the possibility to evaluate some of these DEGs that changed in the same direction (Upregulated or Downregulated) in blood and brain tissue data sets as a possible diagnostic biomarkers and new therapeutic targets for PSP.

Disclosures: J. Rivera-Osorio: None. **V. Muley:** None. **M. Cardenas-Aguayo:** None.

Digital Abstract Session

P120. Cellular and Molecular Mechanisms in Neurodegenerative Disease

Program #/Poster #: P120.04

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: CIHR
FRQS
NINDS 1F32NS096803-01 and 1U01NS086659-01
NIH/NIA P30-AG13846
BU CTSI UL1 TR001430
BU ADC 5P30AG013846-23
Andlinger Foundation

Title: Clinicopathological associations for psychiatric phenotypes across brain regions in chronic traumatic encephalopathy

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Abstract: BACKGROUND
Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease with cognitive, behavioral, and psychiatric symptoms, with depression and suicidality present in a large proportion of pathologically validated CTE cases to date. Pathologically it is defined by a distinctive accumulation of hyperphophorylated tau (pTau) in the frontal and temporal cortex, medial temporal lobe and brainstem. However, the association between pathology in particular brain regions and psychiatric phenotypes in CTE is currently unknown.
METHODS AND RESULTS
Human postmortem brains from CTE cases and controls were obtained from the Veterans Affairs - Boston University - Concussion Legacy Foundation (VA-BU-CLF) Brain Bank (N=320). A team of trained neuropathologists examined each brain (blinded to identity) for gross and microscopic pathology, and provided semi-quantitative assessments. Brain regions of interest were isolated and sectioned as reported previously (Mez et al. 2015 Alzheimers Res Ther 7:62), including frontal, temporal, limbic, and brainstem structures. Sections were stained for pathological markers of interest (e.g. pTau, β-amyloid, α-synuclein, pTDP43, etc). Stained slides were scanned and traced digitally, with staining quantified using a Leica Aperio system. Preliminary analyses suggest that alterations in specific limbic, midbrain, and white matter structures may associate with specific depressive and suicidal phenotypes in CTE. In particular, we have found white matter loss and hippocampal and nigral pathology were increased in depressed cases. We have also found myelination-, gliosis-, and inflammation-associated changes for white matter in the anterior cingulate cortex of depressed CTE cases.
CONCLUSIONS
This ongoing investigation is the first to explore the association between neuropathology in particular brain regions and specific psychiatric phenotypes in CTE. Preliminary results suggest that increased neurodegenerative pathology, loss of white matter, and neuroinflammation could underlie depressive symptoms in CTE. We are currently adding additional cases and markers in order to validate and extend our initial analyses and findings.

Disclosures: I. Mahar: None. **S.E. Chancellor:** None. **B.R. Huber:** None. **T.D. Stein:** None. **V. Alvarez:** None. **A.C. McKee:** None.

Digital Abstract Session

P120. Cellular and Molecular Mechanisms in Neurodegenerative Disease

Program #/Poster #: P120.05

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: Grant DGAPA/PAPIIT number IA210620

Title: Analysis in silico to identify neurodegenerative pathways related to Niemann-Pick disease as potential new biomarkers

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Abstract: Analysis in silico to identify neurodegenerative pathways related to Niemann-Pickdisease as potential new biomarkersIntroduction:Niemann-Pickdisease(NP)is arare(1:5000births)neuroviscerallysosomallipidstoragedisorder,classifiedinto3differenttypesbase dongeneticorigin,typeA andB (NPAandNPB)arecausedbyadefect inthe geneencodingthe lysosomalsphingomyelin(SM)-degradingenzymeacidsphingomyelinase(ASM)andthe typeC (NPC)thatresults frommutationinthe genethatencodesendolysosomalcholesterol-transportproteinsNPC1orNPC2(Toledano-Zaragoza,A. y Ledesma,M. D. 2019).NP is characterizedbythe accumulationofunesterifiedcholesterolandotherlipidswithinthe cell, the functionoflysosomesusuallyis impairedaswell asthe autophagyflux andthe processofrecyclinganddegradationofcompounds.Tobetterunderstandthe molecularpathwaysaffectedin this disease, we perform a bioinformatic study.Methods:We used insilicomethodstoidentifythe principalgenesaffectedinthisdisease.The first approachwasananalysisofmicroarraydatafromthe publicarchiveArrayExpress,accessionnumber:E-MEX-2280.We studied the geneexpressionof11brainsamples,5controlsandother6with NPC, the sampleswere fromthe medialtemporallobe,patient´sagewere between64-83yearsandthe relationofsex is 4:2forNPC and3:3forcontrols(maletofemale)ineverygroup.The differentialexpressionanalysiswasperformedusinglimmapackage,geneswith significantadjustedpvalueandfoldchange> 1.5and< -1.5wereselected, we used topGOpackagetoobtainthe GO andlimmapackagefortheKEGG pathway.We analyzed284upregulatedDEGs and181downregulatedDEGs. Results: The analysisrevealedthatcellularcomponentswith mostdifferentiallyexpressedgenes(DEG)were dendrite,nuclearbodyandendoplasmicreticulum togetherwith the molecularfunctionmoreaffectedwasionbindingandthe cellularmatrixstructuralconstituent,inbiologicalprocessandKEGG pathwayshowedthatthe metabolismofglucose,aminoacidsandcholesterolwerederegulated. Conclusions:Our results were consistentwith changesinneuronhomeostasisreportedincells fromNPC patients,thatmight contributetothehigher level ofmortalityinthis disease.We proposethatsomeofthe DEGs thatwere

morerelatedtoNP diseaseaccordingtoourstudy, couldhavevalueaspotential biomarkers of this neurodegeneration.

Disclosures: T. Pradel-Bernal: None. **J. Rivera-Osorio:** None. **M. Cardenas-Aguayo:** None.

Digital Abstract Session

P120. Cellular and Molecular Mechanisms in Neurodegenerative Disease

Program #/Poster #: P120.06

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: Spatio-temporal mapping of 3R and 4R Tau isoforms during mouse brain development using BaseScope™ in situ hybridization technology

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Abstract: The microtubule-associated protein tau (MAPT) gene encodes a multifunctional protein that is predominantly expressed in neurons where it has a role in microtubule assembly and stability, axonal transport and neurite outgrowth. Neurodegenerative diseases such as Alzheimer's disease are a result of toxic tau aggregates often referred to as tauopathies. Alternative splicing of exon 10 that encodes for the microtubule-binding repeat sequence gives rise to two protein isoforms with either three or four microtubule-binding repeats in the 3R Tau (exon 10 exclusion) and 4R Tau (exon 10 inclusion) splice variant, respectively. The expression of both isoforms is developmentally regulated with each of these isoforms likely to have distinct physiological roles. While both isoforms are balanced in the normal adult brain, this balance or 3R:4R ratio can be skewed in diseased brains like Frontotemporal dementia (FTD). Also, the isoform content of Tau aggregates differs amongst various tauopathies. In order to elucidate the regulatory mechanisms and functional intricacies of Tau isoforms and develop effective Tau-based therapies, there is a critical need to map the spatial and temporal expression patterns of Tau isoforms at a single-cell resolution with the morphological context during brain development and disease progression. Here we report the development of an *in situ* hybridization (ISH) assay for the isoform-specific detection of 3R and 4R Tau mRNA isoforms and their profiles during mouse brain development. The BaseScope ISH technology was employed in this study to detect the alternative splicing of MAPT exon 10 at the single cell level. BaseScope probes were designed to target the exon 9 and exon 10 junction and the exon 9 and exon 11 junction to specifically detect 4R and 3R Tau, respectively. We used transfected cells as a model system to optimize the probe designs to achieve highly specific detection of these highly homologous isoform-specific junctions. These probes were then used to evaluate the neuroanatomic expression of 3R and 4R Tau variants during mouse brain development in postnatal ages P1, P10, P30, and P56 of adult C57Bl/6J mouse. Combining these probes with an antibody for NeuN in an ISH/IHC dual assay, our results revealed a clear opposing dynamic between these two isoforms over the course of development in the cortex and hippocampus regions. To conclude,

the unique BaseScope probe designs used in this study provide a powerful approach to study the spatial and temporal expression patterns of various Tau splice variants in various cell-based and animal models.

Disclosures: **J. Sheldon:** None. **E. Doolittle:** None. **H. Jarnagin:** None. **N. Zhaveri:** None. **B. Nyugen:** None. **W. Wei:** None. **A. Dikshit:** None. **M. Qutaish:** None. **B. Zhang:** None. **K.C. Bradley:** None. **X. Ma:** None.

Digital Abstract Session

P120. Cellular and Molecular Mechanisms in Neurodegenerative Disease

Program #/Poster #: P120.07

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH GRANT R01AG054008-01

Title: Comprehensive gene expression and transcript analysis in post-mortem autopsy-validated progressive supranuclear palsy

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Abstract: Progressive supranuclear palsy (PSP) is a neurodegenerative disease characterized by movement disorder, cognitive impairment, and other symptoms. Neuropathologically, PSP patients display neurofibrillary and gliofibrillary tangles prominently in the substantia nigra, subthalamic nucleus, globus pallidus, neocortex, and other brain regions. Prior studies have demonstrated that sporadic PSP is associated with the 17q21.31 *MAPT* H1 haplotype, but the impact of this complex genetic locus on gene expression remains unclear. Here we examine the transcriptomic changes in post-mortem brain tissue in a novel cohort of PSP cases compared to controls. Next-generation RNA sequencing (RNA-seq) of the transcriptome was performed using the frontal cortex from a cohort of sporadic PSP cases (n = 16) and controls (n = 24) using Illumina HiSeq. These data were aligned using STAR, with library sizes > 30M reads per sample. Transcript quantification was performed using RSEM. Differential gene expression was analyzed using R Bioconductor Package, and visualized with ggplot2. Differential splice junction analysis using Leafcutter, differential transcript usage, and differential transcript expression were performed. Differential expression analysis was repeated in an independent publicly available dataset of PSP cases (n = 84) and controls (n = 80) from two different brain regions. We obtained an average of 38.6 million surviving reads across 40 samples with an average read length of 134 bp. There were 1,576 differentially expressed genes between PSP and control samples ($p < 0.05$, unadjusted). We identified 34 genes differentially spliced between cases and controls, and 10 transcripts were differentially expressed with genome-wide significance ($p < 0.05$, adjusted). Expression analysis was repeated in a publicly available RNA-seq dataset from the cerebellum and temporal cortex. 11 genes were differentially expressed

across all cohorts and brain regions. This included *PANK2*, the gene that causes pantothenate kinase-associated neurodegeneration (PKAN), a progressive neurodegenerative disease affecting regions that are also highly vulnerable in PSP, including the globus pallidus and substantia nigra. Pending further validation, these findings highlight a set of genes that may help elucidate the mechanisms underlying disease risk in PSP and other neurodegenerative disorders.

Disclosures: H.A. Walsh: None. **R. Vialle:** None. **K. Farrell:** None. **M.A. Iida:** None. **A. Casella:** None. **T. Raj:** None. **J.F. Crary:** None.

Digital Abstract Session

P120. Cellular and Molecular Mechanisms in Neurodegenerative Disease

Program #/Poster #: P120.08

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIA/NIH Grant P30 AG013854
NIA/NIH Grant R01 NS085770
NIA/NIH Grant R01 NS075075
NIA/NIH Grant R01 AG056258
NIA/NIH Grant R01 AG062566
NIDCD/NIH Grant R01 DC008552
NACC New Investigator Award U01 AG016976

Title: Clinical features of limbic and subcortical Pick body distribution in behavioral variant Frontotemporal Dementia (bvFTD)

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Abstract: Introduction: Behavioral variant frontotemporal dementia (bvFTD) is an early-onset dementia syndrome characterized by progressive decline in executive functioning and comportment. Several pathologies may underlie the pattern of bilateral, frontotemporal cortical atrophy typically observed in this syndrome, including Pick's disease ("PiD"), a 3R tauopathy. This study examined the clinical features of bvFTD with PiD. Unbiased stereological counting permitted clinicopathologic correlations between Pick bodies and antemortem features in cortical, limbic, and subcortical brain regions.
Methods: Six right-handed participants with antemortem clinical diagnoses of bvFTD and autopsy confirmed PiD were selected from the Northwestern University Alzheimer's Disease Center brain bank. Twelve neuropsychiatric symptoms (NPS) were assessed longitudinally

(M=1.10 years between visits) with the Neuropsychiatric Inventory—Questionnaire (NPI-Q), an informant-based screener. AT-8 immunostaining identified Pick bodies, which were quantified via unbiased stereological analysis across bilateral middle frontal gyrus (MFG), inferior parietal lobule (IPL), superior temporal gyrus (STG), dentate gyrus (DG) and CA1 region of the hippocampal complex, and unilateral caudate, amygdala, and anterior cingulate cortex (ACC). **Results:** Average age of disease onset was 59.5 years (range, 46-70 years). Mean disease duration was 11.5 years (range, 8-14 years), with average age of death at 71 years (range, 58-82 years). Wilcoxon signed-rank test revealed a significant increase in total NPS from initial visit to final visit before death (p<0.05). Pick body density was highest in DG (M=~71652 per mm^3), followed by caudate (M=~58951per mm^3), then CA1 (M=~42994 per mm^3). Pearson correlations showed a significant, positive correlation between age of onset and Pick bodies in ACC (r = 0.94, p<0.01). Interestingly, densities of Pick bodies in caudate were positively associated with disease duration (r = 0.98, p<0.01); and, though this did not reach statistical significance, Pick body densities in the caudate were also positively related to total NPS observed at final visit prior to death (Kendall's τ = 0.77, p=0.08).
Conclusions: Later disease onset seems to be associated with greater Pick bodies in the ACC. The vulnerability of the caudate to pathology appears to be related to disease duration and the emergence of NPS over time. While frontotemporal cortical atrophy is typically associated with clinical symptoms in bvFTD, this study demonstrates that at the cellular level, Pick bodies in limbic and subcortical brain regions also have significant clinical relevance.

Disclosures: R.M. Keszycki: None. **A. Kawles:** None. **G. Minogue:** None. **S. Juthapan:** None. **M. Riley:** None. **C. Spencer:** None. **J. Lilek:** None. **I. Ayala:** None. **R. Shepard:** None. **K. Ajroud:** None. **A. Feldman:** None. **Q. Mao:** None. **M.E. Flanagan:** None. **S. Weintraub:** None. **M.M. Mesulam:** None. **E. Bigio:** None. **C. Geula:** None. **T. Gefen:** None.

Digital Abstract Session

P121. Motor Neuron Disease: Defects and Therapies For ALS

Program #/Poster #: P121.01

Topic: C.06. Neuromuscular Diseases

Support: Presbyterian Health Foundation (PHF)

Title: OKN-007 slows down disease progression in the G93A ALS mouse model

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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that leads to death of motor neurons (MNs) in the brain and the spinal cord. There are currently four FDA-approved treatments for ALS, however there is still no cure for the disease. In our study, we investigated if treatment with OKN-007, a compound with antioxidant and anti-inflammatory

properties, can modulate ALS onset and progression. We treated female Sod1 G93A mutant mice and wildtype mice with OKN-007 in drinking water starting at 60 days of age and the mice were sacrificed at day 145, before disease end stage. Disease progression was monitored and scored based on hindlimb tremor and progressing hindlimb dysfunction leading to paralysis. Although the treatment did not delay the disease onset (treated G93A mice became symptomatic on day 97 (median), while the untreated G93A mice reach disease onset on day 96 (Mantel-Cox test, $p = 0.19$)), the treatment significantly slowed disease progression. The treated G93A mice reached a score of 2 on day 127.5, while untreated G93A mice progressed to stage 2 on day 113.5 ($p = 0.015$). Strikingly, by 145 days, the treated group had not progressed beyond the score of 2, whereas G93A mice in the untreated group had reached as high as a score of 4 at this age. OKN-007 also prevented α-motor neuron loss. Untreated G93A mice had 10.7 ± 1.9 α-MNs per ventral horn, while the OKN-007-treated G93A mice had 19 ± 4.6 α-MNs, which is comparable to the α-MN counts in WT mice (untreated: 15.9 ± 2.2, treated: 20.2 ± 6.2). OKN-007 treatment did not affect astrocyte proliferation, but significantly reduced microglia proliferation and activation. Moreover, treatment with OKN-007 changed the tissue structure, as the apparent diffusion coefficient (ADC) measured using diffusion-weighted MRI (DWI) was lower in the lumbar spinal cord in OKN-007-treated G93A mice comparing to untreated G93A, indicating higher tissue cellularity, which is consistent with higher α-motor neuron count. Perfusion MRI revealed that the relative tissue blood flow in the spinal cord is lower in control G93A mice than in WT mice, suggesting a decreased vascularization, and the OKN-007 treatment significantly increased the blood flow in the G93A mice. Moreover, the MR spectroscopy data indicates that the level of myo-inositol decreases in control G93A mice, which is indicative of neuroinflammation, and OKN-007 treatment increases the level of this metabolite. Overall, our results suggest that, due to its neuroprotective and anti-inflammatory effect, OKN-007 may be a potential treatment for slowing down ALS progression.

Disclosures: K.M. Piekarz: None. **D. Saunders:** None. **R. Gulej:** None. **M. Zalles:** None. **R. Towner:** None. **H. Van Remmen:** None.

Digital Abstract Session

P121. Motor Neuron Disease: Defects and Therapies For ALS

Program #/Poster #: P121.02

Topic: C.06. Neuromuscular Diseases

Title: Reducing tau and DRP1 prevents mitochondrial fragmentation in Amyotrophic Lateral Sclerosis

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Abstract: Although several genes have been implicated in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS), mutations in these genes account only for a minority of cases, and the etiology of the disease remains to be elucidated. Therefore, understanding the exact pathogenic mechanisms leading to motor neuron loss is crucial for the development of new therapeutic approaches. Several studies demonstrate that alterations in mitochondrial morphology, dynamics and function, a common and early pathogenetic event in neurodegeneration, are also involved in ALS pathogenesis. Accordingly, deficits in bioenergetics and mitochondrial dysfunctions have been described in ALS patient samples as well as in animal and cellular models of the disease. Here, we hypothesized that the accumulation of dynamin-related protein 1 (DRP1), the GTPase involved in mitochondrial fission, and the microtubule binding protein tau may lead to mitochondrial fragmentation and dysfunction. Our findings using a large cohort of post-mortem samples demonstrated a significant decrease in both mitochondria number and length in ALS post-mortem motor cortex (mCTX) as measured by electron microscopy. Furthermore, there was a significant increase in DRP1 and phosphorylated-tau (pTau) in synaptoneuronsomes (SNs) derived from ALS mCTX and co-IP experiments demonstrated that DRP1 interacts with pTau in these samples. Lastly, the treatment of SH-SY5Y cells with ALS SNs, enriched in both pTau and DRP1, induced a significant decrease in mitochondrial length, volume, and networks. Importantly, knocking down DRP1 using a siRNA approach or reducing tau levels by the specific tau degrader, QC-01-175, significantly mitigated alterations in mitochondrial length, volume and networks induced by ALS SNs. Collectively, our results suggest that increases in DRP1 and pTau may cause mitochondria fragmentation in ALS, leading to an unfavorable energetic state. Importantly, targeting this molecular pathway reverses mitochondrial deficits in vitro. ***Acknowledgements.*** *T.P. was supported by an award from the Judith and Jean Pape Adams Charitable Foundation and Byrne Family Endowed Fellowship in ALS Research. S.J.H. was supported by the Alzheimer's Association/Rainwater Foundation Tau Pipeline Enabling program.*

Disclosures: T. Petrozziello: None. **E.A. Bordt:** None. **A. Amaral:** None. **A. Mills:** None. **S. Kim:** None. **S. Farhan:** None. **E. Sapp:** None. **S. Dujardin:** None. **P. Dooley:** None. **C. Henstridge:** None. **T. Gomez-Isla:** None. **M. Silva:** None. **M. Silva:** None. **B. Hyman:** None. **J. Berry:** None. **M. Cudkowicz:** None. **S. Bilbo:** None. **S. Haggarty:** None. **T. Spires-Jones:** None. **M. DiFiglia:** None. **K. Vakili:** None. **G. Sadri-Vakili:** None.

Digital Abstract Session

P121. Motor Neuron Disease: Defects and Therapies For ALS

Program #/Poster #: P121.03

Topic: C.06. Neuromuscular Diseases

Support: US NIH R01 NS644912-4
US NIH RC2 NS69476-01
Packard Center for ALS Research Grant P2ALS
Helping Link Foundation
Swiss National Science Foundation
Marie Curie Fellowship
Muscular Dystrophy Association

Title: Rapid reprogramming method differentiates CuATSM responders/nonresponders from ALS patient population.

Authors: *C. N. DENNYS[1], F. ROUSSEL[1], R. RODRIGO[1], X. ZHANG[1], A. SIERRA DELGADO[1], A. HARTLAUB[1], S. KOLB[2], J. BECKMAN[3], M. FRANCO[3], K. MEYER[1]; [1]Nationwide Children's Hosp., Columbus, OH; [2]Ohio State Univ., Columbus, OH; [3]Oregon State Univ., Corvallis, OR

Abstract: Patient diversity and unknown disease cause are major challenges for drug development and clinical trial design for neurodegenerative diseases. Moreover, the heterogeneity of the Amyotrophic Lateral Sclerosis (ALS) patient population is not reflected in currently available transgenic animal models. Hence, the direct translation of potential therapeutics tested in such models to the clinic has proven difficult. To address this, we utilize direct conversion technology to transform skin biopsies from ALS patients into neuronal progenitor cells (NPC). Using induced astrocytes (iAs) differentiated from these NPCs in co-culture with mouse embryonic motor neurons, we developed an *in vitro* ALS model to screen potential therapeutics. We have screened numerous compounds on multiple sporadic (sALS) and familial (fALS) iAs. Our data indicate a diverse patient response to different therapeutic agents, suggesting shared pathways of interest between patient subgroups. Here we utilize the compound CuATSM, in clinical trial for the treatment of ALS, to investigated its effects on iAstrocyte mediated motor neuron toxicity in both sALS and fALS (mtSOD1 and C9ORF72 patients) lines. We identified responders and nonresponders in co-culture assay for each patient subpopulations. We then performed a detailed analysis of the effects of CuATSM on known ALS disease markers (oxidative stress, mitochondrial dysfunction, elevation of stress response systems). We identified one shared parameter in mitochondrial activity present in all ALS patient CuATSM responders, that was nonexistent in nonresponders. Treatment of iAs with CuATSM restored this activity to healthy control levels. Together these findings suggest that patient iAstrocytes can be used to identify disease modifiers influencing therapeutic response and be sub-grouped based on targetable dysregulated pathways. Moreover, shared pathways amongst patient responders implicated CuATSM as a potential therapeutic strategy for additional disorders. Thus, these results indicate that enhanced understanding of cellular profiles could aid clinicians in determining the best treatment approach for patients in the future.

Disclosures: C.N. Dennys: None. **X. Zhang:** None. **R. Rodrigo:** None. **F. Roussel:** None. **A. Sierra Delgado:** None. **A. Hartlaub:** None. **S. Kolb:** None. **J. Beckman:** None. **M. Franco:**

None. **K. Meyer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amicus. F. Consulting Fees (e.g., advisory boards); Alcyone.

Digital Abstract Session

P122. Huntington's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P122.01

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant NS111202

Title: Dynamic analysis of huntingtin interactomes, normal and diseased, in response to proteotoxic stress

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Abstract: Huntingtin protein (Htt) is a large protein (~350KDa) whose exact function is unknown. It is generally recognized that Htt is a scaffold protein that forms protein complexes under various physiological conditions. In Huntington's disease (HD), mutant huntingtin (muHtt) is implicated in perturbing protein-protein interactions (PPI) in association with normal Htt. Neurons are selectively venerable to lowered stressor-thresholds in neurodegenerative diseases. We and others have shown that HD cells are more susceptible to cellular stresses. To identify the dynamic interacting partners, for both normal and mutant Htt, in response to cellular stresses, we established an ascorbate peroxidase (APEX2)-based proximity labeling platform to spatiotemporally label Htt-interacting proteins in live cells in order to map the dynamic Htt/muHtt interactome in response to proteotoxic stress by unbiased quantitative proteomic and bioinformatic analyses. Using the human neuroblastoma SH-SY5Y cells line, we first generated stable cell lines expressing Flag-APEX2-Htt23Q, Flag-APEX2-Htt145Q and NES (nucleus export sequence)-APEX2. NES-APEX2 randomly labels cytosolic proteins that are in close proximity with APEX2, thus serves as the reference probe for background proteins identified in Htt samples. The clones were verified for Htt and Flag epitope expression by Western blot and immunostaining using specific antibodies against Htt and Flag, respectively. *In vivo* biotinylation was performed to demonstrate that APEX2-Htt can successfully label Htt-interacting proteins. A known Htt-interacting protein p62 was used for assay validation. Biotinylated proteins were pulled down by streptavidin beads, trypsin-digested and concentrated using ZiptipC18. The resulting samples were subjected to further characterization and analysis using LC-MS/MS. We have identified ~500 proteins from each group without stress treatment. We used the spectral count, defined as the total number of spectra identified for a protein, to semi-quantitively measure protein abundance in different samples. For common proteins present in all three groups, Foldchange was calculated as the ratio of total spectral counts of proteins identified from Flag-APEX2-Htt23Q and Flag-APEX2-Htt145Q to those of NES-APEX2. Proteins with a foldchange above 2 and unique proteins identified in Flag-APEX2-Htt23Q and Flag-APEX2-

Htt145Q samples were selected for further analysis. We will build dynamic interaction networks of Htt/muHtt under various stress conditions which could be the key to understand the molecular pathogenesis of HD.

Disclosures: J. Wei: None. **H. Xu:** None. **M. Lu:** None. **E. Capobianco:** None.

Digital Abstract Session

P122. Huntington's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P122.02

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Profiling of an orally active small molecule Huntingtin lowering splicing modulator demonstrates systemic mHTT lowering in BACHD mice

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Abstract: Several modalities aimed at lowering levels of pathogenic mutant HTT (mHTT) are currently in development for the treatment of Huntington's disease. Positive HTT lowering results have been recently shown in clinical trials with the use of antisense oligonucleotides (ASO) targeting HTT mRNA.
Recently, small molecules have been identified targeting HTT mRNA splicing, that result in nonsense mediated decay (NMD) decreasing HTT mRNA and thus lowering HTT protein production. The use of orally bioavailable, brain penetrant small molecules for therapeutic intervention represents a potential advantage over modalities currently in development.
Here we describe the *in vitro* and *in vivo* profile of one such splicing modulator, Compound X. Compound potency was assessed and mutant HTT protein lowering, splicing modulation, mRNA lowering and window over cell toxicity demonstrated. Moreover, Compound X exhibited acceptable ADME properties making it potentially suitable for *in vivo* evaluation. Indeed, *in vivo* PK studies in mice confirmed that Compound X is orally bioavailable and highly brain penetrant, reaching levels in brain tissue that would be expected to generate splicing modulation and HTT lowering.
Sub-chronic oral dosing of Compound X was carried out in the BACHD mouse model, with once daily dosing given for 21 days. After 7-, 14-, and 21-day dosing, tissues and plasma samples were collected at 8 hrs post-dose for assessment of compound concentration, splicing modulation of HTT mRNA and mHTT protein levels. Compound X showed a significant time- and dose-dependent HTT protein reduction in brain, muscle, and liver. In this study, mHTT lowering was

observed to be greater in peripheral tissues compared to brain tissue at all doses and time points. Additionally, detectable level of mHTT protein in plasma was also significantly reduced upon compound treatment.
These data demonstrate that orally dosed splicing modulators targeting HTT mRNA is a feasible therapeutic approach to lowering mHTT protein systemically *in vivo*.

Disclosures: **M.E. Herva Moyano:** None. **D. Magnani:** None. **K. Malagu:** None. **S. Clifton:** None. **H. Atton:** None. **H. Patel:** None. **A. Barnard:** None. **D. Todd:** None. **M. Stebbeds:** None. **M. Chambers:** None. **P. Mitchell:** None. **I. Mancini:** None. **A. Kyrizaki:** None. **M. Visser:** None. **A. Bresciani:** None. **M. Daldin:** None. **N. Gennari:** None. **V. Fodale:** None. **G. McAllister:** None. **L. Liu:** None. **V. Khetarpal:** None. **D. Macdonald:** None. **C. Dominguez:** None. **I. Muñoz-Sanjuan:** None.

Digital Abstract Session

P122. Huntington's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P122.03

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NINDS R24 201603716
NINDS 1R01NS102486
NIH NIGMS Predoctoral Fellowship T32GM099608
CIRM Bridges Trainee Funding
A Stewart's and Dake Family Gift
HELP4HD International
WeHaveAFace.org

Title: Use of novel cell-based delivery platform for the evaluation of dxCas9 for allele-specific silencing in Huntington's disease

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Abstract: Huntington's disease (HD) is a rare, autosomal dominant neurodegenerative disorder caused by a trinucleotide expansion in exon 1 of the Huntingtin gene (HTT), which leads to neuronal dysfunction and cell death. Healthy huntingtin is implicated in axonal trafficking and trophic factor regulation, making targeted allele-specific reduction important. This study aimed to target single nucleotide polymorphisms (SNPs) in HD patient-derived cells to reduce expression of mutant HTT using dxCas9 fused to repressive domains. This study also evaluated a

novel delivery modality harnessing mesenchymal stem cells (MSCs).Heterozygous SNPs near regulatory regions of the Huntingtin promoter were confirmed in HD patient cells, allowing for the design of allele-specific gRNAs. Our novel vector (dxiCas9) containing repressive effector domains allowed for broader PAM site coverage and higher binding specificity compared to spdCas9. dxiCas9 and gRNA plasmids were introduced to an HD patient fibroblast line and knockdown of total HTT transcript levels was assessed to screen for gRNA selection. Knockdown was assessed at multiple loci in the HTT gene and significant downregulation was achieved using several of our gRNAs that were designed to be allele specific. Targeting of rs762855 or rs3856973 resulted in significant downregulation compared to a non-treated control. Interestingly, synergy between multiplexed gRNAs was not observed. A gRNA targeting rs762855 also showed significant downregulation in primary cortical neurons derived from YAC128 mice.Delivery of large proteins into the CNS continues to be a barrier to success when using these gene editing tools. By harnessing the packaging mechanism of a lentivirus, cells can be engineered to secrete large proteins into extracellular vesicles to deliver the protein of interest into recipient cells. MSCs have a strong clinical safety profile and are easily engineered, making them a top candidate for cell-based therapies. This technology has been termed Cell based Nanoengineered X Therapies (Cell NeXT). MSCs were transduced with packaging components and GFP fused to a packaging signal. These MSCs successfully produced NeXT particles containing GFP that were able to infect recipient cells. HEK293T production of NeXT particles containing dxiCas9-KRAB has also shown successful recipient cell infection as a proof of principle. Our current efforts are to assess knockdown of HTT using the Cell NeXT delivery platform as well as assessing the allele-specificity of our gRNAs.These studies support the potential of targeted allele-specific strategies paired with the Cell NeXT non-invasive delivery platforms for CNS disorders.

Disclosures: **J.J. Waldo:** None. **P. Deng:** None. **J.A.N.M. Halmai:** None. **I. Villegas:** None. **F.K. Buchanan:** None. **J. Carter:** None. **C.E. Gonzalez:** None. **D.L. Cameron:** None. **D. Segal:** None. **J. Nolta:** None. **K. Fink:** None.

Digital Abstract Session

P122. Huntington's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P122.04

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NeuroCure (DFG)
German Research Foundation (DFG)

Title: Assessment of a selective histone deacetylase 1 and 3 inhibitor as a therapeutic candidate in Huntington’s disease mice

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Abstract: Progressive transcriptional dysregulation in brain is an early and central feature of Huntington's disease (HD) pathogenesis. Using cell and mouse models, we and others have previously demonstrated genome-wide changes in transcription, DNA methylation and histone modification patterns that may underlie transcriptional dysregulation in HD. Thus, targeting epigenetic mechanisms for rescue of aberrant gene expression is a promising therapeutic strategy for HD. Here, we investigate potential therapeutic effects of a histone deacetylase inhibitor (HDACi) targeting selectively HDAC1 and HDAC3 in a mouse model of HD. Using transcriptional profiling and behavioral testing, we found that selective inhibition of HDAC1 and HDAC3 repaired the expression of a number of genes and about half of the gene sets that were dysregulated by mutant Huntingtin expression in the striatum and improved motor skill learning deficit in the R6/1 mice. We also found, by volumetric MRI, a widespread brain atrophy in the R6/1 mice at the symptomatic disease stage, on which the selective HDACi treatment showed a modest effect. Collectively, our combined work presents new evidence for specific HDAC 1 and 3 inhibition as a therapeutic strategy for alleviating the phenotypic and molecular features of HD.

Disclosures: K. Hecklau: None. **S. Mueller:** None. **S. Koch:** None. **M. Mehkary:** None. **B. Kilic:** None. **C. Harms:** None. **P. Boehm-Sturm:** None. **F. Yildirim:** None.

Digital Abstract Session

P122. Huntington's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P122.05

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Brain Canada Foundation (RES0030547)
Faculty of Medicine & Dentistry Dean's Doctoral Student Award

Title: Microglia response to polarizing agents in Huntington's disease and modulatory effects of ganglioside GM1

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Abstract: Huntington disease (HD) is a neurodegenerative disease caused by mutation of the *huntingtin* (*HTT*) gene, which results in the production of a protein, mutant HTT, that misfolds and aggregates within brain cells. In addition to the loss of neurons, growing evidence suggests that non-neuronal cells are also affected in HD. Microglia are the immune cells of the brain, that not solely fight pathogens but also play homeostatic roles in brain health. In HD, microglia activation was proposed to occur from early disease stages. However, a broader characterization of HD microglia phenotype is still missing, and the overall extent of microglia dysfunction in the disease onset and progression remains controversial. The aim of our study was to investigate the

response of Q140 primary microglia and human HD macrophages to polarizing agents, including LPS and necrotic cells (M1-like polarization) and IL4/IL13 (M2-like polarization). We also tested whether GM1 - a ganglioside with neuroprotective activity in HD models - affects polarization of HD microglia and macrophages. Measurement of RNA and protein levels for pro-inflammatory cytokines showed that Q140 primary microglia respond to pro-inflammatory stimuli in a similar manner as wild-type (WT) cells. In human HD macrophages, stimulation with IFNγ and LPS resulted in higher transcription of pro-inflammatory genes, but the levels of the corresponding proteins were not significantly different from control cells. Polarization toward M2 state resulted in the transcription of anti-inflammatory cytokines to a similar extent in cells (microglia and macrophages) of both genotypes. In Q140 microglia, however, transcription of the M2 marker Ym-1 was higher than in WT cells. Finally, Q140 and WT microglia showed a similar inflammatory profile upon uptake of necrotic cells. Treatment with GM1 decreased inflammatory responses in HD microglia and macrophages. In the latter, it also increased transcription of M2 markers upon polarization with IL-4. Overall, our studies suggest that the HD mutation does not significantly affect the magnitude of microglia and macrophage response to polarizing factors. This is in contrast with previous studies that have shown increased inflammatory response to LPS. We speculate that the reasons for this discrepancy may rely on differences in experimental conditions and/or initial microglia state. Our data show that GM1 has anti-inflammatory activity and promotes the transcription of anti-inflammatory molecules, at least in HD macrophages. These beneficial effects exerted by GM1 directly on HD microglia might contribute to the neuroprotective activity of the ganglioside in HD models.

Disclosures: **N. Steinberg:** None. **D. Galleguillos:** None. **A. Zaidi:** None. **S. Sipione:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; University of Alberta.

Digital Abstract Session

P122. Huntington's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P122.06

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Brain Canada Foundation (RES0030547)
Alberta Prion Research Institute (RES0039430)
Glyconet Grant (RES0050801)
SynAD (RES0049270)
Canadian Institutes of Health Research Frederick Banting and Charles Best Canada Graduate Scholarship
SynAD ADRD Trainee Grant
University of Alberta FoMD Graduate Recruitment Scholarship

Title: An unbiased proteomic analysis to identify neuroprotective pathways elicited by ganglioside GM1 in Huntington's disease

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Abstract: Gangliosides are glycosphingolipids that participate in cell signaling, cell-cell interactions and modulate immune cell signaling. In Huntington's disease (HD), a protein misfolding neurodegenerative disease caused by the expansion of an N-terminal polyglutamine stretch in the protein huntingtin, levels of ganglioside GM1 are reduced. In mouse models of HD, administration of GM1 is a disease-modifying treatment: it significantly reduces levels of both soluble and aggregated mutant huntingtin (mHTT) in the striatum and cortex of HD mice, and corrects all disease symptoms. We have shown that mHTT-lowering effects of GM1 are independent of *Htt* gene transcription. To uncover neuroprotective pathways elicited by GM1 treatment, we performed an unbiased label-free proteomics screen. Wild-type (WT) or knock-in Q140 HD mice were administered artificial CSF (vehicle) or GM1 for 42 days, by intraventricular infusion. Proteomics analysis of lysates from striata and cortices was performed by Gel-LC-MS/MS. We then performed pairwise comparisons of WT mice treated with CSF (n=9) or GM1 (n=8) and HD mice treated with CSF (n=7) or GM1 (n=11) to identify significant changes in protein levels in the striatum and cortex. In line with the disease-modifying effects observed in GM1-treated mice, a significant proportion of the proteins affected by GM1 was represented by known HTT interactors and/or proteins previously found to be affected in HD. In addition, Gene Ontology cell compartment analysis revealed the extracellular vesicle (EV) compartment was significantly enriched in our datasets, regardless of genotype and tissue. EVs are membrane-enclosed nanoparticles that play roles in cell-cell communication and proteostasis. Using a combination of imaging flow cytometry, fluorometry and immunoblotting, we confirmed that GM1 treatment significantly increases EV secretion from both WT and mHTT-expressing cells. Importantly, GM1 increases the secretion of mHTT via EVs, potentially explaining the mHTT-clearing effect of GM1 treatment *in vivo* and *in vitro*. Altogether, our data suggest that GM1-mediated potentiation of EV secretion may underlie the mHTT-lowering effects we observe in HD mice. Furthermore, our data provide insights into a novel role of ganglioside GM1 in proteostasis and EV secretion with important potential implications for other misfolded protein diseases.

Disclosures: J. Monyror: None. **V. Kadam:** None. **M. Alpaugh:** None. **L.C. Morales:** None. **D. Galleguillos:** None. **M. Stenlund:** None. **J. Huang:** None. **D. Kramer:** None. **A. Maguire:** None. **E. Posse de Chaves:** None. **R. Fahlman:** None. **S. Sipione:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Alberta.

Digital Abstract Session

P122. Huntington's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P122.07

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: John G. Kulhavi Professorship in Neuroscience at CMU
The Field Neurosciences Institute
Central Michigan University Undergraduate Research and Creative Endeavors Grant

Title: Use of Ovine-sourced GM1 ganglioside in R6/2 Huntington's mouse model to Prolong the Lifespan

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Abstract: Huntington's disease (HD) is a genetically inherited progressive neurodegenerative disease caused by a mutation in the trinucleotide repeats of cytosine, adenine, and guanine (CAG) of the *huntingtin* (*HTT*) gene. The mutation in the *HTT* gene increases the number of CAG repeats, leading to increased production of the huntingtin protein. Healthy individuals typically exhibit 16 to 20 CAG repeats on the *HTT* gene, while those with HD exhibit 35 or greater CAG repeats, with an increase in CAG repeats corresponding to increased severity in HD symptoms, including motor and cognitive declines, personality changes, involuntary movements, weight loss, muscle loss, and a shorter lifespan. The R6/2 mouse model of HD has a mutated gene that exhibits 160 CAG repeats, serving as a severe model of patients with HD. R6/2 mice display similar symptoms to HD patients, such as severe weight loss, muscle loss, deficits in motor function, and reduced lifespan. Individuals with HD show a decrease in GM1 ganglioside levels, severely impacting neurons, and ultimately leading to a shorter lifespan in the affected individual. Treatment of mouse models of HD with bovine-sourced GM1 gangliosides leads to a decrease in disease symptoms. Ovine-sourced GM1 gangliosides are one-fourth the cost and thus may serve as a cost-efficient alternative to bovine-sourced GM1 gangliosides. The aim of this project was to determine the effectiveness of ovine-sourced GM1 ganglioside treatment on R6/2 mouse models by studying survivability and quality of life of the mouse model. The results of our study indicated that HD mice treated with ovine-sourced GM1 ganglioside showed a significant increase in weight over their lifespan, compared to bovine-sourced GM1 ganglioside treated HD mice and the vehicle-treated HD group. Ovine-sourced GM1-treated HD mice had a prolonged lifespan of ten days compared to HD mice treated with bovine-sourced GM1 ganglioside and vehicle-treated HD mice.

Disclosures: **N.B. Fettinger:** None. **S. Koneru:** None. **M.R. Resk:** None. **P. Otero:** None. **J. Rossignol:** None. **G.L. Dunbar:** None.

Digital Abstract Session

P122. Huntington's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P122.08

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: John G. Kulhavi Professorship in Neuroscience at CMU
The Field Neurosciences Institute

Title: Comparison of Ovine and Bovine sources of GM1 Ganglioside for treating behavioral deficits in the R6/2 mouse model of Huntington's disease

Authors: *S. KONERU[1,2,3], M. R. RESK[1,2,3], K. A. OLSON[1,2,3], N. B. FETTINGER[1,2,3], O. KHACHERIAN[1,2,3], R. M. BRISTOW[1,2,3], C. E. MALKOWSKI[1,2,3,4], P. OTERO SEQUEIROS[1,2,3,4], K. M. BELGYA[1,2,3], M. I. SANDSTROM[1,2,5], J. ROSSIGNOL[1,2,3,4], G. L. DUNBAR[1,2,3,5,6];
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Abstract: Huntington's disease (HD) is a genetic autosomal dominant neurodegenerative disease caused by mutations in the *HTT* gene containing a long polyglutamine (CAG) stretch. This leads to excessive production and accumulation of mutant huntingtin protein (mHTT) in the brain. This excessive mHTT protein accumulation causes motor, psychiatric, cognitive impairments and, ultimately, neuronal death. Currently, no effective treatment has been approved for HD. However, a promising line of research in therapeutics for HD, involves the use of GM1 ganglioside, as levels of GM1 are reduced in HD patients, which is thought to contribute to the disease progression. GM1 ganglioside is a sialic acid-containing glycosphingolipid that is found abundantly in the outer leaflet of the neuronal membrane in the brain. The initial source for GM1 came from bovine brains, but new sources, including synthetic as well as those obtained from the bovine brain, have been developed subsequently. Treatments with synthetic- and bovine- sourced gangliosides have been shown to reduce motor dysfunction in HD rodent models. However, the efficacy of treatment using ovine-sourced GM1, which is much more economical, has yet to be tested. The goal of this study was to compare the efficacy of ovine-sourced GM1 with that obtained from bovine sources on the motor and cognitive dysfunctions in the R6/2 mouse model of HD. In the current study, we used intraventricular osmotic pumps to deliver the different-sourced GM1 gangliosides for four weeks in five-weeks-old R6/2 mice. Rotarod-, open field-, catwalk-, Barnes maze- and novel-object-recognition- tasks were used to measure motor and cognitive abilities in the treated and non-treated mice. Results indicated that ovine-sourced GM1 gangliosides significantly reduced behavioral deficits in R6/2 mice on the rotarod-, open field-, catwalk-, but not on Barnes maze-, and novel-object-recognition- tasks, compared to vehicle-treated and bovine-sourced-GM1-treated R6/2mice.

Disclosures: **S. Koneru:** None. **M.R. Resk:** None. **K.A. Olson:** None. **N.B. Fettinger:** None. **O. Khacherian:** None. **R.M. Bristow:** None. **P. Otero Sequeiros:** None. **C.E. Malkowski:** None. **K.M. Belgya:** None. **M.I. Sandstrom:** None. **J. Rossignol:** None. **G.L. Dunbar:** None.

Digital Abstract Session

P122. Huntington's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P122.09

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: John G. Kulhavi Professorship
Field Neurosciences Institute

Title: Effects of Ovine and Bovine sources of GM1 Ganglioside for treating Circadian rhythm deficits in the YAC128 and R6/2 mouse models of Huntington's disease

Authors: *O. KHACHERIAN[1], G. L. DUNBAR[2];
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Abstract: O.KACHERIAN[1,2] S. KONERU[1,2], M. RESK[1,2], K. OLSON[1,2], B. McDonald, J. ROSSIGNOL[1,2,3], G. L. DUNBAR[1,2,4,5]
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Abstract:
Huntington's disease (HD) is neurodegenerative disease with progressive motor and cognitive impairment caused by a mutation in the HTT gene. The mutation causes abnormal elongation of the polyglutamine (CAG) repeats. This leads to over-production and accumulation of huntingtin protein (mHTT) in the brain. Furthermore, the excessive mHTT protein accumulation causes various impairments in sleep, activity levels, and circadian rhythms, all of which would further impact behavior and quality of life. Currently, no treatment has been approved to help with sleep and activity problems in HD. GM1 ganglioside has significant promise as a potential therapeutic for HD, given that its levels are reduced in HD patients. Treatments with synthetic- and bovine-sourced gangliosides have been conducted with these types of GM1 gangliosides, and no studies have been conducted with the less expensive, ovine-sourced GM1 on the reduction of motor deficits, activity levels, and circadian rhythms in rodent models of HD. The goal of this study was to examine the effect of both ovine-sourced and bovine-sourced GM1 on activity and circadian rhythms in R6/2 and YAC128 mice models of HD. We used intraventricular osmotic pumps for the delivery both sources of GM1 gangliosides for six weeks in 15-month-old YAC128 mice and four weeks treatment for five-week-old R6/2 mice. We monitored the GM1- and vehicle-treated activity levels and circadian rhythms of the mice in an open-field apparatus equipped with infrared light beams. Although no treatments affected the YAC128 mice, the R6/2

mice who were treated with ovine-sourced GM1 tended to be less hypoactive and maintain a more normal circadian rhythmicity than their vehicle-treated and bovine-sourced-GM1-treated counterparts.

Disclosures: O. Khacherian: None. **G.L. Dunbar:** None.

Digital Abstract Session

P122. Huntington's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P122.10

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: The John G. Kulhavi Professorship in Neuroscience at CMU
The Field Neurosciences Institute

Title: Effects of ovine- and bovine-sourced GM1 ganglioside treatments on proliferation and differentiation of neuronal progenitor cells from the Yac 128 mouse model of Huntington's disease

Authors: *R. BOSE[1,2,3], O. KACHERIAN[1,2], S. BANERJEE[1,2,3], J. ROSSIGNOL[1,3,4], G. DUNBAR[1,2,3,5];
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Abstract: Huntington's Disease (HD) is a devastating neurodegenerative disorder that clinically manifests as motor dysfunction, cognitive impairment, and psychiatric symptoms. The disease is caused by an expansion of cytosine-adenine-guanine trinucleotide repeats in exon 1 of the huntingtin (HTT) gene. The mutation of HTT forms abnormal aggregates, which affect cellular proteostasis, axonal transport, transcription, translation, as well as mitochondrial and synaptic functions. Medium spiny neurons of the striatum are selectively vulnerable to the HTT gene mutation. Gangliosides are cell membrane components that are important for the survival and functioning of cells. GM1 ganglioside expression is decreased in HD cells and exogenous treatment of GM1 can reduce cell death. However, the effects of GM1 exposure in brain development, such as neural progenitor cell proliferation and differentiation, remains to be elucidated. Here we show that ovine- or bovine-sourced GM1 exposure can modulate proliferation and differentiation in neural progenitor cells (NPCs). Primary cultures of NPCs were obtained at embryonic day 18 from individual embryonic cortices dissected from timed-pregnant YAC 128 mice, which carry the HD transgene. Based on the genetic characteristics, cells were divided into wild and HD groups, which were exposed to ovine- or bovine-sourced GM1 (100, 200, and 400 ng/ml) for 8 days. We found upregulation of differentiating markers, Tuj1 and GFAP in NPCs given bovine or ovine-sourced GM1. Our findings suggest that ovine- or bovine-sourced GM1 may alter cell proliferation and differentiation, and further investigation may provide new insights into the molecular mechanisms of GM1-induced effects on NPCs.

Disclosures: **R. Bose:** None. **O. Kacherian:** None. **S. Banerjee:** None. **J. Rossignol:** None. **G. Dunbar:** None.

Digital Abstract Session

P122. Huntington's Disease: Candidate Therapeutic Strategies

Program #/Poster #: P122.11

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: John G. Kulhavi Professorship in Neuroscience at CMU
Field Neurosciences Institute
Central Michigan University-Undergraduate Summer Scholars Grant

Title: Utilizing Liraglutide and HM15211 as a Treatment for HD to Reduce Behavioral Deficits in YAC128 Huntington's Mouse Model

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Abstract: Huntington's disease (HD) is an inherited autosomal dominant disease that is caused by a mutation on the huntingtin gene (*HTT*) which is located on the short arm of chromosome 4. Multiple polyglutamine (CAG) repeats lead to the overproduction of mutant huntingtin protein (mHTT). The overproduced mHTT protein accumulates in the nuclei of neuronal cell bodies which causes cell dysfunction and death in the affected brain region(s), most heavily in the caudate nucleus and putamen. HD symptoms are progressive and are characterized by behavioral, cognitive, and motor deficits, especially chorea. Over 50% of HD patients develop type-2 diabetes, which is a metabolic disease that is caused by defective insulin receptor substrate (IRS) proteins. The function of IRS proteins is to monitor glucose levels and signal to/activate beta pancreatic cells to release insulin; but these defective IRS proteins are unable to effectively send signals to the beta pancreatic cells which release the insulin needed to restabilize blood glucose levels. It is hypothesized that the overproduction of mHTT impairs the cells that are responsible for insulin signaling and eventually causes apoptosis of neurons in the affected region of the cortex. The YAC128 mouse model was used to conduct this study. This specific mouse model was used because they show significant striatal degeneration, and increased insulin resistance which mimics the diseased human condition. Liraglutide and HM15211 are GLP-1 analogues, both of which have been proven to restore insulin sensitivity in IRS proteins and demonstrate neuroprotective effects in previous studies. Liraglutide (25 nmol/kg/day) and HM15211 (10 nmol/kg/day) were administered intraperitoneally for 12 weeks in 12-month-old

YAC128 mice. Blood glucose levels were measured once every two weeks throughout the study. It was found that the glucose levels of the liraglutide- and HM15211-treated groups were unaffected. Behavioral assessments using rotarod-, open field-, and water-T-maze -tasks indicated that both liraglutide and HM15211 treatments did not reduce motor behavioral deficits in YAC128 mice on the rotarod or open fields- tasks. However, Liraglutide- and HM15211-treated YAC128 mice showed trends of marginal improvements in cognitive deficits in learning and memory during water-T-maze testing.

Disclosures: **J. Wassell:** None. **S. Koneru:** None. **P. Otero:** None. **O. Khacherian:** None. **N. Wedster:** None. **E. Lauzon:** None. **L. Paladugu:** None. **N. Fettinger:** None. **A. Poudel:** None. **D. Dolland:** None. **S. Banerjee:** None. **J. Rossignol:** None. **G. Dunbar:** None.

Digital Abstract Session

P123. Huntington's Disease: Animal Model Characterization

Program #/Poster #: P123.01

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: FDN-143210
UBC Four Year Fellowship
Canadian Open Neuroscience Platform Scholar Award

Title: In vivo striatal neural activity during motor learning and spontaneous behaviour in Huntington's disease mice

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Abstract: Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder characterized by motor, cognitive and psychiatric deficits. The dorsal striatum is the major site of neurodegeneration in HD, particularly the spiny projection neurons, along with atrophy of other areas including the cortex. The YAC128 and zQ175 mouse models of HD both show progressive deficits in the accelerating rotarod motor learning task as well as changes to locomotor activity in the open field. *In vitro* studies have revealed that YAC128 mice display aberrant cortico-striatal signalling, including changes to glutamate release and deficits in cortico-striatal plasticity. Although there has been extensive research on changes to cortico-striatal signalling *in vitro* in HD models, there has been relatively little research on how these changes to signalling correlate with behavioural deficits *in vivo*. Here, we have combined the accelerating rotarod and open field tasks with GCaMP7f imaging using fiber photometry to correlate activity in striatal neural populations with task performance and motor learning. We are also using machine learning software to analyze behaviour on the rotarod and open field in detailed ways, such as looking at the positioning and kinetics of the paws during rotarod and open field, and identifying the frequency of different behaviours in the open field. In our wild-type mice, we found that as mice learn the rotarod task, the mean GCAMP7f activity in the striatum decreases, as well as mean

peak amplitude of Ca2+ events. Mean GCAMP7f activity was thus negatively correlated with latency to fall on the rotarod task. Early stage 2-3 month old YAC128 mice did not have a deficit on the accelerating rotarod, however the correlation between mean GCAMP7f activity and performance on the rotarod was disrupted in these mice. Overall, early stage YAC128 HD mice show changes to GCAMP7f activity including reduced mean activity levels, and changes to peak kinetics such as frequency, amplitude, and width, during open field and rotarod. This research contributes to our understanding of the changes to striatal signalling that may contribute to motor and cognitive symptoms in HD.

Disclosures: E.T. Koch: None. **M.D. Sepers:** None. **R. Yeung:** None. **L.A. Raymond:** None.

Digital Abstract Session

P123. Huntington's Disease: Animal Model Characterization

Program #/Poster #: P123.02

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R21NS115041

Title: Effects of circadian intervention on sleep and EEG in the BACHD mouse model of Huntington’s disease

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Abstract: Sleep and circadian disturbances are common features of neurodegenerative diseases, such as Huntington’s disease (HD). Since sleep disruptions manifest early in the disease pathogenesis, we examined whether these can serve as early biomarkers for disease. In this study, EEG was used to characterize sleep-wake states and spectral power in the BACHD mouse model of HD. Our findings show that temporal patterning of the sleep-wake cycle is disrupted in BACHD mice. They exhibit more wake during the light phase, and less wake during the dark phase than wildtypes ($F(11,187)=4.374$, $p<0.001$). The diurnal rhythm of NREM sleep was also significantly disrupted ($F(11,187)=4.273$, $p<0.001$). In order to examine sleep homeostatic regulation, mice underwent 6 hrs of sleep deprivation by gentle-handling. There were no significant differences in wake amount ($F(11,154)=1.8$, $p=0.0583$), NREM ($F(11,176)=1.613$, $p=0.0986$), or REM sleep amount ($F(11,176)=1.459$, $p=0.1506$) during recovery from sleep loss. This suggests that the homeostatic regulation of sleep may be preserved in BACHD mice. Next we sought to determine if the BACHD mutation alters EEG spectral power. Examination of the 24-hr rhythm of delta power (0.5-4 Hz) revealed that BACHD mice are unable to dissipate delta power during NREM sleep in the dark phase ($F(11,176=1.854$, $p=0.0484$) at rates similar to wildtype controls. Additionally, BACHD mice exhibit a distinct phase difference and a reduction in the amplitude of gamma (20-40 Hz) power during the 24-hr recording period ($F(11,88)=1.723$, $p=0.0811$). These results suggest that the BACHD mutation is sufficient to cause impairments in the sleep-wake cycle early in disease pathogenesis, and prior to the onset of more severe

behavioral phenotypes. They also suggest that EEG may be a valuable diagnostic tool in revealing and monitoring progression of HD. To explore the therapeutic potential of a circadian-based treatment, we implemented a time-restricted feeding (TRF) protocol (6-hr feeding/18-hr fasting). TRF improved the temporal patterning of wake ($F(11,66)=2.949$, $p=0.0031$), and NREM ($F(11,66)=2.633$, $p=0.0075$) in BACHD mice. Thus, a circadian-based treatment, such as scheduled feeding, may be an effective therapeutic tool to alter the trajectory of disease progression.

Disclosures: E. Chiem: None. **C. Ghiani:** None. **C. Colwell:** None. **K. Paul:** None.

Digital Abstract Session

P123. Huntington's Disease: Animal Model Characterization

Program #/Poster #: P123.03

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH R01NS089750
NIH 5R25NS089463-05
NINDS P30 NS47466
The Dixon Family Foundation
NIH 1R01NS089750-01A1
NIH 2R01NS089750-06

Title: Reducing astrocytic SNARE-dependent exocytosis affects neuropathological phenotypes in a mouse model of Huntington's disease

Authors: *A. KING[1,2], T. WOOD[1,2], E. RODRIQUEZ[1,2], E. PAYNE[1,2], E. STEPHENS[1,2], M. GRAY[1,2];
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Abstract: Huntington’s disease (HD) is a devastating, progressive, neurodegenerative disorder caused by a CAG expansion in the *Huntingtin (HTT)* gene which encodes the widely expressed protein huntingtin. Neuropathologically, HD causes brain atrophy with overt degeneration of medium spiny neurons and to lesser extent cortical pyramidal neurons. We have shown that reduction of mHTT in astrocytes of BACHD mice results in a significant improvement in behavioral phenotypes, brain weight, striatal volume and the expression of synaptic proteins thus suggesting that mHTT expression in astrocytes is critical in HD. In primary astrocyte cultures, we observed increased extracellular glutamate levels after evoking SNARE-dependent exocytosis. An increase in extracellular glutamate levels could lead to excitotoxicity and contribute to behavioral abnormalities. In order to determine whether SNARE-dependent exocytosis from astrocytes contributed to neuropathological changes *in vivo*, we crossed BACHD mice to dominant negative SNARE (dnSNARE) mice and performed behavioral analyses. We observed an improvement in motor coordination, worsening of the anxiety-like phenotype but found no effect on the depressive-like phenotype when astrocytic SNARE-

dependent exocytosis was reduced. In the study presented here, we determined whether SNARE-dependent exocytosis from astrocytes contributes to the neuropathological changes observed in BACHD mice. We analyzed mice at 13-15 months of age when neuropathological changes are present. We did not observe a significant increase in the total brain weight in the BACHD/dnSNARE mice. However, we observed a significant increase in the striatal volume with no significant change observed in the cortical volume when compared to BACHD mice. Our analyses of post-synaptic proteins in the striatum shows that reducing astrocytic SNARE-dependent exocytosis had no effect on the expression of these post-synaptic proteins. Although astrocyte morphology is altered in HD patients and some mHTT expressing mouse models, we did not observe any overt changes in the BACHD mice or BACHD/dnSNARE mice. Thus, the modulation of astrocyte SNARE-dependent exocytosis in BACHD mice is beneficial for striatal volume while not causing overt changes in astrocyte number or morphology. Along with our previous behavioral data, we can conclude that modulating SNARE-dependent exocytosis globally has benefits in motor coordination and striatal volume as well as adverse effects in the anxiety-like phenotype. This work reveals that broadly targeting this cell type is not the most appropriate approach for alleviation of disease relevant phenotypes.

Disclosures: **A. King:** None. **T. Wood:** None. **E. Rodriquez:** None. **E. Payne:** None. **E. Stephens:** None. **M. Gray:** None.

Digital Abstract Session

P124. Ataxia and Dystonia: Molecular, Cellular and Circuit Mechanisms

Program #/Poster #: P124.01

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NINDS Grant R01NS050808
NINDS Grant R01NS105470

Title: The role of adrenergic transmission in stress-induced cerebellar dysfunction

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Abstract: Stress is the most common trigger among episodic channelopathies, such as periodic paralyses, and Episodic Ataxia. In fact, in some cerebellar disorders, such as many ataxias, dystonias, and tremors, motor symptoms are either triggered, or worsen with stress. The mechanism by which stress elicits or exacerbates these symptoms remains unknown. Episodic ataxia type 2 (EA2) is a channelopathy that arises from mutations in the *CACNA1A* gene encoding for the α1 pore forming subunit of P/Q-type voltage-gated calcium channels. Patients with this disorder exhibit baseline ataxia, and motor attacks in the form of ataxia and dyskinesia, which are brought about by physical or emotional stress, or consumption of caffeine or alcohol.

We used the *tottering* mouse, a faithful animal model of Episodic Ataxia Type 2, to dissect the mechanisms underlying stress-induced motor attacks. Previously, our lab showed a decrease in calcium activated potassium (SK) channel activity in Purkinje cells is the underlying mechanism for the baseline ataxia. Cerebellar Purkinje cells (PCs) are required for the expression of attacks in *tottering* mice. Pharmacologically activating α1 adrenergic receptors in the cerebellum was sufficient to induce attacks, and was required for stress-induced attacks. This receptor can regulate SK channel activity through casein kinase II (CK2), which phosphorylates SK channels decreasing its activity. Using slice electrophysiology, we show that bath application of norepinephrine (NE) increased PCs irregularity from *tottering* and wild type slices. Knock down of casein kinase II (CK2) using shRNAs prevented stress-induced attacks and NE- induced irregularity of *tottering* PCs. Additionally, pharmacologically blocking CK2 with CX-4945, an FDA approved blocker of CK2, prevented stress-induced attacks and NE induced irregularity in PCs. In *tottering* and wild type PC, block of α1 adrenergic receptors attenuated but failed to abolish NE-induced irregularity, suggesting involvement of an additional adrenergic receptor. While the mechanism of action remains unknown, β-adrenergic receptor blockers, such as propranolol, are used to treat stress/anxiety-induced tremor and Essential tremor. Bath application of the β-adrenergic agonist isoproterenol caused an increase in the irregularity of firing of wild type PC that was prevented by blockers of fast synaptic transmission. These data suggest irregular firing of PC could contribute to stress-induced motor disorders through the modulation of cerebellar adrenergic receptors, and offers insight into the mechanism of stress-induced attacks in EA2 and potentially other stress-induced motor disorders.

Disclosures: H.D. Snell: None. **A. Vitenzon:** None. **J. Bryen:** None. **K. Khodakhah:** None.

Digital Abstract Session

P124. Ataxia and Dystonia: Molecular, Cellular and Circuit Mechanisms

Program #/Poster #: P124.02

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: MJF 13445
NIH R01NS08852
NIH R01MH073676
NIH K99NS110878

Title: Selective M_4 muscarinic acetylcholine receptor antagonists demonstrate efficacy in parkinsonian and dystonia models.

Authors: K. J. STILLWELL[1], J. W. DICKERSON[1], M. S. MOEHLE[2], A. M. BENDER[1], D. J. FOSTER[1], Y. DONSANTE[3], S. CHANG[1], A. RODRIGUEZ[1], C. M. NISWENDER[1], C. W. LINDSLEY[1], E. J. HESS[3], P. J. CONN[1], *J. M. ROOK[1];
[1]Vanderbilt Univ., Nashville, TN; [2]PHARMACOLOGY / THERAPEUTICS, Univ. of Florida, Gainesville, FL; [3]Emory Univ., Atlanta, GA

Abstract: Non-selective antagonists of muscarinic acetylcholine receptors (mAChRs) that broadly inhibit all five mAChR subtypes provide an efficacious treatment for some movement disorders, including Parkinson's disease and dystonia. Despite their efficacy in these and other central nervous system disorders, anti-muscarinic therapy has limited utility due to severe adverse effects that often limit their tolerability by patients. Recent advances in understanding the roles that each mAChR subtype plays in disease pathology suggest that highly selective ligands for individual subtypes may underlie the anti-parkinsonian and anti-dystonic efficacy observed with the use of non-selective anti-muscarinic therapeutics. Our recent work has indicated that the M_4 muscarinic acetylcholine receptor has several important roles in opposing aberrant neurotransmitter release, intracellular signaling pathways, and brain circuits associated with movement disorders. This raises the possibility that selective antagonists of M_4 may recapitulate the efficacy of non-selective anti-muscarinic therapeutics and may decrease or eliminate the adverse effects associated with these non-selective drugs. However, this hypothesis has not been directly tested due to lack of selective antagonists of M_4. Here we utilize genetic mAChR knockout animals in combination with non-selective mAChR antagonists to confirm that the M_4 receptor underlies the locomotor-stimulating and anti-parkinsonian efficacy in rodent models. We also report the synthesis, discovery, and characterization of the first-in-class selective M_4 antagonists VU6013720, VU6021302, and VU6021625. These novel compounds have ideal pharmacokinetic properties for in vivo evaluations, and we have confirmed that these optimized compounds have anti-parkinsonian and anti-dystonic efficacy in pharmacological and genetic models of movement disorders. These data provide critical pre-clinical rationale for the development of M_4 antagonists and represent a potential novel treatment mechanism to meet the unmet clinical need across several movement disorders.

Disclosures: K.J. Stillwell: None. **J.W. Dickerson:** None. **M.S. Moehle:** None. **A.M. Bender:** None. **D.J. Foster:** None. **Y. Donsante:** None. **S. Chang:** None. **A. Rodriguez:** None. **C.M. Niswender:** None. **C.W. Lindsley:** None. **E.J. Hess:** None. **P.J. Conn:** None. **J.M. Rook:** None.

Digital Abstract Session

P124. Ataxia and Dystonia: Molecular, Cellular and Circuit Mechanisms

Program #/Poster #: P124.03

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: no number; Hess, PI; Smith, co-I
P51OD011132

Title: Nigrostriatal dopamine terminals in DYT1 dystonia: a 3D ultrastructural analysis in DYT1 knock-in mice

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Abstract: The most common form of inherited dystonia is DYT1 dystonia, a form of generalized dystonia caused by a GAG deletion (ΔE) in the *TOR1A* gene. Mouse models of DYT1 dystonia exhibit a profound decrease in striatal dopamine (DA) release. Although dysregulation of vesicular function/trafficking has been suggested, the underlying substrate(s) of reduced striatal DA release in DYT1 dystonia remain unknown. To study potential ultrastructural changes of nigrostriatal DA terminals that could contribute to this reduced striatal DA release in DYT1 dystonia, we used serial electron microscope images generated with a Serial-Block Face/Scanning Electron Microscope (SBF/SEM) and the 3D reconstruction software *Reconstruct* (NIH) to analyze the ultrastructure of striatal tyrosine hydroxylase-immunoreactive (TH+) terminals and their synapses in one wild type (WT) and one DYT1(ΔE) knock-in (KI) mouse. Furthermore, to study possible changes in vesicle packaging capacity of DA, we used transmission electron microscope (TEM) to assess changes in the surface area of synaptic vesicles in striatal DAT-immunoreactive (DAT+) terminals between the WT and the DYT1 KI mouse. Quantitative analysis of 100 fully reconstructed TH+ terminals in the WT (n=50) and DYT1 KI (n=50) mice indicate: 1) The volume of TH+ terminals ranged from 0.1 to 0.8 μm^3 without any major difference in the average terminal volume between WT (0.41+/-0.04 μm^3) and DYT1 KI (0.48 +/-0.03 μm^3) mice. 2) However, when pooled into small-sized (0.1-0.4 μm^3) and large-sized (>0.5μm^3) terminals, the relative proportion of large-sized TH+ terminals were higher in the DYT1 KI mouse than in the WT animal (36% vs 16% of total TH+ terminals, respectively). 3) No major change was found in the proportion of axo-spinous (50% in WT vs 40% in DYT1 KI) vs axo-dendritic (39% in WT vs 38% in DYT1 KI) synapses formed by TH+ terminals between the two mice, but the number (46 in WT vs 26 in DYT1 KI) and surface area (0.014+/-0.002μm^2 in WT vs 0.0084+/-0.002 μm^2 in DYT1 KI) of the postsynaptic densities of axo-spinous synapses were smaller in the DYT1 KI mouse than in control. 4) No significant difference was found in the surface area of synaptic vesicles (250 in WT and 250 in KI mice) in dopaminergic (DAT+) terminals between WT and DYT1 KI mice (487.43 +/- 16.1 nm^2 in WT vs 499.31 +/- 13.7 nm^2 in DYT1 KI) . These ultrastructural data provide a solid foundation for further studies of the synaptic mechanisms that underlie decreased striatal DA release in DYT1 dystonia. Further analyses of a larger sample of terminals from additional animals are needed to confirm these preliminary data.

Disclosures: K. Pan: None. **J. Pare:** None. **X. Fan:** None. **A.M. Downs:** None. **E. Hess:** None. **Y. Smith:** None. **R.M. Villalba:** None.

Digital Abstract Session

P124. Ataxia and Dystonia: Molecular, Cellular and Circuit Mechanisms

Program #/Poster #: P124.04

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CIHR PJT-153150

Healthy Brains for Healthy Lives PhD fellowship (A.A.C.)
McGill Biology excellence doctoral award (A.A.C.)
NSERC Undergraduate Student Research Award (K.C.J.S)

Title: Exercise acts via BDNF-TrkB signaling to rescue deficits in a mouse model of spinocerebellar ataxia type 6

Authors: *A. A. COOK[1], S. JAYABAL[1,2], K. C. J. SHENG[1], A. J. WATT[1];
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Abstract: Spinocerebellar ataxia type 6 (SCA6) is a hereditary, progressive ataxia, presenting with mid-life onset of motor coordination difficulties and eventual cerebellar degeneration. We used a knock-in mouse model ($SCA6^{84Q/84Q}$) that carries a CAG expansion mutation in the *CACNA1A* gene to characterise the pathophysiology of SCA6 and identify potential treatments. We have previously shown that at 7 months these mice display deficits in motor coordination, as well as abnormalities in the intrinsic firing patterns of the Purkinje cells in the cerebellum. A 1 month program of voluntary exercise was able to rescue both motor coordination deficits and the frequency of intrinsic Purkinje cell firing. In order to identify the mechanism by which exercise acts therapeutically in the $SCA6^{84Q/84Q}$ model, we investigated brain-derived neurotrophic factor (BDNF) since BDNF levels are reduced in post-mortem brain tissue from SCA6 patients[1] and BDNF administration has been found to delay disease onset in a model of SCA1[2]. We found that $SCA6^{84Q/84Q}$ mice had reduced levels of cerebellar BDNF, but this could be reversed by exercise. To determine whether exercise acts therapeutically in this model via BDNF-TrkB signaling, we administered 7,8-dihydroxyflavone, a small molecule that mimics BDNF as an agonist of the TrkB receptor. Chronic oral administration of 7,8-DHF rescued both motor coordination and Purkinje cell firing frequency deficits in the $SCA6^{84Q/84Q}$ mouse. The beneficial effect on motor coordination was sustained over several months of administration, demonstrating its potential as a long term treatment for SCA6. By identifying a pathway by which exercise exerts its positive effect on cerebellar function, we have identified novel therapeutic strategies for SCA6.
References 1. Takahashi, M. et al. Reduced brain-derived neurotrophic factor (BDNF) mRNA expression and presence of BDNF-immunoreactive granules in the spinocerebellar ataxia type 6 (SCA6) cerebellum. Neuropathology 32, 595-603 (2012). **2.** Mellesmoen, A., Sheeler, C., Ferro, A., Rainwater, O. & Cvetanovic, M. Brain Derived Neurotrophic Factor (BDNF) Delays Onset of Pathogenesis in Transgenic Mouse Model of Spinocerebellar Ataxia Type 1 (SCA1). Front. Cell. Neurosci. 12, 509 (2019).

Disclosures: **A.A. Cook:** None. **S. Jayabal:** None. **K.C.J. Sheng:** None. **A.J. Watt:** None.

Digital Abstract Session

P124. Ataxia and Dystonia: Molecular, Cellular and Circuit Mechanisms

Program #/Poster #: P124.05

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Impaired vestibular reflexes response and vestibulo ocular reflex adaptation in a mouse model of spinocerebellar ataxia type 6

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Abstract: Spinocerebellar Ataxia Type 6 (SCA6) is a mid-life onset neurodegenerative disease that affects motor coordination. This autosomal dominant disease is caused by the expansion of a CAG repeat tract in a CACNA1A gene that encodes the α1A subunit of the P/Q type voltage-gated Ca^{2+} channel. A hyper-expanded polyglutamine (84Q) mouse model of SCA6 ($SCA6^{84Q/84Q}$), is characterized by impaired locomotive function. Using both *in vitro* and *in vivo* recordings, we have previously shown that, in this same mouse model, the firing precision of cerebellar Purkinje cells in lobule 3, areas of the cerebellum generally associated with locomotion, is significantly reduced (Jayabal et al., 2016). In addition, $SCA6^{84Q/84Q}$ mice showed reduction in complex spike firing rate and increase in the duration of pause after the appearance of complex spikes. Previous studies have demonstrated cell death across the entire cerebellar cortex in SCA6 patients. Given the involvement of cerebellum in generation of and motor learning in the vestibulo ocular reflex (VOR) and optokinetic reflex (OKR), we hypothesized that $SCA6^{84Q/84Q}$ mice would likely show deficits during these oculomotor behaviors. To test this hypothesis and to understand the pathophysiology of SCA6 mice in more detail, we quantified their eye movements to characterize their VOR, OKR, and VOR adaptation. Vestibular rotational stimuli were delivered to a head-restrained mouse at frequencies and velocities spanning the range of those comprising natural behaviors (0.2-2 Hz and 16 deg/s). VOR eye movement responses were measured in both dark and light conditions. OKR were evoked by rotation of visual stimulus at the same frequencies and velocities. For VOR adaptation, gain decrease was induced by presenting visual and vestibular stimuli (2Hz and peak velocity of 16 deg/s) that were at the same speed and exactly in phase. The gain-down training lasted for 30 minutes. Our preliminary data show that $SCA6^{84Q/84Q}$ mice had a significant reduction of VOR gain at 0.8, 1 and 2 Hz and a ~35% reduction of OKR gain without a change in phase compared to litter-matched control WT mice. Additionally, during VOR motor learning, $SCA6^{84Q/84Q}$ mice only showed 16% decrease in VOR gain whereases WT mice showed over 40% VOR gain decrease. Finally, we found that $SCA6^{4Q/84Q}$ mice also generated slower saccades than the control mice. Taken together, our results confirm our original hypothesis that neuronal responses are altered in the floccular lobe of $SCA6^{84Q/84Q}$ mice, a region of the cerebellum known to play a vital role in the calibration of the VOR pathway as well as generation of the optokinetic responses.

Disclosures: H.V. Chang: None. **A.J. Watt:** None. **K.E. Cullen:** None.

Digital Abstract Session

P124. Ataxia and Dystonia: Molecular, Cellular and Circuit Mechanisms

Program #/Poster #: P124.06

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: DoD CDMRP W81XWH-17-1-0393
NIH ORDR/NCATS/NINDS NS065701, TR001456

Title: Head tremor in cervical dystonia: the effect of postural maneuvers

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Abstract: Cervical Dystonia (CD) is a common form of focal dystonia characterized by abnormal head posture and head tremor (HT). Many patients with CD develop a sensory trick, which is a maneuver used to control or diminish CD motor symptoms including HT. A variety of tricks (alleviating maneuvers) with common characteristics have been described, however, tactile stimulation is not necessarily a defining feature. The standard CD neurologic exam includes a task in which patients are instructed to raise their arms into a horizontal position in pronation, supination, and the winged posture, normally used to examine for upper limb tremor. The objective of this study was to determine how raising the arms, a postural maneuver with no tactile input, affects HT severity. We analyzed data collected from 206 patients with isolated CD recruited through the Dystonia Coalition. Video-recordings of standard patient examination protocol were reviewed by a movement disorders neurologist (CC) who assessed HT presence and severity during periods of rest and arms up on a 0-10 global scale. Changes in HT severity were assessed between the rest period and arms up period and scored as decreased, unchanged, or increased by arms up activation. Of the 206 patients, 84 were excluded due to the lack of HT in either condition, and two were excluded because they were not prompted to do the arms up maneuver. We found that patients were more likely to exhibit no change in HT severity (N = 72) than change (N = 48) during the arms up period (χ^2 (1, N = 120) = 4.8, p = 0.03). Of those who exhibited change in HT severity with arm activation, decrease in HT severity (N = 35 patients) was more common than HT increase (N = 13, χ^2 (1, N = 48) = 10.1, p = 0.002). Demographic factors (age at onset, disease duration, gender, and the interaction term between age at onset and disease duration) were not found to be significant predictors of whether there would be change or no change in HT severity (χ^2 (4, N = 120) = 0.978, p = 0.913) and an increase or decrease in HT severity (χ^2 (4, N = 48) = 2.51, p = 0.643). This study highlights the effectiveness of proprioceptive modulation or postural change (raising arms) in reducing HT severity in some patients with CD. Further exploration into the effects of the arms up maneuver on other CD motor symptoms as well as physiological investigations are needed to better understand the effectiveness of this maneuver to alleviate HT severity and other CD motor symptoms.

Disclosures: E. Cisneros: None. **J.P. Vu:** None. **H. Lee:** None. **Q. Chen:** None. **C.N. Benadof:** None. **Z. Zhang:** None. **R.L. Barbano:** None. **J. Jankovic:** None. **H.A. Jinnah:** None. **J. Perlmutter:** None. **B.D. Berman:** None. **A. Mahajan:** None. **C.G. Goetz:** None. **G.T. Stebbins:** None. **C.L. Comella:** None. **D.A. Peterson:** None.

Digital Abstract Session

P125. Neuromuscular Diseases -SMBA and Batten

Program #/Poster #: P125.01

Topic: C.06. Neuromuscular Diseases

Support: Center for Chronic Disorders of Aging, PCOM pilot grant
Division of Research, PCOM
DO-PhD program, PCOM
Giunta Family Research Scholarship

Title: Modulating Sirtuin 3 to abrogate toxicity within in vitro and in vivo models of spinal and bulbar muscular atrophy

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Abstract: Spinal and bulbar muscular atrophy (SBMA) is an adult-onset, X-linked neuromuscular disease estimated to affect about 1 in 40,000 men. SBMA is caused by an expansion of a CAG repeat (n > 39) within the androgen receptor (AR) gene, which encodes a glutamine tract in the AR protein. The polyglutamine-expanded AR causes proximal and lower distal limb weakness due to toxicity in both motor neurons and skeletal muscle. To date, there is no cure or therapy. Like other neurodegenerative diseases, there is mitochondria dysfunction in SBMA, which leads to reduced ATP, increased oxidative stress, and diminished mitochondrial biogenesis. Sirtuin 3 (SIRT3) is the main mitochondrial NAD-dependent deacetylase, functioning to regulate mitochondrial homeostasis, biogenesis, and anti-oxidative defense mechanisms. Using PC12 and C2C12 cell models of SBMA, we found that pharmacologic activation of SIRT3 and stable SIRT3 overexpression of the protein abrogated polyQ-expanded AR cytotoxicity and decreased reactive oxygen species. We next tested the effect of SIRT3 overexpression in a transgenic mouse model of SBMA (males only), but failed to identify any significant improvement in motor function. However, there was a trend of increased time on both an accelerating rotarod and a treadmill for the SIRT3-overexpressing SBMA mice compared to control SBMA mice. These data lead us to postulate that the diminished NAD^+ levels in SBMA, as previously reported in another mouse model, may impede effective activity of SIRT3 *in vivo*. We have validated this decrease in NAD^+ in the mouse model utilized in these studies. We are now investigating whether restoring cellular NAD^+ can allow for SIRT3 activation and protection against polyglutamine-expanded AR *in vivo*.

Disclosures: **D. Garcia-Castro:** None. **J. Mazuk:** None. **D. Simpson:** None. **E. Heine:** None. **D. Mathis:** None. **P. Morrin:** None. **T. Farmer:** None. **Q. Tong:** None. **H. Montie:** None.

Digital Abstract Session

P125. Neuromuscular Diseases -SMBA and Batten

Program #/Poster #: P125.02

Topic: C.06. Neuromuscular Diseases

Support: R01NS108114

Title: Role of neurofilament subunit dysregulation in motor neuron degeneration in SBMA

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Abstract: Spinal and bulbar muscular atrophy (SBMA) is an X-linked, slowly progressing neuromuscular neurodegenerative disease that affects 1 in 40,000 men and for which there is no cure. SBMA is caused by polyglutamine repeat expansion in the androgen receptor (AR), and disease progression is dependent on binding of the AR to its ligand hormone testosterone or dihydrotestosterone (DHT). Symptoms such as fasciculations, dysarthria, and dysphagia are caused by pathological motor neuron degeneration in the brainstem and spinal cord. Previous work by Chevalier-Larsen et al (2004) showed that, in a transgenic mouse model of SBMA, spinal cord motor neurons exhibit decreased levels of unphosphorylated neurofilament heavy chain (NFH) compared to non-transgenic controls. NFH is one of four proteins that make up the neuronal structural protein neurofilament, which is critical in maintaining axonal structural integrity and determining axon diameter in peripheral neurons (the other three proteins being neurofilament medium chain, NFM; neurofilament light chain, NFL; and peripherin). However, the role of neurofilament subunit dysregulation in pathological motor neuron degeneration in SBMA has yet to be fully explored. Moreover, SBMA patients show skeletal muscle fiber type grouping and atrophy of fast twitch muscle fibers, indicative of enhanced vulnerability of fast twitch motor units. We hypothesize that neurofilament subunit expression is altered in SBMA, contributing to differential motor unit vulnerability. Our data reveal substantial deficits in phosphorylated NFH staining intensity at the NMJ of motor neurons innervating fast twitch gastrocnemius muscle of 6-month-old transgenic mice, without a significant difference in endplate fragmentation or pre- and post-synaptic colocalization (slow-twitch experiments ongoing). However, preliminary data show that endplate fragmentation in the gastrocnemius is evident in 9-month-old mice. These data provide evidence for neurofilament subunit dysregulation prior to denervation in a mouse model of SBMA. In addition, we found a considerable deficit in NFL and phosphorylated NFH in DHT-treated SBMA patient iPSC-derived motor neurons compared to control iPSC-derived motor neurons. Notably, we did not see a difference in levels of phosphorylated NFM. These studies show a DHT-dependent alteration in neurofilament subunit levels in SBMA patient derived motor neurons. Together, these findings reveal neurofilament subunit dysregulation in SBMA models, *in vitro* and *in vivo,* and provide further context for the potential role of neurofilaments in differential vulnerability of fast twitch motor units in SBMA.

Disclosures: **E. Molotsky:** None. **C. Grunseich:** None. **D.E. Merry:** None. **K.H. Fischbeck:** None.

Digital Abstract Session

P125. Neuromuscular Diseases -SMBA and Batten

Program #/Poster #: P125.03

Topic: C.06. Neuromuscular Diseases

Support: Cinque Foundation

Title: Force dependent recruitment of intrinsic versus extrinsic hand muscles to assess upper motor neuron function

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Abstract: Techniques to effectively assess upper motor neuron (UMN) function and dysfunction are key to understanding motor neurophysiology in healthy individuals and creating biomarkers of pathology in diseases such as Amyotrophic lateral sclerosis (ALS). Transcranial magnetic stimulation (TMS) offers a unique opportunity to non-invasively inspect the function of UMNs, however novel experimental protocols are needed to dissociate UMN neurophysiology from the rest of the corticospinal pathway. In this study, known differences in the recruitment of the first dorsal interosseous muscle with increasing finger force were leveraged in an attempt to create a direct measure of UMN function. Five young healthy subjects participated in the study following institutionally approved informed consent. Four intrinsic and four extrinsic hand muscles were recorded with EMG during an index finger flexion force production task. TMS was triggered at 110% of the resting motor threshold when the subject produced a force that matched a visual target representing 5, 10, 25, or 50% of their maximal voluntary contraction (MVC). One-way ANOVAs were used to compare the activation ratios of each muscle tested to the FDI. Results indicated a limited contribution of extrinsic hand muscles to finger flexion force below 10% of MVC. The ratio of flexor digitorum superficialis (FDS), the primary extrinsic finger flexor, to FDI activation (FDS:FDI ratio) was found to be monotonically increasing with increased force. The difference between force levels for the FDS:FDI ratio was found to be significant ($F(3,15) = 6.86$, $p = .03$, Greenhouse-Geisser corrected). Given known differences in the UMN contribution to FDI and FDS activation this ratio could serve as a marker of UMN function. Further investigation will explore whether this ratio can be used as a sensitive marker of changes in UMN function with learning or disease.

Disclosures: **N. Pinkes:** None. **M. Yarossi:** None. **O. Soto:** None. **E. Tunik:** None.

Digital Abstract Session

P125. Neuromuscular Diseases -SMBA and Batten

Program #/Poster #: P125.04

Topic: C.06. Neuromuscular Diseases

Support: R01 NS644912-4
RC2 NS69476-01
Felowship from the Swiss National Science Foundation
Marie Curie Fellowship
Families of SCN2A
MDA
THE CHARLOTTE AND GWENYTH GRAY FOUNDATION

Title: A novel in vitro modeling system for CLN3 Batten disease

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Abstract: Batten Disease or Neuronal Ceroid Lipofuscinosis (CLN) encompasses fatal lysosomal storage disorders characterized by cognitive and motor deficits, vision impairments, and seizures. Juvenile Neuronal Ceroid Lipofuscinosis (JCLN), is an autosomal recessive subtype caused by loss of function (LOF) mutations in the CLN3 gene. However, the cellular mechanisms by which CLN3 mutations cause Batten disease are poorly understood. Moreover, most research focuses on the function and expression of CLN3 in neurons, with little known about the contributions of other cell types to disease mechanisms. To address this, we used a direct reprogramming method to generate Neural Progenitor Cells (iNPCs) from primary fibroblasts derived from CLN3 patients. We then differentiated the iNPCs into specific cell types of the nervous system to evaluate markers known to be affected by neurological disease. We found cell-type and patient specific alterations in mitochondrial morphology. Interestingly, mitochondrial activity of CLN3 cells did not differ from controls, suggesting that changes in mitochondrial networks did not impact mitochondrial energy production in our assays. Our results indicate that patient specific mutations may have a differential effect on the mitochondria. This suggests that current mouse models may be limited in their evaluation of potential therapeutic strategies. To combat this, we developed a co-culture system using healthy mouse GFP+ neurons and CLN3 patient derived cells. Using this system, we found that CLN3 patient derived cells induced a high degree of neuronal death, with some lines showing a more toxic phenotype than others. Combined, we have developed a patient specific human model of CLN3 Batten's disease that allows investigation of the impact of specific CLN3 mutations on cell mediated disease pathology. In addition, we have developed an assay conducive to drug

screening to evaluate potential therapeutic strategies for patients with diverse genetic backgrounds.

Disclosures: **J.A. Sierra Delgado:** None. **C.N. Dennys:** None. **S. Sinha Ray:** None. **R. Rodrigo:** None. **J.M. Weimer:** None. **K.C. Meyer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Receiving royalties for Batten disease programs licensed to Amicus. F. Consulting Fees (e.g., advisory boards); Scientific Advisor for Alcyone.

Digital Abstract Session

P126. Animal Models and Tau Pathology

Program #/Poster #: P126.01

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: CNRM - 70-9922.

Title: How typical is healthy, adult, male ferret behavior?

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Abstract: Gyrencephalic animals are becoming increasingly critical to study mechanisms of brain injuries and test therapies. The ferret is the smallest mammal with a gyrencephalic cerebral cortex and their small size enables group housing and imaging in preclinical MRIs, which is beneficial compared to larger gyrencephalic animals. However, the behavioral profile of normal, healthy ferrets is not well known. In this study, we compared the behavior of a number of normal, healthy, young-adult ferrets and report the variability of the results. We investigated motor, social, memory, sleep, and headache behavioral tests. In addition, we followed a small group of ferrets over time to determine the effects of repeated testing. We used 3 cohorts of young-adult male ferrets in 3 age groups (who were an average of 34, 50, or 81 weeks old at testing). We evaluated motor behaviors (open field, beam walk), sociability (socialization, eye contact), memory (novel object recognition), headache (grimace, light/dark box), and sleep (actigraphy). Some of these behaviors required pre-training. We followed a subset of ferrets over time with testing at 1 week and 4 weeks, and an additional group also at 4 months and 6 months. Several behavioral tests were only done at the final time-point. The youngest cohort (average 34 weeks old) had several behaviors that differed significantly from one or more of the other groups, suggesting that there is an age threshold for consistent results. Studies being planned should consider using ferrets of 50 weeks or older prior to injury. Some tests, like eye contact, showed a different amount of variability between cohorts. Eye contact expectedly increased with time and repeated testing, whereas open field test parameters decreased as animals habituated to the test, space and investigators. This investigation provides data assessing normal behavioral measures in healthy, young-adult, male ferrets using multiple behavioral tests. The results will be

helpful in planning future ferret experiments either in healthy ferrets or in studies using interventions.

Disclosures: **A.A. Obasa:** None. **S.C. Schwerin:** None. **T. Haight:** None. **M. Strayhorn:** None. **M. Ray:** None. **S.L. Juliano:** None.

Digital Abstract Session

P126. Animal Models and Tau Pathology

Program #/Poster #: P126.02

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: NIH Grant R01AG065830
NIH Grant R01GM133032
NIH Grant R01MH102261
NIH Grant T32GM086252,
NIH Grant F99NS11874301

Title: Circadian-clock gating of memory recall is disrupted in a mouse model of tauopathy

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Abstract: The aggregation of tau protein and the resulting formation of neurofibrillary tangles are thought to trigger a range of pathological processes which ultimately result in the profound cognitive deficits associated with tauopathies. Additionally, circadian-regulated physiological processes, including sleep, are disrupted in tauopathies, notably Alzheimer's disease and frontotemporal dementia. Given both cognition and circadian timing are disrupted in tauopathies, we sought to examine the relationship between tau aggregation and the dysregulation of the circadian timing system, and also to assess potential consequences on cognitive capacity. Here, we examined clock-gated cognition in the Tau P301S transgenic mouse model of human tauopathy. This transgenic mouse harbors the PS301S mutation in the human MAPT gene and develops filamentous tau lesions at 6 months of age, which is followed by marked synaptic loss, cognitive impairment, and neurodegeneration within cortico-limbic brain regions. We first tested the fidelity of the circadian timing system located within the suprachiasmatic nucleus (SCN), the locus of the master circadian clock. Using wheel running activity as a functional output of the SCN, we found no difference between P301S transgenic mice and WT littermates (7-8 months old) in clock phasing or periodicity. This indicates that key functional properties of the SCN clock are intact in the P301S mouse line. Next, we assessed whether circadian clock-gated cognition was altered in the P301S mice. To this end, mice were entrained to a standard light cycle, and then transferred to constant red-light illumination, which uncouples the circadian clock from the light/dark cycle. Mice were then assessed for (1) working memory (t-maze task), (2) intermediate term memory (novel object location test) and (3) long-term memory (contextual fear conditioning recall) during the circadian day and night. Overall, the performance of P301S

mice (8 months old) on these cognitive tests was inferior to WT mice. Interestingly, time-of-day profiling revealed that the relative efficacy of memory recall over the circadian cycle was diminished in P301S mice relative to WT mice; hence, the clock-gating of cognition appeared to be damped as a result of aberrant tau accumulation. Our results raise the prospect that the disruption of circadian time-keeping properties within cortico-limbic circuits that underlie learning and memory could contribute to the diminished/dysregulated cognitive capacity resulting from tauopathies. Studies are underway to identify the potential neuronal signaling pathways that could contribute to this clock-gated cognitive phenotype.

Disclosures: **A. Kalidindi:** None. **H. Yoon:** None. **A. Joshi:** None. **K.R. Hoyt:** None. **K. Obrietan:** None.

Digital Abstract Session

P126. Animal Models and Tau Pathology

Program #/Poster #: P126.03

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: Canadian Institutes of Health Research Project Fund (PJT-162124) to QY

Title: The effects of chronic phasic and tonic locus coeruleus activation patterns on tau pathology and cognitive function in a pre-tangle tau rat model

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Abstract: Braak and colleagues have proposed that the appearance of soluble hyperphosphorylated, pre-tangle tau in the brainstem structure, locus coeruleus (LC), marks the beginning of the tau pathology that may eventually develop into Alzheimer's disease (AD). Although pre-tangle tau is prevalent in human brains around mid-age, the selective vulnerability of a sub-population of people developing AD is unknown. LC neurons exhibit both phasic and tonic firing modes, which differentially modulate behavior. We hypothesize that LC activity itself may influence tau pathology development. Here we study whether and how phasic and tonic LC activation by optogenetic stimulations alters cognitive function in an LC pre-tangle tau model. Viral vectors carrying a human pseudophosphorylated tau gene (AAV9-DIO-htauE14-EGFP) and an opsin channel (AAVdj-DIO-eChR2-mCherry) were infused bilaterally into the LC of 2-3-month-old TH-Cre rats, mimicking the onset of tauopathy in humans. Control rats were infused with htauE14 and reporter AAV without ChR2 channels. Six-seven months after viral infusion, rats underwent optical cannula implantation. They then received either a 10 Hz phasic (300 msec every 2 sec) or a 25 Hz tonic LC activation for 20-30 min daily for six weeks. Two months following LC light stimulation, rats underwent a battery of behavioral tests, followed by immunohistochemistry. Preliminary results showed that phasic LC activation improved spatial

and olfactory discrimination, paralleled by higher LC fiber densities in both the piriform cortex and dentate gyrus. These results show that phasic and tonic LC activation have distinct effects on cognitive functions in the pre-tangle tau model.

Disclosures: **T. Omoluabi:** None. **A. Ghosh:** None. **S.E. Torraville:** None. **K.D. Power:** None. **C. Reinhardt:** None. **L.A. MacGowan:** None. **Q. Yuan:** None.

Digital Abstract Session

P127. Motor Neuron Disease: ALS and SMA

Program #/Poster #: P127.01

Topic: C.06. Neuromuscular Diseases

Support: NIH grant AG051513
NIH grant AG047612

Title: Early physiological defects in motor neurons of Drosophila ALS-related SOD1 mutants

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Abstract: Cu/Zn superoxide dismutase (SOD1) is a cytoplasmic antioxidant enzyme. In humans, mutations in the SOD1 gene are associated with familial cases of amyotrophic lateral sclerosis (ALS), a lethal degenerative disease in motor neurons. The *Drosophila* SOD1 gene (*Sod*) shares a highly conserved sequence with the human homolog, and we have shown that *Sod* mutant *Drosophila* display high mortality rates during larval and pupal development, decreased adult lifespan, and attenuated motor function. The *Drosophila* larval neuromuscular junction (NMJ) is well-adapted for the study of early physiological defects underlying later motor neuron degeneration, due to its accessibility for *in vivo* studies, and a long history of study of neuromuscular transmission-altering genetic mutations. This study includes examinations of the established hypomorphic *n108* allele (Sod^{n108}), alongside knock-in constructs of alleles found in human ALS patients: *G85R* and *H71Y* (Sod^{G85R} and Sod^{H71Y}). Immunohistostaining (anti-horseradish peroxidase, anti-HRP) of neuronal membrane at neuromuscular synapses in *Sod* mutant larvae revealed presynaptic terminals of abnormal, swollen morphology. In Sod^{G85R} larvae, *in vivo* mitochondrial staining (tetramethylrhodamine, TMRM) demonstrated the presence of aggregated mitochondria inside the swollen synaptic terminals. To overcome poor penetration of the larval motor axon bundle by TMRM, a genetically encoded GFP construct targeted to mitochondria was employed to reveal that aggregates of mitochondria also appear inside the axon bundles of Sod^{G85R} larvae. In whole-cell recordings of neuromuscular transmission, both Sod^{n108} and Sod^{G85R} exhibited lower muscle input resistance and smaller miniature excitatory junction potentials (mEJPs) compared to WT. However, evoked EJPs were similar to those of WT, indicating homeostatic adjustment of transmitter release. Focal electrophysiological recording showed that both Sod^{n108} and Sod^{G85R} NMJ terminals displayed slightly higher release probability than WT terminals. Treatment of Sod^{G85R} with the *Shaker*

channel (Kv1) blocker 4-aminopyridine (4-AP) and the broad-spectrum K^+ channel blocker tetraethylammonium (TEA) induced prolonged "plateau-like" potentials at the larval NMJ upon electrical stimulation. These potentials were accompanied by extended periods of presynaptic Ca^{2+} influx, made visible by a fluorescent Ca^{2+} reporter (GCaMP6f). Altogether, this study provides a snapshot of the alterations in mitochondrial distribution, synaptic morphology, and neurotransmission that characterize the motor neurons of *Sod* mutants prior to neurodegeneration and death of the organism.

Disclosures: T.C.D.G. O'Harrow: None. **A. Ueda:** None. **X. Xing:** None. **C. Wu:** None.

Digital Abstract Session

P127. Motor Neuron Disease: ALS and SMA

Program #/Poster #: P127.02

Topic: C.06. Neuromuscular Diseases

Support: Maryland Stem Cell Research Fund 2019-MSCRFD-5093
MDA 416750
NIH Grant 2R56NS079339-06

Title: Examining Regeneration Capacity and Innervation of NMJs by iPSC-Derived Motor Neurons

Authors: *K. L. MARSHALL[1], M. E. JAMES[1], L. RAJBHANDARI[2], A. TAGA[2], A. VENKATESAN[2], N. J. MARAGAKIS[2], M. H. FARAH[2];
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Abstract: Distal axon degeneration, dying-back, is a hallmark of motor neuron diseases, such as ALS, that precedes symptom onset and motor neuron death both in human patients and animal models. While motor neurons derived from human iPSCs (hMNs) hold promise for advancing ALS research, the length of axons, regenerative capacity, and mutant-specific innervation of neuromuscular junctions (NMJs) by these human neurons is not well-characterized. hMNs cluster into circular groups as they grow, and extend axons to other clusters, confounding quantification of axon outgrowth from individual hMNs. To address this, we have cultured hMNs from ALS patients and controls in custom microfluidic devices, and sequestered neuronal cell bodies in the main compartment that extended processes through microgrooves into two adjacent axonal compartments. We determined that devices with ample room in the axonal compartments are appropriate for examining axonal outgrowth, and allow for individual tracing of axons that are millimeters in length. We are able to sever axons at the entry point to the axonal compartments, and use time-lapse live imaging to quantify regeneration speed. We have performed axotomies and compared regeneration speed of hMNs harboring ALS-linked mutations, including hMNs with a $SOD1^{A4V}$ mutation to an isogenic corrected control. In co-cultures with primary human myoblast-derived myofibers, hMNs form NMJs. This system lays the groundwork for gathering electrophysiological data from myocytes innervated by hMNs in

the axonal compartment, and introducing relevant cell types. Systematic permutations of this microfluidic culture system have the potential to elucidate the ALS mutation-specific effects on axonal regeneration and structural and functional innervation of NMJs.

Disclosures: K.L. Marshall: None. **M.E. James:** None. **L. Rajbhandari:** None. **A. Taga:** None. **A. Venkatesan:** None. **N.J. Maragakis:** None. **M.H. Farah:** None.

Digital Abstract Session

P127. Motor Neuron Disease: ALS and SMA

Program #/Poster #: P127.03

Topic: C.06. Neuromuscular Diseases

Support: MND Scotland
Sprint MND scholarship

Title: Novel Methodology for Producing Postnatal Spinal Co-Cultures of Astrocytes and Neurons for Use in the Study of ALS

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Abstract: Spinal circuits support a wide range of functions necessary for survival and are composed of neurons, which are supported by glia. Neuro-glial interactions are critical for the healthy function of the nervous system and emerging evidence implicates roles in the pathogenesis of neurodegenerative diseases, including but not limited to Motor Neurone Disease. Currently there are limited tools available to interrogate neuro-glial interactions and respective contributions to function and disease. Here, we present an in vitro primary culture system for neurons and astrocytes derived from the lumbar region of spinal cords of postnatal mice up to 4 days old. This approach allows the generation of cultures obtained from spinal cords of verified, genetically modified mice, which is challenging using traditional approaches derived from embryonic tissue. Neurons and astrocytes can be isolated to produce 'pure' cultures and then be re-combined as mixed-genotype (wildtype and/or genetically modified) co-cultures at later time points to explore neuro-glial interactions. Electrophysiological analysis reveals that primary neuron cultures are functional; cells express sodium and potassium currents and can repetitively fire action potentials. Immunocytochemical analysis indicates neurons in co-culture with astrocytes express postsynaptic markers for glutamatergic (PSD95) and GABAergic (gephyrin) synapses. We also find a proportion of neurons express enriched **motor neuron** marker SMI-32. Glial primary cultures are composed of cells that express glial fibrillary acidic protein (GFAP; 82-96%) and glutamine synthetase (99%) indicating that these cultures are primarily composed of astrocytes. Importantly, GFAP and glutamine synthetase-expressing astrocytes are also colocalized with the excitatory amino acid transporter - 2 and connexin 43; markers of

functionally mature astrocytes, as early as 2 weeks-post plating. Furthermore, little change in marker expression is observed after 2 weeks, allowing co-culture neuron plating to be viable across a wide time-frame. These findings confirm that our protocol reliably produces neurons, astrocytes and co-cultures. Obtaining viable cultures of spinal neurons from postnatal mice has been a challenge in the past, making this approach of interest to the broader motor control and MND communities. We expect that this system, in combination with genetic models in mice, will serve as a valuable tool to study neuro-glial interactions in health and disease.

Disclosures: **S. Burley:** None. **C. Bonthron:** None. **M. Broadhead:** None. **V. Metodieva:** None. **S. Grant:** None. **G. Miles:** None.

Digital Abstract Session

P127. Motor Neuron Disease: ALS and SMA

Program #/Poster #: P127.04

Topic: C.06. Neuromuscular Diseases

Support: NIH DP2 NS106664
NYSCF-I-R44
ALS Association 12-PDF-564
NINDS F32 NS114319
The Cullen Education and Research Foundation

Title: The role of autophagy in ALS pathology in iPSC-derived motor and cortical neurons

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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a devastating and rapidly fatal disorder of motor neurons. 10% of cases have a familial history, with more than 40 individual genes implicated in a broad range of molecular functions, including protein homeostasis. Indeed, cytoplasmic protein aggregates, containing the autophagy protein p62 (SQSTM1/p62) in more than 98% of ALS cases, represent a key pathological hallmark of ALS. This points to autophagy as a conserved disrupted pathway across a broad range of ALS cases. However, the precise role and timing of autophagy disruption in ALS pathology is still controversial. This study aims to characterize the time-dependent function of autophagy in ALS and decipher key molecular mechanisms upstream and downstream of autophagy disruption. We hypothesize that autophagy dysregulation is an early event that centrally contributes to ALS onset and progression. We use three isogenic pairs of iPSC-derived spinal and cortical neurons, each iPSC pair harboring one of three ALS mutations (TDP-43^{G298S}, PFN1^{G118V}, and SOD1^{G85R}) within a single genetic background, and a cell line carrying C9ORF72 hexanucleotide repeat expansion. We employed immunofluorescence, molecular quantification of autophagy proteins, and live imaging of mitochondria, lysosomes, and autophagosomes at different time points in the maturation of the neurons. Our preliminary data show that autophagy is deficient in all of the ALS cell lines, with

evidence of time dependent accumulation of lysosomes, mitochondria, p62, and ubiquitin. Interestingly, the ALS lines differed in the extent and speed of progression of autophagy deficiency, suggesting ALS-mutation dependent effects on autophagy disruption. To investigate the relationship between autophagy and other pathways affected in ALS, we plan to treat the iPSC neurons with autophagy inducers and inhibitors and assess other ALS-associated pathways including mitochondrial function, DNA damage, and inflammation. This project is intended to provide insight into molecular pathways dysregulated in disease and identify pathways and targets for the development of therapeutic strategies for ALS.

Disclosures: **K. Dorfman:** None. **C. Marques:** None. **A. Held:** None. **J. Sung:** None. **M. Adler:** None. **B.J. Wainger:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; BJW is the PI for the Ezogabine (Retigabine) ALS clinical trial (NCT02450552). F. Consulting Fees (e.g., advisory boards); BJW is on the scientific advisory board for Quralis.. Other; BJW is a New York Stem Cell Foundation - Robertson Investigator.

Digital Abstract Session

P127. Motor Neuron Disease: ALS and SMA

Program #/Poster #: P127.05

Topic: C.06. Neuromuscular Diseases

Support: NIH-NIGMS Centers of Biomedical Research Excellence

Title: New TDP-43 Proteinopathy Model for ALS

Authors: *M. DOPLER, M. GITCHO;
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Abstract: New TDP-43 Proteinopathy Model for ALS Amyotrophic Lateral Sclerosis (ALS) is characterized by the death of both upper and lower motor neurons. After diagnosis, the average lifespan of the patient is ~5 years, most commonly due to respiratory failure and pneumonia. There are currently no effective treatments for ALS. In addition, 97% of cases are pathologically characterized by cytoplasmic accumulation of hyperphosphorylated and ubiquitinated transactive response DNA binding protein 43 (TDP-43). Familial mutations in TDP-43 account for ~5-10% of all ALS cases. In order to develop better drug treatments, we must have effective animal and cellular models to represent the disease. To this end, we have inserted three familiar mutations (A315T, M337V, S379P) in a TDP-43 construct (3X-TDP-43) in order to create a new cellular pathological model. Cells transfected with 3X-TDP-43 into HEK293T and SH-SY5Y have many of the hallmark characteristics found in ALS. This cellular model induces cytoplasmic accumulation of phosphorylated TDP-43 and a distinct change in solubility that has only been observed in human cases of TDP-43 proteinopathy. Based on these findings, we hope to develop

a better understanding of the disease process. This cellular model may provide a better avenue in the development of therapeutics targeting aggregation.

Disclosures: **M. Dopler:** None. **M. Gitcho:** None.

Digital Abstract Session

P127. Motor Neuron Disease: ALS and SMA

Program #/Poster #: P127.06

Topic: C.06. Neuromuscular Diseases

Support: 1ZIANS003155-04

Title: Developing a human iPSC-derived model of the neuromuscular junction to study functional phenotypes of neurodegeneration

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Abstract: Despite advances in rodent models of neurodegeneration, the basic mechanisms linking pathology and genetic function remain unknown. This knowledge gap persists because of technical limitations to evaluate the consequences of mutations and protein loss/gain of function in parallel. Human-induced pluripotent stem cells (iPSCs) provide the advantage of systematically testing disease-related mutations on specific cellular phenotypes on an isogenic background. In this study, we engineered two transgene cassettes to differentiate iPSCs into motor neurons (Ngn2-Isl1-Lhx3; hNIL) or skeletal myocytes (MyoD1-shRNAOct4; MyoD-shO). We used the PiggyBac transposase system to rapidly and efficiently deliver the doxycycline-inducible transcription factor technology into iPSCs. Upon doxycycline induction, the hNIL cassette derived a motor neuron phenotype, as previously shown by our group. The MyoD-shO differentiation resulted in a myotube-like morphology at day 5 and skeletal muscle markers expression at day 14 (MHC, SAA, MyoD1). Next, we optimized the cell density and media conditions to obtain a sustainable co-culture system. Phase-contrast microscopy revealed a successful co-culture of motor neuron aggregates and skeletal muscle myoblasts. To investigate functional phenotypes, we developed a ChR2 and a gCaMP7 motor neuron iPSC lines. Short light pulses induced skeletal muscle contraction, suggesting that the co-culture system recreates functional neuromuscular units. Ongoing experiments will assess the consequences of mutations in a multi-cell system, providing new insights into the cell-autonomous mechanisms driving neurodegeneration. With this newly developed tool, these forthcoming studies will allow us to fully characterize phenotypes that evolve from genetic mutations in every aspect of the neuromuscular unit.

Disclosures: **J.M. Colon Mercado:** None. **O.F. Vila:** None. **A. Snyder:** None. **T.C. McDevitt:** None. **M.E. Ward:** None.

Digital Abstract Session

P127. Motor Neuron Disease: ALS and SMA

Program #/Poster #: P127.07

Topic: C.06. Neuromuscular Diseases

Support: Fund "Innovative Medical Research" of the University of Muenster Medical School, PA 5 2 19 01

Title: Infantile superoxide dismutase 1 deficiency syndrome (iSODDES): phenotype of eight children with a novel motor neuron disease

Authors: *J. H. PARK[1], U. NORDSTRÖM[1], A. BOTZEN[2], S. ALBURAIKY[3], M. NOLAN[4], C. ELPERS[5], E. BOLTSHAUSER[7], R. HELLER[3], T. MARQUARDT[6], A. HAHN[2], R. SANTER[8], S. L. MARKLUND[1], P. M. ANDERSEN[9];
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Abstract: Mutations in *SOD1* account for a proportion of adult-onset ALS. The vast majority of these act in a Mendelian-dominant fashion with varying degrees of penetrance although homozygous mutations have been shown to cause ALS in a subset of patients. The homozygous truncating mutation c.335dupG/p.C112Wfs*11 has recently been identified as leading to absence of SOD1 enzymatic function and a severe pediatric motor neuron disease. The revelation of a distinct neurological phenotype in patients with loss of SOD1 function has major implications for currently explored therapeutic approaches to ALS. Here, we report on the phenotype of 8 pediatric patients homozygous for the c.335dupG variant.
Establishing a cohort of eight individuals homozygous for *SOD1* c.335dupG, metabolomic studies relying on mass spectrometry were used to identify markers of oxidative stress and organ damage. MR imaging and -spectroscopy, neurophysiological and in-depth clinical examinations were employed to characterize their phenotype.
The patients showed a remarkably similar course of symptoms with onset of disease within the first year of life. Following the development of truncal muscular hypotonia beginning at age 6-9 months, severe spastic paresis developed first in the limbs and then in bulbar-innervated muscles. Frontal lobe involvement with affective lability has appeared in some. cMRI identified some cortical atrophy. EMG revealed some signs of neurogenic denervation.
In summary, absence of SOD1 enzyme activity caused by the homozygous truncating variant *SOD1* c.335dupG leads to a severe neurodevelopmental phenotype that can be subsumed under the term infantile superoxide dismutase 1 deficiency syndrome (*iSODDES*). Further research is needed to understand the relevance for SOD1 silencing as a treatment of ALS.

Disclosures: **J.H. Park:** None. **U. Nordström:** None. **A. Botzen:** None. **S. Alburaiky:** None. **M. Nolan:** None. **C. Elpers:** None. **E. Boltshauser:** None. **R. Heller:** None. **T. Marquardt:** None. **A. Hahn:** None. **R. Santer:** None. **S.L. Marklund:** None. **P.M. Andersen:** None.

Digital Abstract Session

P127. Motor Neuron Disease: ALS and SMA

Program #/Poster #: P127.08

Topic: C.06. Neuromuscular Diseases

Title: Genetic variants and alterations in WWOX affect mitochondrial function and neuronal survival in amyotrophic lateral sclerosis

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Abstract: The precise molecular mechanisms leading to motor neuron degeneration in amyotrophic lateral sclerosis (ALS) are not yet completely understood. Therefore, identifying the underlying pathogenic mechanisms and disease-modifiers capable of altering the course of ALS is vital for the development of new therapies. One such candidate is the WW domain-containing oxidoreductase (WWOX), which plays a role in DNA damage response, oxidative stress, neuronal differentiation, and neurodegeneration. Here, we investigated whether and how alterations in WWOX signaling may contribute to ALS pathogenesis. Our results demonstrate a significant decrease in WWOX levels in a large cohort of ALS post-mortem motor cortex (mCTX). Genetic analysis also revealed several rare genetic variants in WWOX in 4,366 ALS patients from Project MinE that were completely absent in gnomAD. Four of these variants were associated with a high Combined Annotation Dependent Depletion score (CADD) (>25), including rWWOX^{G57E} in the WW2 domain, WWOX^{STOP261E} in the mitochondria binding region of the short-chain alcohol dehydrogenases (SDR) domain, and rWWOX^{STOP353Q} and rWWOX^{A363P} in the D3 region containing a pro-apoptotic tail. Interestingly, it has been suggested that the SDR domain may regulate the mitochondrial electron transport chain (mtETC). Consistent with previous studies showing changes in the mtETC in ALS, our results revealed a significant decrease in the levels of the ATP synthase subunit alpha of complex V (ATP5A) and the cytochrome c oxidase of complex IV (COX II or MTCO2) in ALS mCTX. Additionally, co-immunoprecipitation experiments revealed that WWOX interacts with ATP5A in ALS post-mortem mCTX. To determine whether the novel ALS mutations in WWOX may affect cell survival or alter mitochondrial function, SH-SY5Y cells were treated with human recombinant rWWOX^{WT}, rWWOX^{G57E}, rWWOX^{STOP261E}, rWWOX^{STOP353Q}, and rWWOX^{A363P} proteins. Our results indicate that the treatment with mutant rWWOX proteins reduced cell

viability in human neuroblastoma SH-SY5Y cells as measured by an MTT assay and the levels of proteins involved in pro-survival pathways. Furthermore, the treatment with rWWOX^{STOP261E} affected the levels of several proteins involved in the mtETC in SH-5YSY cells as well as ATP and reactive oxygen species (ROS) production. Collectively, our findings suggest that alterations in WWOX signaling may lead to mitochondrial dysfunction and motor neuron death in ALS.

Disclosures: S.E. Kim: None. **T. Petrozziello:** None. **A.N. Mills:** None. **S.M.K. Farhan:** None. **K. Vakili:** None. **G. Sadri-Vakili:** None.

Digital Abstract Session

P127. Motor Neuron Disease: ALS and SMA

Program #/Poster #: P127.09

Topic: C.06. Neuromuscular Diseases

Support: R21NS102911
Pick Grant

Title: Uncovering the pathological role of astrocytes in SMA

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Abstract: Spinal muscular atrophy (SMA) is the most common genetic cause of infant mortality and is caused by mutations of the survival motor neuron 1 gene (SMN1) resulting in reduced expression of the SMN protein. Motor neuron loss is the primary phenotypic outcome in SMA patients though it is unclear why motor neurons are particularly impacted. SMA motor neurons show intrinsic deficits in splicing and electrophysiological function, but these defects alone are not sufficient to induce overt motor neuron loss. Data from us and others indicate that there are significant non-cell autonomous astrocyte and microglia influences on motor neuron health and survival, raising the possibility that glia directly contribute to the vulnerability of neurons in SMA.

We have previously found that SMA astrocytes undergo morphological and functional changes very early in disease and are capable of inducing motor neuron pathology. We found that SMA astrocytes have reduced growth factor release and abnormal microRNA production. Additionally, others have shown that SMA microglia exhibit activation later in the disease process and are involved in synapse engulfment. However, the mechanisms of glia-mediated neuron dysfunction and the temporal contributions of astrocyte-microglia crosstalk to SMA pathology have not been elucidated.

We hypothesize that early SMA astrocyte malfunction induces motor neuron pathology and simultaneously activates microglia. In support of this, we found that induced pluripotent stem cell (iPSC)-derived astrocytes exhibit aberrant expression of the transcription factor GATA6, increased NFκB nuclear localization, and increased complement factor C3 release. Moreover, we

found that SMA iPSC-derived astrocyte conditioned medium (ACM) is sufficient to induce motor neuron death. Interestingly, lentiviral knockdown of GATA6 in SMA iPSC-derived astrocytes abrogated ACM-mediated motor neuron toxicity. Separately, we found that SMA iPSC-derived microglia adopted a reactive morphology, increased phagocytosis, and increased expression of complement C1q compared to control microglia. Exposure of SMA iPSC-derived microglia to SMA ACM significantly increased their phagocytic activity compared to untreated microglia, consistent with a role of astrocytes in exacerbating microglial activation in SMA. These data indicate the significant role of astrocyte secreted factors in contributing to disease phenotypes in SMA. Gaining a better understanding of the non-cell autonomous processes involved in SMA will help elucidate the mechanisms of motor neuron malfunction and can help identify targets for therapeutic intervention.

Disclosures: R.L. Allison: None. **G. Khayrullina:** None. **B.G. Burnett:** None. **A.D. Ebert:** None.

Digital Abstract Session

P128. Cellular Stress and Death Mechanisms

Program #/Poster #: P128.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Novel neuroprotective mechanisms of cannabidiol (CBD) against tetramethylene-disulfotetramine toxicity to hippocampal neurons

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Abstract: Tetramethylenedisulfotetramine (TETS), a potent rodenticide and GABAA inhibitor is a chemical terrorist warfare agent that can produce severe seizures, progressive neurodegeneration, and neurocognitive deficits in humans and animals. Cannabidiol (CBD), a non-psychoactive cannabinoid, has anticonvulsant, antioxidant, and antinflammatory effects that may mitigate acute TETS-induced neuropathophysiology. We *hypothesized* that presynaptic Ca^{2+}-dependent neurotransmitter release is a critical target of CBD and that midazolam (MDZ), a GABAergic positive modulator, will facilitate CBD-induced neuroprotection. Therefore, expression of a Ca^{2+} binding protein calmodulin 2 (Calm2), a Ca^{2+} specific controller of several signaling proteins and two high voltage-gated Ca^{2+} channels (VGCC), P/Q and Cav1.2 (L type) to which they bind were immunocytochemically investigated with specific antibodies. At 14 DIV, dissociated hippocampal and sister cortical cultures were exposed to TETS (10 μM) ± CBD (15 μM) ± MDZ (10 μM) for 24 h. $[Ca^{2+}]_i$ was measured with a Flexstation 3 at 25ºC at varied doses of CBD and MDZ following acute TETS application. TETS reduced Calm2 intensely and lightly stained hippocampal neurons; many cells were shrunken. When TETS was co-treated with CBD (15 μM), hippocampal Calm2 and P/Q expression was elevated and intensely-stained neurons were increased; morphology was preserved. In contrast, MDZ reduced Calm2 expression and further reduced P/Q positive cell counts at the concentration tested. Cav1.2

immunostaining increased after TETS in hippocampal neurons; many had compromised morphology which was reversed by either CBD or MDZ. Sister cortical neuronal cultures grown from dissected embryos under the same conditions were nearly unaffected by any of the treatments. Acute application of CBD + MDZ post TETS produced greater suppression of $[Ca^{2+}]_i$ signals than either agent alone suggesting a potential co-treatment strategy. Results suggest a novel mechanism of CBD neuroprotection against severe seizures by suppression of persistent elevations in $[Ca^{2+}]_i$ and upregulation of critical Ca^{2+} binding proteins. Hippocampal neurons were more sensitive to TETS or MDZ toxicity than cortical neurons so that lower non-saturating doses of MDZ would be required to avoid injury to hippocampal Ca^{2+} binding proteins, critical for neuronal survival. At 100 μM CBD, suppression on $[Ca^{2+}]_i$ was maximal in absence of MDZ suggesting CBD alone may improve the standard of care for chemical threats.

Disclosures: L.K. Friedman: None. **M. Lauková:** None. **M.P. Shakarjian:** None.

Digital Abstract Session

P128. Cellular Stress and Death Mechanisms

Program #/Poster #: P128.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Expression of caspase 3, 8, cytochrome C, VDA-C, TUNEL, BAX and BCL-2 by exposure to ozone in rats cerebellum

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Abstract: Ozone (O3) is one of the major air pollutants around the world, it is created by a photochemical reaction of volatile organic compounds, nitrogen oxides and carbon monoxide that accumulates in the troposphere. According to the worldwide organization stipulates that an O3 concentration above 100 μg/m3 (510 parts per million) for 8 hours can be dangerous for humans causing moderate to severe effects in health. Several studies about the exposure to O3 have been made in animal models, concluding to an increase in oxidative stress, resulting in lipoperoxidation, inflammation, metabolic disturbances, alteration in cell signaling and the possible initiation of cell death in vulnerable areas such as the olfactory bulb, cerebellum, striatum, hippocampus and frontal cortex which are not fully described. The inflammatory response can represent an initial factor of cell death since high concentration of TNF-α can activate caspase 3, which is one of the main effectors to bind substrates such as immune molecules that causes cell death. Apoptosis is a programmed cell death, which activates caspases leading to DNA fragmentation, forming and phagocyting apoptotic bodies with or without an inflammatory response. Apoptosis onset can occur through 2 pathways: intrinsic (mitochondrial)

and extrinsic (death receptors), where multiple proteins interfere. Therefore, we aim to evaluate the participation of caspase 3 and caspase 8, BCL-2, cytochrome C, VDA-C, TUNEL and BAX proteins, in the initial damage resulting from ozone exposure in rats cerebellum. We used ten male Wistar rats, who were randomized, in two groups, a control and experimental equally divided. The experimental group was subjected to a hermetic ozone chamber for 12 hours provided with 1 ppm of O3, subsequently they were immediately sacrificed to perform immunohistochemistry analysis. Compared to the control group, we found a decreased in BCL-2, an anti-apoptotic protein, and an increased in TUNEL, caspase 3, 8 and BAX who trigger the cell death, this indicates that the two pathways (extrinsic and intrinsic) were activated. We also found a decreased in VDA-C and cytochrome C, this could be explained because of the release of cytochrome C is independent of its oxidation state and it becomes reduced in the cytosol. O3 induce cell death through the extrinsic and intrinsic apoptotic pathways, this could explain some manifestations in the rats such as motor alterations.

Disclosures: **P.M. Rodríguez Quintero:** None. **W. Moreno:** None. **R. Luna:** None. **L.F. Hernandez:** None. **V. Custodio:** None. **V. Custodio:** None. **J. Brito:** None. **C.R. Osornio:** None.

Digital Abstract Session

P128. Cellular Stress and Death Mechanisms

Program #/Poster #: P128.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Early S phase events protect neurons from amyloid-β toxicity

Authors: *Y. DENG[1], S. IPPATI[2], E. PARIC[1], G. CHAN[1], A. FEITEN[1], T. FATH[1], Y. KE[1], N. K. HAASS[3], L. M. ITTNER[1];
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Abstract: Neurons are post-mitotic cells that do not undergo division after terminal differentiation. Reactivation of neuronal cell cycle has been reported in Alzheimer's disease (AD) brains and models. Accordingly, the hypothesis has been put forward that re-entering the cell cycle renders neurons vulnerable contributing to AD pathogenesis. Here, we observed transient cell cycle activation events in wild-type neurons by using the fluorescent ubiquitination-based cell cycle indicator (FUCCI) mAG-hGem. Interestingly, neurons have increased mAG-hGem activity did not undergo cell death under the oligomeric amyloid-β (oAβ) challenge. We further use two FUCCI reporter mKO2-hCdt1 and mAG-hGem together to visualize stages of the cell cycle in living cells, where the cell will show red color (mKO2) during the G1(G0) phase and green color (mAG) during S/G2/M stage of cell cycle. Consistent with our previous finding, nearly all neurons are mKO2 positive. However, transient mAG activity can also be detected even those neurons continue to express mKO2-hCdt1. It is noted that the transient mAG-positivity was self-limiting in all recorded cells and only lasted for

several hours. Furthermore, even increasing mAG activity is observed under oAβ challenge, there is no DNA synthesis and replication in these cells. Accordingly, we found high-intensity mAG activity in the brains of human mutant Aβ precursor protein transgenic (APP23) mice. Additionally, increased neuronal expression of the endogenous cell cycle control protein geminin in 3-month-old APP23 mice and human AD brains has been detected. Taken together, our data suggest that the post-mitotic state of neurons is a dynamic process that happens naturally and the rapid induction of pathways that lead to early S phase cell cycle events in response to Aβ may protect neurons and prevent or delay cell death in AD.

Disclosures: **Y. Deng:** None. **S. Ippati:** None. **E. Paric:** None. **G. Chan:** None. **A. Feiten:** None. **T. Fath:** None. **Y. Ke:** None. **N.K. Haass:** None. **L.M. Ittner:** None.

Digital Abstract Session

P128. Cellular Stress and Death Mechanisms

Program #/Poster #: P128.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DGAPA PAPIIT IN220120

Title: Static magnetic fields modulate the response of the brain to different oxidative stress markers in a restraint stress model animal

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Abstract: Stress alters physiology and behavioral responses; it can induce oxidative damage in organs, such as the brain. Several investigations reveal that magnetic stimulation could modulate the response to oxidative stress and be an effective complementary therapy in different pathologies. The study aimed to describe the effect of static magnetic fields (SMF) in an animal model of restraint stress, focusing on changes in different markers of oxidative damage: nitric oxide (NO), malondialdehyde (MDA), advanced protein oxidation products (AOPP), superoxide dismutase (SOD), and reduced glutathione concentration (GSH), in the brain. Male Wistar rats were used and separated into seven groups (n = 8 each): control, restriction animals during the 30, 60, or 240-minute/day, and rats with restriction plus SMF exposition (O.8mT) during the 30, 60, or 240-minute/day for 5 days. After this period of exposure, the brain of rats was obtained and measured the oxidative markers. Our results show time dependent SMF effects. We found decreasing brain's lipoperoxidation at 60 and 240 minutes of SMF exposure. The magnetic fields can modulate the oxidative radicals in some tissues. SMF could offer complementary therapy in various pathologies, such as neurodegenerative diseases, to reduce oxidative stress.

Disclosures: **F. Estrada Bernal:** None. **J. Arroyo-Pérez:** None. **F.E. Rojo:** None. **L. Navarro:** None. **L. Verdugo-Diaz:** None.

Digital Abstract Session

P128. Cellular Stress and Death Mechanisms

Program #/Poster #: P128.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: VA merit grant

Title: Genotoxic stress induced by a small interfering peptide derived from heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1)

Authors: ***S. LEE**, Y. SHIN, J. W. TSAO;
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Abstract: Neurodegenerative diseases, such as Alzheimer disease (AD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS) are known to be associated with loss of hnRNPA1, but its detailed role is not well characterized. We confirmed an increase in hnRNPA1 truncated in the central nervous system and a decrease in the expression of hnRNPA1A mRNA in PBMCs with disease progression in the experimental autoimmune encephalomyelitis (EAE) animal model of MS. In order to maintain homeostasis of hnRNPA1 in neurons, the quality and quantity of the protein were controlled by cleavage between $AA^{245-286}$ of hnRNPA1, and a hnRNPA1 N-terminal fragment (AA^{1-267}) of approximately 30kDa was produced. However, the C-terminal fragment ($AA^{268-320}$) of roughly 6kDa could not be confirmed with a commercial antibody. It is thought that the epitope for the antibody is not preserved due to the involvement of various proteases. We have previously confirmed the neuronal cytotoxicity of 6kDa C-terminal fragment ($AA^{268-320}$), and a peptide sequence ($^{306}GGSSSSS^{313}$) truncated from the 6kDa C-terminal fragment ($AA^{268-320}$) was generated using the PeptideCutter online tool to predict the potential cleavage site of the protease. A monoclonal antibody was created and used to confirm the presence of $^{306}GGSSSSS^{313}$. To examine the role of $^{306}GGSSSSS^{313}$ peptide-induced neurotoxicity, PCR array analysis of a panel of apoptosis-related genes (PAHS-012Z, Qiagen) was performed using SK-N-SH cells expressing hnRNPA1 fragments (AA^{1-267}, $AA^{306-313}$). Analysis of the apoptosis process through PCR array technology demonstrated high expression of tumor protein p53(TP53) and caspase3(CASP3) when compared to control with $^{306}GGSSSSS^{313}$-transfected SK-N-SH cells. We conclude that $^{306}GGSSSSS^{313}$ induces neuronal death through the p53 pathway. In addition, in the $^{306}GGSSSSS^{313}$-transfected cells, polyadenylate-binding protein 1 (PABPC1) is relocated to the nucleus. This rearrangement of PABPC1 into the nucleus requires a decrease in protein synthesis by inhibiting protein translation. Thus, $^{306}GGSSSSS^{313}$ derived from hnRNPA1 induces a significant loss of hnRNPA1, which is involved in the stability of RNA and DNA in neurons. This results in mRNA processing disorders and genotoxic stress, suggesting a major role for this protein fragment in neuronal death in neurodegenerative diseases.

Disclosures: **S. Lee:** None. **Y. Shin:** None. **J.W. Tsao:** None.

Digital Abstract Session

P128. Cellular Stress and Death Mechanisms

Program #/Poster #: P128.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Role of related MAP triple kinases DLK and LZK in the vincristine-induced neuronal injury response in human neurons

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Abstract: Dual leucine zipper kinase (DLK; MAP3K12) and leucine zipper kinase (LZK; MAP3K13) arekey mediators of the neuronal injury response shared among neurodegenerative conditionssuch as amyotrophic lateral sclerosis (ALS) and chemotherapy-induced neuropathic pain(CIPN). These related MAP3 kinases initiate a transcriptional cascade resulting inphosphorylation of the transcription factor c-Jun, facilitating the fate of neuronal cell death.Previous studies show that, while DLK disruption is partially protective in models of optic nerveinjury and traumatic brain injury, combined disruption of DLK and LZK is ultimately required toprevent retinal ganglion cell (RGC) death (Welsbie et al, 2017 and 2019). Using human iPSC-derived cortical and sensory neurons, we sought to tease apart the distinctive and synergisticroles of DLK and LZK in the transcriptional response to neuronal injury. Single knockout iPSClines of DLK and LZK, as well as a DLK/LZK double knockout, were generated usingCRISPR/Cas9. Vincristine, a chemotherapeutic agent known to cause CIPN and activate theDLK/LZK pathway, induces partial c-Jun activation in both DLK KO and LZK KO neurons, butthis activation is blocked in DLK/LZK double KO neurons. Transcriptomics in these cell lines willelucidate the relative contributions of DLK and LZK to the vincristine-induced neuronal injuryresponse and potentially reveal new drug targets for neurodegenerative pathologies.

Disclosures: **A. Slavutsky:** None. **J. Gomez-Deza:** None. **C.E. Le Pichon:** None.

Digital Abstract Session

P129. Mechanisms of Neurotoxicity

Program #/Poster #: P129.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Grant-in-Aid for Scientific Research C 15K10993, 19K09442

Title: Demyelination of cerebellum in a murine heatstroke model

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Abstract: Introduction: Selective loss of Purkinje cells has previously been described in heatstroke. There is a report that 22-33 % of survivors were discharged with neurologic disability that remained sustained or worsened at 1- and 2-year follow-up. The damage is maximal in the cerebellum, resulting in marked loss of Purkinje cells, culminating in cerebellar atrophy with debilitating and permanent sequelae for survivors. Thought, it is not well understood the mechanism of delayed cerebellar syndrome. In our study, we investigated the delayed cerebellar syndrome using murine heatstroke model. **Methods:** Male C57BL/6J mice were subjected to heat exposure {(HE), 41 ± 0.5ºC, RH>95%} for 60 min. The rotarod performance test was used to evaluate the motor coordination after HE. Mice were trained once a week for 6 weeks. Then, they were divided into Control (Ct, no heat exposure, n=36) and Heatstroke (HS, n=36) groups depends on the score. The rotarod test was performed at 1, 3, 5, 7 and 9 weeks post HE. Another group (Ct and HS) were prepared. Animals were sacrificed 1, 3, 9 (n=6 in each group respectively) weeks post heat exposure and the brains were collected respectively. Seven sagittal section (5μm) of whole brains were made every 200μm from longitudinal fissure. Klüver-Barrera (KB) stain were performed to quantify the demyelination at cerebellum and corpus callosum. **Results:** The rotarod score initially decreased in the third week compared to the first week in the HS group, then improved from 5 weeks post HE, whereas no decrease was observed in the Ct groups and significant differences were observed at 3 weeks post HE ($p < 0.05$). Demyelination was significantly detected at cerebellum 1, 3 weeks post heat exposure ($p < 0.05$). Then, it improved and there was no differences 9 weeks post heat exposure. However, no significant differences were observed at corpus callosum. **Conclusion:** Motor coordination disorder appeared 1 weeks and it exacerbated to 3 weeks. Demyelination of cerebellum was also detected 1 and 3 weeks at cerebellum. These results indicate demyelination of cerebellum reflect the motor coordination failure of heatstroke.

Disclosures: A. Yoshikawa: None. **K. Miyamoto:** None. **H. Ohtaki:** None. **H. Yamaga:** None. **M. Nakamura:** None. **K. Suzuki:** None. **K. Honda:** None. **K. Dohi:** None.

Digital Abstract Session

P129. Mechanisms of Neurotoxicity

Program #/Poster #: P129.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Oak Ridge Associated Universities

Title: Behavioral Impacts of Pharmacological and Supportive Care in the Treatment of Status Epilepticus in a Rat Model of Soman Poisoning

Authors: *K. A. HUNDERTMARK[1], J. MORGAN[2], J. H. MCDONOUGH, Jr.[3], H. S. MCCARREN[4];
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Abstract: Behavioral Impacts of Pharmacological and Supportive Care in the Treatment of Status Epilepticus in a Rat Model of Soman Poisoning Kathryn A Hundertmark, Julia E Morgan, John H McDonough, and Hilary S McCarrenAbstract Soman (GD) is a highly toxic chemical compound that can result in severe health degradation following exposure. GD works by inhibiting the enzyme acetylcholinesterase from breaking down the neurotransmitter acetylcholine, resulting in status epilepticus (SE). Developing appropriate pharmacological and supportive care measures is important to increase survivability. This experiment used the open field test (OFT) and elevated plus maze (EPM) to evaluate treatment efficacy of animals who were treated for SE in an ICU setting compared to control animals following exposure to GD. Both behavioral tests evaluate the animal's locomotion and anxiety. Behavioral testing was conducted 4 days prior to exposure to GD and 9 days following exposure. Animals who were treated in the ICU (n = 14) were found to spend more time in the closed arms (p = 0.004), while the controls (n = 7) spent more time in the open arms (p = 0.0005). Compared to the ICU-treated group, the control group spent more time immobile (p = 0.011) when in the OFT. The ICU-treated group also made more entries into the closed arms when compared to the control group (p = 0.014). No significant differences were found between groups for total distance traveled in OFT or EPM. These data support ICU treatment efficacy with animals behaving near baseline measurements. The control group, however, experienced behavioral deficits such as lethargy and lack of inhibition following exposure to GD. Further research will be conducted using the novel object recognition test and home cage reactivity evaluation to more fully characterize the effects of GD-induced SE and subsequent treatments on behavior.

Disclosures: K.A. Hundertmark: None. **J.H. McDonough:** None. **H.S. McCarren:** None. **J. Morgan:** None.

Digital Abstract Session

P129. Mechanisms of Neurotoxicity

Program #/Poster #: P129.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: T32 GMO67795
R01 ES029035
R21 AG05700
Graduate College of the University of Iowa

Title: The inactivation of glutathione S-transferase by dopamine and its metabolites

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Abstract: Background. Neurodegenerative disorders such as Parkinson's disease are characterized by disruption of dopamine homeostasis and oxidative stress. The aldehyde metabolite of dopamine, 3,4-dihydroxyphenylacetaldehyde (DOPAL), is known to be cytotoxic through mechanisms of protein modification and oxidative stress. This research has identified glutathione S-transferase (GST) as a protein target of DOPAL; because GST is paramount in the body's antioxidant defense system, this has implications for mechanisms of neuronal injury. Therefore, this research examines the modification of GST by dopamine, DOPAL, and 3,4-dihydroxyphenylacetic acid (DOPAC). Furthermore, we have found that the amino acid L-cysteine is a scavenger of DOPAL and other biogenic aldehydes, forming an identifiable and measurable conjugate. This has implications in neuroprotection as well as the potential for a novel biomarker for dopamine dyshomeostasis. **Methods.** GST was isolated from the dopaminergic N27 cell line and treated with varying μM concentrations of DOPAL, dopamine, or DOPAC. GST activity was measured using a 2,4-dinitrochlorobezene assay. The time dependence and reversibility of this inhibition were evaluated. The ability L-cysteine to protect GST function was assessed. Mass spectrometric methods were employed to assess the mechanism of action of protection by L-cysteine and elucidate the formation of an L-cysteine-DOPAL conjugate. **Results.** DOPAL, dopamine, and DOPAC inhibited N27 GST activity with IC_{50} values of 31.46μM, 82.32μM, and 260.0μM, respectively. This inhibition was irreversible and time dependent. L-cysteine fully protected GST activity from modification by dopamine, DOPAL, or DOPAC. L-cysteine was also found to form a putative thiazolidine conjugate with DOPAL. This conjugate was also found to form in SH-SY5Y neurons after treatment with dopamine and n-acetyl cysteine, or when cells were treated with dopamine and exposed to stress. **Conclusions.** Dopamine and its metabolites inactive GST, which could lead to a breach in the antioxidant defense system. Therefore, we propose that this enzyme inhibition is a mechanism of neurotoxicity relevant to diseases that are marked by dopamine disruption, such as Parkinson's disease. Further, we propose that the L-cysteine-DOPAL conjugate may be a useful biomarker for dopamine dyshomeostasis.

Disclosures: R. Crawford: None. **K. Bowman:** None. **E. Gilardoni:** None. **B. Cagle:** None. **J. Doorn:** None.

Digital Abstract Session

P129. Mechanisms of Neurotoxicity

Program #/Poster #: P129.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIEHS Grant R01-ES024812
NIH/NIEHS Grant R01ES024812-03S1 (S.M.)
The University of Texas at Austin Graduate School Continuing Fellowship

Title: Activity of the Manganese Efflux Transporter SLC30A10 in Catecholaminergic Neurons Protects Against Manganese-induced Motor Dysfunction

Authors: *C. TAYLOR[1], S. HUTCHENS[2], T. JURSA[3], W. SHAWLOT[2], M. ASCHNER[4], D. R. SMITH[5], S. MUKHOPADHYAY[2];
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Abstract: Manganese (Mn) is an essential metal required for normal brain development and function. However, elevated levels are neurotoxic and lead to an incurable movement disorder. The specific neuronal subtypes most effected by Mn are still unknown, hindering therapeutic progress. To understand Mn neurotoxicity and subsequent motor deficits, our lab studies the primary Mn efflux transporter, SLC30A10. Our recent work revealed that activity of SLC30A10 in the brain reduced Mn levels in the basal ganglia and thalamus during overexposure (Taylor et al., 2019). Here, we use various *Slc30a10* knockout mice to selectively increase Mn levels throughout the brain (pan-neuronal/glial), in catecholaminergic neurons, or in GABAergic neurons. We found that pan-neuronal/glial *Slc30a10* knockouts exhibited hypolocomotion that is exacerbated by a human disease-relevant Mn exposure regimen. This hypolocomotion is not associated with changes in extracellular levels of striatal dopamine, the dopamine metabolite DOPAC, or GABA. However, catecholaminergic, but not GABAergic, knockouts also exhibited hypolocomotion. Thus, while elevated Mn may not lead to GABAergic or dopaminergic neurodegeneration, it may lead to dopaminergic dysfunction. Future work will use immunohistochemistry to confirm our hypothesis that elevated Mn does not lead to dopaminergic or GABAergic neurodegeneration. Future work will also use in vivo microdialysis to determine how Mn impacts dopaminergic function.

Disclosures: C. Taylor: None. **S. Hutchens:** None. **T. Jursa:** None. **W. Shawlot:** None. **M. Aschner:** None. **D.R. Smith:** None. **S. Mukhopadhyay:** None.

Digital Abstract Session

P129. Mechanisms of Neurotoxicity

Program #/Poster #: P129.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Circulating Biomarkers of CNS Damage: Translating Preclinical Endpoints to Clinical Outcomes

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Abstract: Neurotoxicity has been linked to exposure to a number of common drugs and chemicals, yet efficient and minimally-invasive methods for prediction of neurological changes are lacking. Fluid-based biomarkers such as those found in serum, plasma, urine, and cerebrospinal fluid (CSF) have great potential due to the relative ease of sampling, but data on their expression and clinical translation are lacking or inconsistent. As part of the HESI Neurotoxicity Biomarker Subcommittee, we present preclinical data on biomarkers that have some promise for detection and characterization of neurotoxicity and validate such data for their predictive potential. Trimethyltin is known to cause CNS neuropathology in species from mouse to man. A single dose of trimethyltin, TMT (7 mg/kg, ip) to the rat, led to significant alterations in lipid homeostasis, circulating interleukins and related factors, and markers of neuroinflammation in CSF, serum, plasma and urine. A significant correlation of these fluid biomarkers was observed with traditional neuropathology and magnetic resonance imaging (MRI) endpoints that served to define TMT-induced neurotoxicity. Our data demonstrate a novel correlations of several potential neurotoxicity biomarkers and MRI-based endpoints with TMT-induced neuropathology. These findings suggest of an involvement of specific pathways that can be assessed using peripheral fluids in a preclinical model. A clinical translation of these findings can be investigated by analyzing a blood-based safety biomarker panel of five nervous system-derived proteins in the serum of healthy volunteers and of patients with neurological disorders. As part of the IMI-2 TransBioLine Consortum, GFAP, UCH-L1, NFL, pNFH and Tau will be measured in the serum (or plasma if appropriate) of patients with multiple sclerosis (MS), those with non-inflammatory traumatic brain injury (TBI) and those undergoing chemotherapy. Data will be compared with the ranges in serum samples from healthy volunteers. In addition, these proteins will also be measured in the CSF of MS and TBI patients to enable possible correlations with serum data. If successful, regulatory qualification of GFAP, UCH-L1, NFL, pNFH and Tau as fluid-based safety biomarkers of drug-induced neurotoxicity will add to a better insight into the underlying mechanisms of neurotoxicity and its early detection, and to the safety of healthy volunteers in phase I clinical trials and patients treated with potentially neurotoxic drugs.

Disclosures: **S. Imam:** None. **R.A. Roberts:** None. **Z. He:** None. **S. Rogstad:** None. **J. Hanig:** None. **S. Burks:** None. **J. Raymick:** None. **B. Robinson:** None. **E. Cuevas:** None. **S. Sarkar:** None. **C. Law:** None. **D.W. Herr:** None. **D. Macmillan:** None. **A. Smith:** None. **S. Liachenko:** None. **J.P. O'Callaghan:** None. **C. Somps:** None. **I. Pardo:** None. **C. Aluise:** None. **G. Tuens:** None. **K. Ruprecht:** None. **M. Kothe:** None. **C. Otto:** None. **J. Pierson:** None. **B. Gong:**

None. **W. Tong:** None. **M. Aschner:** None. **M. Paule:** None. **M. Kallman:** None. **W. Slikker:** None.

Digital Abstract Session

P129. Mechanisms of Neurotoxicity

Program #/Poster #: P129.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NCRR Grant 5P20RR016469
NGIMS Grant 2P20GM103427-19

Title: Secondary structural analysis of enetrovirus D68 5'UTR to identify neurotropism determining structural elements

Authors: *P. OJHA, W. TAPPRICH;
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Abstract: Enterovirus D68 is a single-stranded positive-sense RNA virus of the picornaviridae family which utilizes its 5' untranslated region (5'UTR) to recruit ribosome and undergo cap-independent translation. First isolated in 1962 in California, EV-D68 only had minor cases of respiratory illness until 2014. Since the summer of 2014, reported outbreaks for EV D68 have been increasing with a strong association with polio-like acute flaccid myelitis (AFM). Ample evidence suggests that the 750 nucleotides long 5'UTR of enteroviruses include the internal ribosome entry site (IRES) which plays an important role in determining their virulence. Neurotropic strains of EV-D68 have an approximate 20 nucleotide deletion in the spacer region of their 5'UTR. Understanding the structural changes in 5'UTR of current EV D68 strains from the ones in 1962 can help determine the reason for its newly gained neurotropism. We hypothesize the interactions of 5'UTR of neurotropic strains with cell-specific host proteins and non-coding RNAs dictates its ability to exhibit neurovirulence. A robust secondary structure of the 5'UTR is being generated by using the SHAPE- MaP analysis. This method involves chemical modification of the 2' hydroxyl group of nucleotides in the RNA molecules based on their position and flexibility. These modified molecules are converted into cDNA to create high-quality mutational profiles (MaP), which are then subjected to massively parallel sequencing. By using computational tools like the *shapemapper2* and *superfold* to analyze the NGS data, a secondary structure of the 5'UTR can be generated. Elucidating novel 5'UTR secondary structures of EV D68- Fermon (1962) and EV D68- KT347251.2 (2014) strains can reveal the structural changes leading to neurotropism. These novel structures of 5'UTR of EVD68 can also be utilized for comparative studies of 5'UTRs between other neurotropic enteroviruses like EV 71- KF312457.1 (1998), Polio Virus, and non-neurotropic enteroviruses like CVB3 to find key shared structures involved in determining the virulence of enteroviruses. To better characterize the specific roles of key conserved structural elements between neurotropic enteroviruses, a set of in-vivo experiments involving mutated EV-D68 strains are being designed.

Disclosures: **P. Ojha:** None. **W. Tapprich:** None.

Digital Abstract Session

P129. Mechanisms of Neurotoxicity

Program #/Poster #: P129.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: James and Esther King Biomedical Research Grant 9JK08

Title: Chronic nicotine exposure increases blood-brain barrier permeability and decreases tight junction protein levels in rats.

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Abstract: Tobacco use is one of the leading causes of preventable death. Tobacco use not only increases the risk of but also worsens outcomes following ischemic and hemorrhagic stroke in both men and women. The blood-brain barrier (BBB) separates the circulating blood from the central nervous system (CNS) by capillary endothelial cells sealed together with tight junction proteins, and any disruptions of the BBB could significantly impact the CNS. Our hypothesis is that chronic nicotine increases the risk of cerebrovascular diseases by increasing BBB permeability. To determine the effect of nicotine on the BBB, we evaluated BBB disruption using Evans blue dye (EBD) extravasation and measured the tight junction protein levels in animals of both sexes treated with nicotine. Young Sprague-Dawley rats of both sexes were implanted with an osmotic pump containing either nicotine (4.5 mg / kg / day) and saline (vehicle group) and treated for two to three weeks. After, EBD was injected into the animals intravenously. One hour later, animals were perfused with saline, and brains were harvested, weighed, homogenized, and spectrophotometrically analyzed for EBD. In addition, microvessels were isolated from brain tissue, and tight junction protein levels were measured using Western blot. Brain tissue from female animals was collected during the diestrous stage of the estrous cycle to avoid hormonal fluctuation. EBD content in male animals was significantly higher in nicotine-treated rats (800 ± 100 ng / g of brain tissue) than saline-treated rats (284 ± 74 ng / g of brain tissue) by 182% ($p<0.01$). EBD content in female animals was also significantly higher in nicotine-treated rats (800 ± 100 ng / g of brain tissue) than saline-treated rats (284 ± 74 ng / g of brain tissue) by 98% ($p<0.05$). In male animals, Claudin 3, Claudin 5, and occludin levels were significantly lower by 48% ($p<0.05$), 37% ($p<0.05$), and 39% ($p<0.01$), respectively, in nicotine-treated rats than in saline-treated rats. In female rats, Claudin 3, claudin 5, and occludin protein levels were also significantly lower in nicotine-treated rats than in saline-treated rats by 31% ($p<0.05$), 44% ($p<0.05$), and 38% ($p<0.01$), respectively. We are in the process of evaluating the protein levels of other BBB proteins. Our results, so far, show that nicotine exposure increases BBB permeability and decreases tight junction protein levels in animals of both sexes. This increased BBB permeability may be responsible for the increased risk of and worsened outcomes following stroke.

Disclosures: S. Cho: None. **A.K. Rehni:** None. **A.P. Raval:** None. **M.A. Perez-Pinzon:** None. **K.R. Dave:** None.

Digital Abstract Session

P130. Neuroinflammation: Neurodegeneration

Program #/Poster #: P130.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Immunohistochemical detection of $ChAT^+$ cells in and around multiple sclerosis lesions

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Abstract: Recently, clemastine was found to have clinical benefit in human multiple sclerosis (MS) patients (Green et al., 2017). Using rodent models of MS and selective knockout of muscarinic receptor subtypes, it was established that clemastine induces OPC differentiation and remyelination via inhibition of the muscarinic acetylcholine receptor M1 (M1R; Mei et al., 2016). Direct evidence for M1R protein expression on cells of oligodendroglial lineage, however, has been lacking in human tissue. Using an M1R selective fluorescent probe, we observe $M1^+/Olig2^+$ cells in adult human cortex, optic nerve, corpus callosum, spinal cord, and cerebellum. Further, using adult human organotypic slice cultures, we show that an M1R selective antagonist, PIPE-307, is sufficient to induce oligodendrocyte markers by PCR and immunohistochemistry. These suggest that M1R overactivation by acetylcholine (ACh) may impede remyelination. Coincidentally, Jiang et al., 2017 reported upregulation of ACh and the ACh-synthesizing enzyme choline acetyltransferase (ChAT) in the MOG-EAE mouse model of MS. In the same study, they observed elevated ACh and $ChAT^+$ natural killer cells in human MS tissue. To expand on these findings, we performed immunohistochemistry on human MS tissue and observe the presence of $ChAT^+/HLA\text{-}DR^+$ (Human Leukocyte Antigen - DR isotype) cells at the border of and within lesions. These $ChAT^+$ cells also express the macrophage/microglial marker $IBA1^+$ (ionized calcium binding adaptor molecule 1), but are negative for the microglia specific marker, TMEM119 (transmembrane protein 119), suggesting that the aberrant ACh is, in part, a result of ChAT-expressing infiltrating immune cells. The expression of M1R on $Olig2^+$ cells and $ChAT^+$ immune cells in proximity of human MS lesions support a model whereby excess ACh released by $ChAT^+$ immune cells leads to increased M1R activity in effect hindering OPC differentiation (and subsequent myelin repair) at the lesion. Further studies will be needed to ascertain if there is a correlation between lesion status and ChAT expression. Nonetheless, this data provides an impetus for the use of an M1R selective antagonist as a remyelinating therapy for multiple sclerosis.

Disclosures: M.M. Poon: A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); Pipeline Therapeutics. **J. Chan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. F. Consulting Fees (e.g., advisory boards); Pipeline Therapeutics. **A.J. Green:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. F. Consulting Fees (e.g., advisory boards); Pipeline Therapeutics. **E. Peles:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. F. Consulting Fees (e.g., advisory boards); Pipeline Therapeutics. **J. Baccei:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **A.C. Chen:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **D.S. Lorrain:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics.

Digital Abstract Session

P130. Neuroinflammation: Neurodegeneration

Program #/Poster #: P130.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Discovery of a Novel M1 Selective Antagonist, PIPE-307, for the Treatment of Multiple Sclerosis

Authors: K. I. LORRAIN[1], M. M. POON[1], A. BROADHEAD[1], K. J. STEBBINS[1], A. CHEN[1], J. BACCEI[1], A. J. GREEN[2], J. R. CHAN[2], ***D. S. LORRAIN**[1];
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Abstract: Multiple sclerosis (MS) is an inflammatory demyelinating disease that results in the disruption of neuronal transmission and ultimately neurodegeneration. Current treatments focus on suppressing the immune system to limit inflammation and the further loss of the myelin sheath. The next advance in the treatment of MS has focused on molecules that regulate remyelination. The M1 muscarinic acetylcholine receptor (M1R) has been shown to be a key regulator in the maturation of oligodendrocyte precursor cells (OPCs) into oligodendrocytes (OLs), the cells that make myelin. This discovery was based on non-selective anti-muscarinic compounds such as Clemastine and Benzetropine and subsequently validated through cell type specific M1R knockout studies. Building from this initial discovery, Pipeline Therapeutics initiated a medicinal chemistry effort to discover a novel M1R selective antagonist. These efforts resulted in PIPE-307, a novel, potent, and selective, first-in-class small molecule antagonist of the M1 receptor. Significantly, PIPE-307 produces robust effects in OPCs driving them towards differentiation and expression of myelin basic protein. Furthermore, PIPE-307 elicited positive

results in a diverse set of *in vitro* assays, including OPC differentiation, cortical myelination, and organotypic brain slice. *In vivo* visual evoked potential and MOG-EAE studies have confirmed that PIPE-307 induces functional remyelination as evidenced by positive results in these models. Taken altogether PIPE-307 represents a promising approach for treating demyelinating diseases such as multiple sclerosis.

Disclosures: D.S. Lorrain: A. Employment/Salary (full or part-time):; Pipeline Therapeutics. **K.I. Lorrain:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. **M.M. Poon:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. **A. Broadhead:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. **K.J. Stebbins:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. **A. Chen:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. **J. Baccei:** None. **A.J. Green:** F. Consulting Fees (e.g., advisory boards); Pipeline Therapeutics. **J.R. Chan:** F. Consulting Fees (e.g., advisory boards); Pipeline Therapeutics.

Digital Abstract Session

P130. Neuroinflammation: Neurodegeneration

Program #/Poster #: P130.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Pipe-307 a novel, potent and selective m1 receptor antagonist as a therapeutic approach for remyelination in multiple sclerosis

Authors: *K. J. STEBBINS[1], M. M. POON[1], A. BROADHEAD[1], G. EDU[1], A. LORENZANA[1], K. I. LORRAIN[1], J. BACCEI[1], A. CHEN[1], C. BACCEI[1], A. J. GREEN[2], J. CHAN[2], D. S. LORRAIN[1];
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Abstract: Novel small molecule approaches aimed at stimulating remyelination would greatly complement immunotherapies and provide significant neural protection in demyelinating conditions such as multiple sclerosis (MS). Recently, we described the muscarinic M1 receptor (M1R) as an important regulator of oligodendrocyte precursor cell (OPC) differentiation and a promising target for drug discovery and reported proof of concept preclinical data with the novel, potent and selective M1 antagonist PIPE-359 (SfN 2019, posters # 206.18, 206.19). We developed PIPE-307, a novel, potent and selective M1R antagonist and highlight the potential for remyelination. PIPE-307 binds with high affinity to the M1 receptor and demonstrates selectivity relative to the other muscarinic receptors (M2, M3, M4 and M5). PIPE-307 promotes differentiation of rodent OPCs and increases myelination in cultured rat brain slices. This compound has good oral exposure and brain penetration. PIPE-307 demonstrates good in vivo M1R occupancy in rat and mouse and functional inhibition of M1R signaling as determined by IP1 accumulation. At dose levels which occupy the M1R, we demonstrate remyelination potential in myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune

encephalitis (EAE) model of demyelination where there is an improvement in clinical behavior score and g-ratios in the spinal cord. Furthermore, PIPE-307 exhibits marked improvement of EAE-induced changes in visual evoked potential N1 latency and optic nerve axon g-ratios. These data highlight the therapeutic potential of a selective M1R antagonist to benefit conditions such as MS in which demyelination plays a role. Entry into human-enabling studies with PIPE-307 are ongoing and clinical trials are expected to commence in early 2021.

Disclosures: **K.J. Stebbins:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **M.M. Poon:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **A. Broadhead:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **G. Edu:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **A. Lorenzana:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **K.I. Lorrain:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **J. Baccei:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **A. Chen:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **C. Baccei:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **A.J. Green:** A. Employment/Salary (full or part-time):; UCSF. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **J. Chan:** A. Employment/Salary (full or part-time):; UCSF. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **D.S. Lorrain:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics.

Digital Abstract Session

P130. Neuroinflammation: Neurodegeneration

Program #/Poster #: P130.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Institute Of Neurological Disorders and Stroke of the National Institutes of Health R21 NS098170
Kentucky Spinal Cord and Head Injury Research Trust 16-3A
NeuroCures Foundation, Inc.

Title: Divergent roles of CXCR4 antagonism in Dectin-1 and TLR2-driven microglia/macrophage activation and neurodegeneration

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Abstract: Innate immune activation develops in neurodegenerative diseases and promotes pathology. The innate ability of microglia/macrophage (M/M) to sense and respond to microbial and host-derived danger molecules relies on its pattern recognition receptors (PRRs). C-type lectin family members like Dectin-1 and toll-like receptors (TLRs) like TLR2 are major PRRs that trigger phagocytosis and neuroinflammation, orchestrating the early host defense to infections and damages. Though they collaborate on M/M, the activation of Dectin-1 or TLR2 shows divergent consequences. The neuroinflammatory response of Dectin-1 or TLR2 activation and their involved signaling during neurodegeneration are not fully understood. To determine the features of activating these receptors in neuroinflammation and axonal degeneration, selective agonists of Dectin-1 or TLR2 (alone or in combination) were microinjected into the dorsal funiculus of spinal cord in female C57BL/6 mice (n=6/group, aged 8-12w). AMD3100 (CXCR4 chemokine receptor antagonist) was injected after surgery to reveal the roles of CXCL12/CXCR4 axis. Detailed pathological changes in axonal degeneration, glial response, M/M phenotype, and somatosensory evoked potential were analyzed and compared between groups. Selective Dectin-1 activation or combined activation results in a sharply demarcated lesion characterized by a dense core of activated M/M, evident axonal injury, and astroglial loss. Oligodendrocyte progenitor cells surround the M/M core, while mature oligodendrocytes disperse outside the epicenter. Selective TLR2 activation elicits abundant M/M scattering throughout the lesion with no axonal loss. The M/M pool is predominantly filled with cells labelled by CD45, Iba1, CXCR4, but not TMEM119, indicating its hematogenous origin. Significant up-regulation of CXCL12 in M/M suggests that CXCL12/CXCR4 signaling may be required for lesion evolution for both receptor agonists. CXCR4 antagonism alleviates M/M response, limits axonal degeneration, and alters the phenotype ratio of proinflammatory/immunoregulatory M/M ($iNOS^+/Arg1^+$) in selective Dectin-1 activation or combined activation, whereas those are exacerbated in selective TLR2 activation. These data indicate a functional dichotomy of Dectin-1 and TLR2 in M/M activation and neurodegeneration. CXCL12/CXCR4 axis plays major but diverse roles when different PRRs are activated, affecting the neuroinflammatory and neurodegenerative process. Receptor dependent effects should be considered in signaling study and regenerative strategy development that target on innate immune receptors like PRRs.

Disclosures: **T. Chu:** None. **Y.P. Zhang:** None. **W. Zeng:** None. **L.B.E. Shields:** None. **G.N. Barnes:** None. **C.B. Shields:** None. **J. Cai:** None.

Digital Abstract Session

P130. Neuroinflammation: Neurodegeneration

Program #/Poster #: P130.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 1F31NS117085-01

Title: B cell depletion reduces glial reactivity in an animal model of multiple sclerosis

Authors: ***J. AHN**, R. H. MILLER;
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Abstract: Multiple sclerosis (MS) is an autoimmune, demyelinating disease of the central nervous system (CNS), characterized by myelin damage, infiltration of peripheral immune cells and severe functional deficits. Antibody-mediated peripheral B cell depletion therapies have been shown to significantly reduce the volume of MS lesions and formation of new lesions, suggesting a pathogenic role for B cells in MS. However, long-term systemic B cell depletion can severely immunocompromise the patient, and a better understanding of how B cells contribute to neurological dysfunction in MS is needed. In the current study, we aim to define the pathogenic role of both peripheral and CNS infiltrating B cells in MS by spatial depletion of B cells. For peripheral B cell studies, we used a combination of anti-CD19 and anti-B220 to deplete peripheral B cells in mice induced with experimental autoimmune encephalomyelitis (EAE), an animal model for MS. For CNS B cell studies, we utilized an inducible caspase 9 (iCP9) mouse with a CD19 promoter to selectively ablate CNS infiltrating B cells in EAE mice. We report that peripheral B cell depletion reduced splenic and circulating B cells, and iCP9 activation in CNS CD19+ B cells selectively ablated CNS B cells with no effect on the peripheral B cell population. Elimination of peripheral B cells reduced Iba1+ microglial and GFAP+ astrocyte reactivity as shown by morphological changes and decreased expression of neurotoxic factors. Similarly, CNS specific B cells reduced glial reactivity, which was associated with reduced myelin and axonal damage and functional impairment compared to control EAE animals. To determine if EAE B cells directly contribute to increased CNS glial cell reactivity, B cells were isolated from EAE animals and co-cultured with spinal cord glial cells. Glial cells stimulated with EAE B cells showed increased astrocyte and microglia reactivity compared to cells co-cultured with healthy B cells. This was associated with oligodendrocyte death and disrupted myelin sheaths. Our data suggest that pathogenic B cells in EAE invade the CNS and contribute to glial reactivity and myelin damage and as such provide novel insight into the underlying mechanism of MS pathology.

Disclosures: **J. Ahn:** None. **R.H. Miller:** None.

Digital Abstract Session

P130. Neuroinflammation: Neurodegeneration

Program #/Poster #: P130.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant EY018606
Research to Prevent Blindness
BrightFocus Foundation
NIH T32 EY007125

Title: The role of proinflammatory cytokines in retinal ganglion cell death

Authors: *K. M. ANDERSH[1,2,3], O. J. MAROLA[1,4,3], R. T. LIBBY[1,5,3];
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Abstract: Glaucoma is a neurodegenerative disease that leads to the death of retinal ganglion cells (RGCs), the main output neurons of the retina, and is the second leading cause of blindness in the world. Therefore, identifying the molecular mechanisms underlying how glaucomatous injury leads to RGC death is crucial. Recent studies have supported a novel role for inflammatory extrinsic signaling as an early driver of glaucomatous neurodegeneration. Specifically, two cytokines have been implicated as potential early drivers of RGC death: interleukin 1 alpha (IL1A) and tumor necrosis factor (TNF). Intravitreal injection of TNF is sufficient to drive RGC death at a delayed rate, ~8 weeks post-injection. However, the potential contribution of IL1A in driving RGC death has not been assessed.
To assess whether these cytokines alone or in combination are sufficient to drive RGC loss, eyes of 2-5-month-old C57BL/6J male and female mice were intravitreally injected with 2 µL of IL1A alone (1 mg/mL), TNF (100 µg/mL), IL1A+TNF, or PBS. Combined injection of TNF and IL1A resulted in 20% RBPMS+ RGC loss 14 days post-injection (n=10, PBS vs IL1+TNF, $p<0.01$). Injection of TNF (n=9, PBS vs TNF, $p>0.05$) or IL1A alone (n=9, PBS vs IL1A, $p>0.05$) did not result in RGC loss at this early timepoint. Thus, IL1+TNF, but not IL1A or TNF alone, was sufficient to drive RGC loss 14 days post injection.
To determine the downstream signaling required for IL1+TNF-induced degeneration, mice with homozygous *Jun*^{fl} alleles that were conditionally recombined in retinal neurons and macroglia with Six3-cre and mice deficient in *Sarm1* were injected with IL1+TNF at 2-5 months of age. Six3-cre^{+} *Jun*$^{fl/fl}$ RGCs were not protected from IL1+TNF insult (n=6, PBS vs IL1+TNF, $p<0.05$). However, *Sarm1* deficient animals had no significant loss of RGCs 14 days post-IL1+TNF (n=3, PBS vs IL1+TNF, $p>0.05$).
In summary, combined intravitreal injection TNF and IL1A was sufficient to drive RGC loss 14 days after injury. Studies have shown that TNF is sufficient to kill 15-20% RGCs 8 weeks post-injection, but our findings show that combined application of TNF and IL1A acted in a rapid and synergistic manner to drive RGC death. Furthermore, we show that this death was SARM1

dependent and JUN independent, which is in contrast to their roles in RGC death after axonal injury.

Disclosures: K.M. Andersh: None. **O.J. Marola:** None. **R.T. Libby:** None.

Digital Abstract Session

P130. Neuroinflammation: Neurodegeneration

Program #/Poster #: P130.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: LSU Health Shreveport COVID-19 research award

Title: Sars-cov-2 spike glycoprotein mediated neuronal infection and dissemination through axonal transport in vivo

Authors: K. KEYS, X.-L. TIAN, X.-H. LU;
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Abstract: Given the increasing global spread of SARS-CoV-2, many questions remain regarding the clinical outcomes of COVID-19, including the possibility of the development of neurological disorders. Evidence of the severity and persistence neurologic manifestations of SARS-CoV-2 infection have been recognized, and is increasing. Virus infection has been speculated as the trigger of neurodegenerative diseases such as Parkinson's disease (PD). In order to provide causal evidence and to study the viral infectivity/cell-entry in CNS in vivo, we are developing a CRISPR/Cas mediated humanized transgenic mouse model with full-length human ACE2 regulatory regions to faithfully recapitulate structure, tissue distribution, and gene regulation of human ACE2. Furthermore, a recombinant pseudotyped vesicular stomatitis virus (rVSV) was developed in which the glycoprotein of VSV was replaced by the spike protein of the SARS-CoV-2, with a Red fluorescent Protein (RFP), mCherry to trace the routes of infection. The expression and presentation of the spike protein on the viral membrane and the antigenicity are similar to SARS-CoV-2. We observed striking neuronal cell invasion accompanied by neuroinflammation. We describe for the first time the presence along axons of viral particles, suggesting axonal transport to distant brain regions is a valid propagation strategy. The constant presence of the virus in the neurons and axons and the concomitant inflammation is destined to cause long-term or chronic sequelae related to the development or aggravation of chronic neurological diseases. Exploiting knowledge on neuroinvasion and dissemination of SARS-CoV-2 will shed light on underlying mechanisms of neuropathogenesis and uncover potential druggable molecular virus-host interfaces.

Disclosures: K. Keys: None. **X. Tian:** None. **X. Lu:** None.

Digital Abstract Session

P130. Neuroinflammation: Neurodegeneration

Program #/Poster #: P130.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Association between cognitive deficit and mitochondrial DNA in Tabasco patients with schizophrenia

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Abstract: Association between cognitive deficit and mitochondrial DNA in Tabasco patients with schizophreniaGuillermo Efrén Villar-Juárez[1], Dulce Dajheanne García-de la Cruz[2], Carlos Alfonso Tovilla-Zarate[3], Alma Genis M[4], Hidemi Aguilar-Mariscal[5], Mario Villar-Soto[2], Isela E. Juárez-Rojop[5].[1]Universidad Anáhuac Querétaro, Escuela de Medicina[2]Hospital Regional de Alta Especialidad de Salud Mental. Villahermosa, Tabasco, México[3]División Académica de Multidisciplinaria de Comalcalco, Universidad Juárez Autónoma de Tabasco. Comalcalco,Tabasco, México.[4]Instituto Nacional de Medicina Genómica. Laboratorio de Enfermedades Psiquiátricas y Neurodegenerativas. Ciudad de México, México.[5]División Académica de Ciencias de la Salud, Universidad Juárez Autónoma de Tabasco. Villahermosa, Tabasco, México

Schizophrenia is a psychiatric disease with a neurodegenerative course. There is evidence link mtDNA alterations and neurodegenerative diseases such as Alzheimer and Parkinson disease. We consider that mtDNA could be associated with the CNS biochemical and structural changes causing cognitive impairment in schizophrenia. The total sample consisted of 95 individuals (56 cases and 39 controls). Cases were grouped according to the severity of symptoms (PANSS) in mild, moderate and marked intensity. Also, the cases were regrouped according to MoCA scores in mild and severe deficits. Spearman correlations were made in order to assess the relationship between mtDNA concentration and MoCA scores (significant $p<0.05$. Circulating cell free mitochondrial DNA was identified in plasma of 39 cases with schizophrenia and 3 health subjects of the control group ($P <0.0001$). MoCA scores were significantly lower in cases

compared to the control group (15.5 vs. 26; P <0.0001). No association was found between mtDNA and cognitive deficit. This is the first study to analyze the association of plasma mtDNA with cognitive impairment of patients with schizophrenia in mexican population. Our findings suggest that circulating cell free mtDNA identified in plasma of individuals with schizophrenia is not related to cognitive deficits or any clinical characteristic. Longitudinal studies in larger samples are needed, in order to explore possible changes in mtDNA levels at the different stages of the illness.

Disclosures: I.E. Juarez-Rojop: None. **G. Villar-Juárez1:** None. **D. García-de la Cruz:** None. **H. Aguilar-Mariscal:** None. **M. Viilar-Soto:** None. **C. Tovilla-Zarate:** None. **A. Genis-Mendoza:** None.

Digital Abstract Session

P131. Cellular Mechanisms

Program #/Poster #: P131.01

Topic: C.03. Parkinson's Disease

Support: FWO Grant G094219N
FWO Grant G092714
FWO Grant G080517N
FWO Grant S006617N
FWO Grant 1503117N
KU Leuven Grant C16/15/073
KU Leuven Grant OT/14/120

Title: Mitochondrial oxidative stress is mitigated by ATP13A2-driven polyamine transport

Authors: *S. VRIJSEN[1], L. BESORA-CASALS[2], S. VAN VEEN[1], J. ZIELICH[2], C. VAN DEN HAUTE[1], N. N. HAMOUDA[1], C. FISCHER[2], B. GHESQUIÈRE[3], I. TOURNEV[4], P. AGOSTINIS[3], V. BAEKELANDT[1], J. EGGERMONT[1], E. LAMBIE[2], S. MARTIN[1], P. VANGHELUWE[1];
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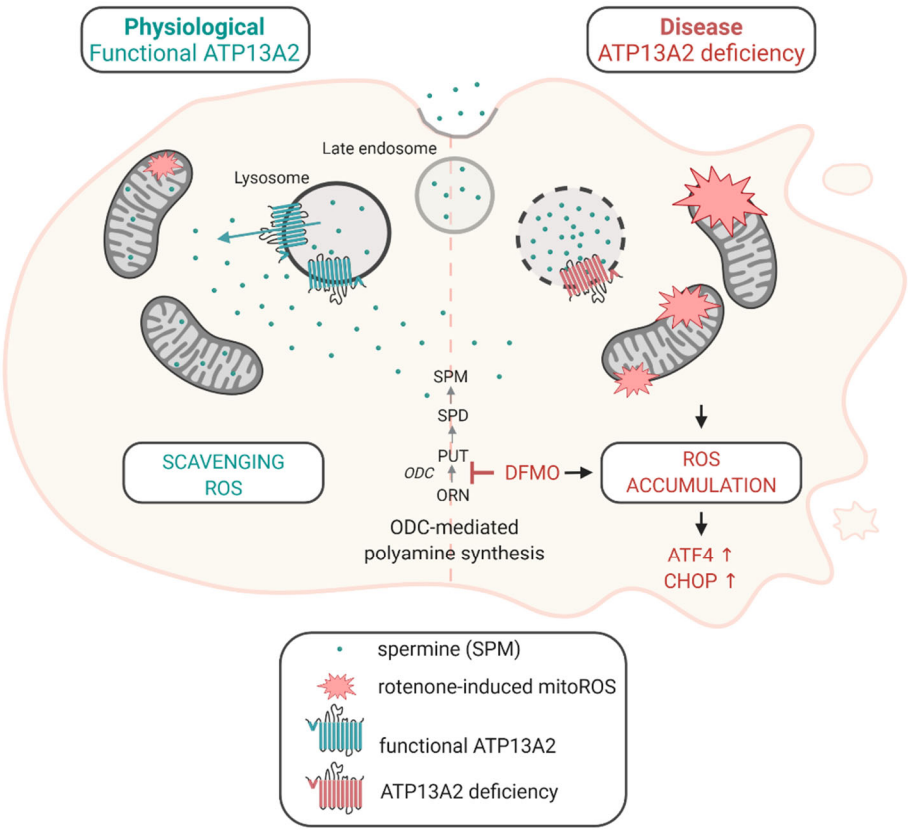
Abstract: Loss-of-function (LOF) mutations in *ATP13A2 (PARK9)* lead to a broad spectrum of neurodegenerative disorders. Functional ATP13A2 protects cells against environmental Parkinson's disease risk factors, such as the pesticide rotenone and heavy metals like zinc and manganese. Our group recently identified ATP13A2 as a primary active transporter present in the lysosomal membrane that transports the endocytosed polyamines spermine and spermidine from the lysosomal lumen to the cytosol, thereby preventing lysosomal polyamine accumulation and maintaining lysosomal health and integrity. Since polyamines are potent antioxidants, we here tested the hypothesis that polyamines transported by ATP13A2 may counter mitochondrial-generated reactive oxygen species (mitoROS) induced by rotenone. Indeed, rotenone induced a

steep increase in mitoROS in ATP13A2 deficient cells, whereas mitoROS levels were reduced in cells overexpressing wild-type ATP13A2, but not a transport dead mutant D508N. Interestingly, D508N ATP13A2 expressing cells also displayed lower mitochondrial polyamine levels. In ATP13A2 deficient cells, high mitoROS induced an ATF4-CHOP marked stress response and cell death, which could be reversed by the mitochondrial superoxide scavenger MitoTEMPO. Also heavy metal exposure and inhibition of polyamine synthesis with α-difluoromethylornithine (DFMO) resulted in increased mitoROS in ATP13A2-deficient cells, indicating that ATP13A2 mediated polyamine transport effectively counters oxidative stress. Importantly, we recapitulated key results in patient-derived fibroblasts with LOF mutations in *ATP13A2* and *in vivo*, in a *Caenorhabditis elegans* strain deficient in the ATP13A2-orthologue CATP-6. Our data on ATP13A2-mediated polyamine transport reveal a highly conserved cell protective pathway that lowers mitochondrial-derived oxidative stress representing a novel form of lysosomal/mitochondrial communication that may be impaired in neurodegenerative disorders.



Disclosures: **S. Vrijsen:** None. **L. Besora-Casals:** None. **S. van Veen:** None. **J. Zielich:** None. **C. Van den Haute:** None. **N.N. Hamouda:** None. **C. Fischer:** None. **B. Ghesquière:** None. **I. Tournev:** None. **P. Agostinis:** None. **V. Baekelandt:** None. **J. Eggermont:** None. **E. Lambie:** None. **S. Martin:** None. **P. Vangheluwe:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent WO-2018002350-A1.

Digital Abstract Session

P131. Cellular Mechanisms

Program #/Poster #: P131.02

Topic: C.03. Parkinson's Disease

Support: NIH R01 ES029035
NIH P30 ES005605

Title: Environmental organochlorines disrupt dopamine homeostasis.

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Abstract: Background. Disruption of dopamine (DA) homeostasis is a proposed mechanistic link between environmental insults and disease. Environmental insults include dieldrin (DI), which is an organochlorine (OC) pesticide. Another class of OCs are polychlorinated biphenyls (PCBs). Both PCBs and DI persist in the environment posing a public health concern. Specifically, DI has been association with an increased risk of Parkinson's disease, while PCBs have been linked to neurodevelopmental conditions. These neurological conditions - both neurodevelopmental and neurodegenerative - are characterized by an imbalance of DA, including metabolism and trafficking. DA is metabolized to 3,4-dihydroxphenylacetaldehyde (DOPAL) by monoamine oxidase (MAO), producing reactive oxygen species. DOPAL is converted to 3,4-dihydroxyphenylacetic acid (DOPAC) by aldehyde dehydrogenase (ALDH). Recent in vitro and in vivo data implicate DOPAL, a biogenic aldehyde, as a toxic factor relevant to disease. This includes increased DOPAL:DOPAC ratios in PD patients revealing an imbalance in DA metabolism. Here, the purpose of this study was to characterize the mechanistic link between OCs and DA disruption. **Methods**. The toxicity of OCs was determined using a dopaminergic cell culture model. Additionally, both cellular and mitochondrial ROS was analyzed using MitoSox and CellRox staining. To study DA metabolism, N27 cells were stably transfected with the human dopamine transporter (hDAT), which allowed for rapid uptake of DA and turnover. **Results.** PCB-52, a major component of PCBs in indoor and outdoor air was studied along with its metabolite 4-OH PCB-52. The metabolite was more toxic and increased mitochondrial ROS more than the parent PCB compound. DI was also found to increase mitochondrial ROS at non-toxic concentrations in dopaminergic cells. Overexpression of hDAT in N27 cells was found to be an efficient model for studying DA metabolism and to assess changes to DA homeostasis in the presence of OCs. Future work will characterize potential changes in DA metabolism, identify protein targets of DOPAL and/or OC agents, and explore the role of glia in maintaining DA homeostasis.

Disclosures: **B. Cagle:** None. **M. Sturgeon:** None. **R. Cornell:** None. **D. Roman:** None. **H. Lehmler:** None. **J. Doorn:** None.

Digital Abstract Session

P131. Cellular Mechanisms

Program #/Poster #: P131.03

Topic: C.03. Parkinson's Disease

Support: Canadian Institute of Health Research
Heart and Stroke Foundation of Canada
Ontario Graduate Scholarship

Title: Activating transcription factor 4 regulates dopaminergic neuron death induced by parkinson's disease neurotoxins and alpha synuclein aggregates

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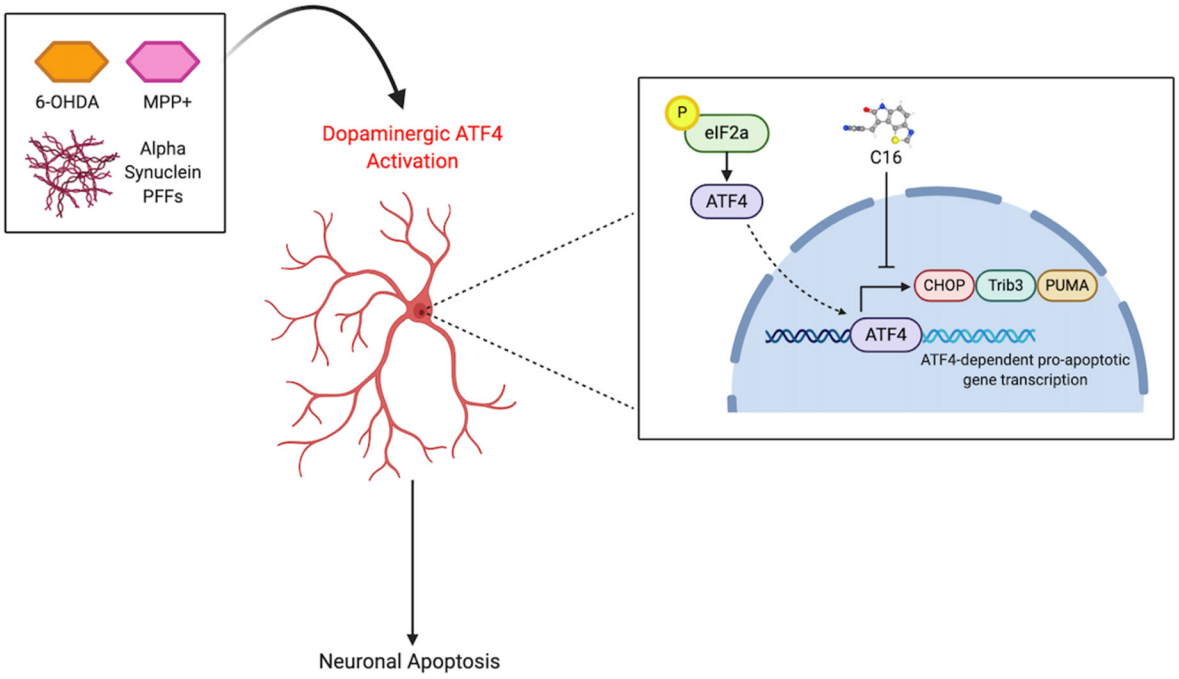
Abstract: Parkinson's Disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra resulting in severe and progressive motor impairments. However, the mechanisms underlying this neuronal loss remain largely unknown. Oxidative stress and ER stress have been implicated in PD and these factors are known to activate the Integrated Stress Response (ISR). Activating Transcription Factor 4 (ATF4), a key mediator of the ISR, and has been reported to induce the expression of genes involved in cellular homeostasis. However, during prolonged activation ATF4 can also induce the expression of pro-death target genes. Therefore, in the present study we investigated the role of ATF4 in neuronal cell death in models of PD. We demonstrate that PD neurotoxins (MPP+ and 6-OHDA) and α-synuclein aggregation induced by pre-formed human alpha-synuclein fibrils (PFFs) cause sustained upregulation of ATF4 expression in mouse cortical and mesencephalic dopaminergic neurons. Furthermore, we demonstrate that PD neurotoxins induce the expression of the pro-apoptotic factors Chop, Trb3 and Puma in dopaminergic neurons in an ATF4-dependent manner. Importantly, we have determined that PD neurotoxin and α-synuclein PFF induced neuronal death is attenuated in ATF4-deficient dopaminergic neurons. Furthermore, ectopic expression of ATF4 but not transcriptionally defective ATF4ΔRK restores sensitivity of ATF4-deficient neurons to PD neurotoxins. Finally, we demonstrate that the eIF2α kinase inhibitor C16 suppresses MPP+ and 6-OHDA induced ATF4 activation and protects against PD neurotoxin induced dopaminergic neuronal death. Taken together these results indicate that ATF4 is a key regulator of dopaminergic cell death induced by PD neurotoxins and pathogenic α-synuclein aggregates and highlight the ISR factor ATF4 as a potential therapeutic target in PD.



Disclosures: **M.D. Demmings:** None. **S.P. Cregan:** None.

Digital Abstract Session

P131. Cellular Mechanisms

Program #/Poster #: P131.04

Topic: C.03. Parkinson's Disease

Support: 5R01NS092823-03

Title: Investigation and rescue of SNARE-mediated macroautophagy inhibition in Parkinson's disease

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Abstract: Parkinson's disease (PD) is the second most common age-related neurodegenerative disease and is characterized pathologically by insoluble protein aggregates of alpha-synuclein (a-syn). Genetic studies have demonstrated that changes in the autophagic-lysosomal pathway (ALP) are a strong risk factor for developing PD, and this finding has been corroborated by biochemical studies showing a-syn is degraded by the ALP in health and disease. Investigation and facilitation of the endogenous ALP is thus one promising approach to developing disease-modifying therapies. While a-syn has been shown to inhibit macroautophagy (MA) - the ALP process by which bulk cytoplasmic aggregates are transported to lysosomes for degradation - molecular mechanisms explaining this inhibition remain undetermined.
Our previous findings indicate that a-syn may cause disruption of the ALP through binding to the

SNARE protein ykt6. This v-SNARE mediates vesicular trafficking, autophagosome formation, and autophagosome-lysosome fusion by facilitating membrane fusing events, and we found that ykt6 overexpression in cultured PD neurons partially rescues lysosomal activity. We therefore investigated the influence of ykt6 and pathogenic a-syn specific to MA in human cell lines and patient-derived midbrain neurons carrying familial triplication of the gene encoding a-syn. We found that wild type a-syn overexpression decreases autophagic flux but not baseline LC3 levels, suggesting a block of autophagosome-lysosome fusion. This inhibition is associated with decreased interaction between ykt6 and two of its autophagy-related SNARE binding partners: SNAP-25 and SNAP-29. Overexpression of a constitutively active ykt6 mutant and pharmacologic activation with a farnesyltransferase inhibitor were each sufficient to rescue autophagic flux in diseased cells. These data suggest that a-syn inhibits MA by reducing ykt6 participation in autophagosome-lysosome fusion SNARE complexes. Ykt6 activation can amplify both cargo delivery into lysosomes through MA and protein degradation. Because ALP dysregulation is a common feature of neurodegenerative diseases, these findings may contribute to disease-modifying therapies in PD and provide insights into the pathogenesis of proteinopathy more generally.

Disclosures: C. Pitcairn: None. **J.R. Mazzulli:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lysosomal Therapeutics Inc.

Digital Abstract Session

P131. Cellular Mechanisms

Program #/Poster #: P131.05

Topic: C.03. Parkinson's Disease

Support: Tucker Couvillon III Memorial Fund in Parkinson's Disease Research
The Almar Foundation

Title: Dysregulation of PLA2G6 activity induce degeneration in human dopaminergic neurons

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Abstract: Deleterious effects produced by the removal or failure to curb PLA2G6 activity from brain cells include neuroaxonal degeneration with iron accumulation, which is lethal during childhood and may induce Parkinsonian-like disease in adulthood (Guo et al, 2018. Front Neurol. 9: 1100). Recently, it was found that fibroblasts of idiopathic or sporadic PD patients showed impaired store-operated calcium entry (SOCE) (Zhou, et al 2016. Nature Communications volume 7, Article number: 10332). SOCE controls the activity of Calmodulin, a negative

regulator of PLA2G6, so the impairment of intracellular calcium flux suggests a role for PLA2G6 in the PD pathology. We determined that the silencing or overexpression of PLA2G6 induce a decreased content of DHA containing phospholipids concomitantly with a reduced amount of RvD5, a derivative of DHA, by Mass Spec. To determine the damage produced by the dysregulation of PLA2G6 in dopaminergic neurons, a culture of pluripotent cells in vitro differentiated into human dopaminergic neurons was exposed to Bromoenol lactone (BEL), an inhibitor of PLA2G6, and SKF96365, an inhibitor of the store-operated calcium entry (SOCE), in the presence or absence of lipid mediators derived from docosahexaenoic acid (DHA), eicosapentanoic acid (EPA), and arachidonic acid (AA). Immunocytochemistry targeting Tyrosine Hydroxylase (TH) and imaging analysis in IMARIS was used to determine the cell death and integrity of the surviving neurons. Maresin 1, RVD1, and RVD5, bioactive lipids derived from DHA, rescued the deficits in PLA2G6 activity induced by BEL. PLA2G6 is proposed to hydrolyze DHA to be converted into Maresin1, RVD1, and RVD5. LipoxinA4, a derivative of AA, and RVE1, a derivative of EPA, did not rescue PLA2G6 deficiency. Maresin 1 strongly reversed the neurodegeneration induced by SKF96365 and, to a lesser extent, RVD1. Future studies are required; however, these results suggest a role of PLA2G6 dysfunction in dopaminergic neurons and point to bioactive lipid messengers as candidate molecules to be used therapeutically to prevent or halt the neurodegeneration induced by PLA2G6 in PD.

Disclosures: J. Calandria: None. **S. Kala-bhattacharjee:** None. **S. Bhattacharjee:** None. **N.G. Bazan:** None.

Digital Abstract Session

P131. Cellular Mechanisms

Program #/Poster #: P131.06

Topic: C.03. Parkinson's Disease

Support: R01ES027245
R01ES026892
R01NS100090
R01NS088206
Eugene and Linda Lloyd Endowment
Armbrust Endowment

Title: Mitochondrial Dysfunction Induces Epigenetic Dysregulation by H3K27 hyperacetylation to Perturb Active Enhancers in Parkinson's Disease Models

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Abstract: Genetic mutations explain only 10-15% of cases of Parkinson's disease (PD), while an overriding environmental component has been implicated in the etiopathogenesis of PD. But

regardless of where the underlying triggers for the onset of familial and sporadic PD fall on the gene-environment axis, mitochondrial dysfunction emerges as a common mediator of dopaminergic neuronal degeneration. Herein, we employ a multidisciplinary approach to convincingly demonstrate that neurotoxicant exposure- and mitochondrial transcriptional factor A (TFAM) knockout-driven mitochondrial dysfunction share a common mechanism of epigenetic dysregulation. Under both scenarios, lysine 27 acetylation of likely variant H3.3 (H3.3K27ac) increased in dopaminergic neuronal models of PD, thereby opening that region to active enhancer activity via H3K27 hyperacetylation. These vulnerable epigenomic loci represent potential transcription factor motifs for PD pathogenesis. We further confirmed the mitochondrial dysfunction induced H3K27ac in *ex vivo* neurodegenerative models of PD. Our results reveal an exciting axis of 'exposure/mutation-mitochondrial dysfunction-metabolism-H3K27ac-transcriptome' for PD pathogenesis. Collectively, the novel mechanistic insights presented here links mitochondrial dysfunction to epigenetic transcriptional regulation in dopaminergic degeneration as well as offer potential new epigenetic intervention strategies for PD. **Support:** R01ES027245, R01ES026892, R01NS100090 and R01NS088206, Eugene and Linda Lloyd Endowment and Armbrust Endowment.

Disclosures: **M. Huang:** None. **D. Lou:** None. **A. Charli:** None. **D. Kong:** None. **H. Jin:** None. **V. Anantharam:** None. **A. Kanthasamy:** None. **A. Kanthasamy:** None. **Z. Wang:** None.

Digital Abstract Session

P132. Mechanisms of Neurodegeneration in Animal and Other Models of Disease and Injury

Program #/Poster #: P132.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2020R1A2C2005021
NRF-2020M3E5D9079908
2017M3A9G7073521
2020R1A5A1019023
20-BR-02-05

Title: Nonmuscle myosin IIB regulates Parkin-mediated mitophagy associated with amyotrophic lateral sclerosis-linked TDP-43

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Abstract: C-terminal fragments of Tar DNA-binding protein 43(TDP-43) have been identified as the major pathological protein in several neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). However, how they affect cellular toxicity and neurodegeneration remain largely unknown. Furthermore, how this process could be modulated is largely unknown. In this study, we found that C-terminal fragment of TDP43 (TDP25) mostly was localized to mitochondria and caused abnormal mitochondria morphology and parkin recruitment, leading to parkin-mediated mitophagy. We further found that knockdown of selective autophagy receptors such as Tax1BP1, Optineurin, or NDP52 accumulated TDP25, indicating that TDP25 is degraded by mitophagy. Very intriguingly, myosin IIB, a non-muscle-type of myosin as an actin-based motor protein, was mostly co-localized to TDP25 associated with abnormal mitochondria. In addition, inhibition of myosin IIB by siRNA or by blebbistatin accumulated insoluble TDP25, Tom20 in mitochondria, abnormal mitochondria and reduced neuronal cell viability. Taken together, our study suggests a novel role of myosin IIB on TDP25 mediated mitophagy. Therefore, our study proposes that regulation of myosin IIB activity might be a potential therapeutic target for neurodegenerative diseases associated with TDP-43 pathology.

Disclosures: **J. Lee:** None. **P. Jeon:** None. **J. Jang:** None. **M. Jun:** None. **S. Lee:** None. **S. Lee:** None. **H. Choi:** None. **Y. Lee:** None. **H. Choi:** None. **S. Park:** None. **J. Kim:** None. **C. Lim:** None. **D. Jang:** None.

Digital Abstract Session

P132. Mechanisms of Neurodegeneration in Animal and Other Models of Disease and Injury

Program #/Poster #: P132.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Supported by the Intramural Research Program of NINDS, NIH ZIA NS003029 and ZIA NS002946 (Z-H. Sheng)

Title: Lipid-mediated sequestration of motor-adaptor proteins impairs axonal lysosome delivery leading to autophagic stress and axonal dystrophy in Niemann-Pick disease type C neurons

Authors: ***J. C. RONEY**[1,2], T. FARFEL-BECKER[1], S. LI[1], T. SUN[1], N. HUANG[1], Y. XIE[1], M.-Y. LIN[1], F. M. PLATT[2], Z.-H. SHENG[1];
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Abstract: Neurons face unique challenges in maintaining cellular homeostasis in axons far removed from the cell body where lysosomes are enriched. Niemann-Pick disease type C (NPC) is a neurodegenerative lysosomal storage disorder characterized by lipid accumulation in endolysosomes. An early pathologic hallmark of NPC is axonal dystrophy with bulbous swellings that contain accumulated organelles at presymptomatic stages prior to neuron loss in

NPC mice. However, the mechanisms underlying these axonal pathologic changes remain obscure. Here, we demonstrate that organelles of the endocytic and autophagic pathways accumulate in NPC dystrophic axons. Using STED super-resolution and live-neuron imaging, we reveal that elevated cholesterol on NPC lysosome membranes in the soma leads to sequestration of kinesin-1 and Arl8, resulting in impaired anterograde transport of mature degradative lysosomes to distal axons, contributing to axonal autophagosome accumulation. Pharmacologic reduction of lysosomal membrane cholesterol with 2-hydroxypropyl-β-cyclodextrin (HPCD) or elevated expression of Arl8b rescues lysosome transport into axons, thereby reducing axonal autophagic stress and neuron death in NPC. Collectively, these findings suggest a new pathological mechanism by which altered membrane lipid composition impairs lysosome delivery into axons of NPC neurons and provide biological insights into the translational application of HPCD in restoring axonal homeostasis at early stages of NPC disease.

Disclosures: **J.C. Roney:** None. **T. Farfel-Becker:** None. **S. Li:** None. **T. Sun:** None. **N. Huang:** None. **Y. Xie:** None. **M. Lin:** None. **F.M. Platt:** None. **Z. Sheng:** None.

Digital Abstract Session

P132. Mechanisms of Neurodegeneration in Animal and Other Models of Disease and Injury

Program #/Poster #: P132.03

Topic: C.10. Brain Injury and Trauma

Support: CNRM-70-9922
CNRM-70-9242
PAT-747-1098

Title: Altered tau expression, astrocytosis, and behavior following injury in a gyrencephalic animal

Authors: S. C. SCHWERIN[1], A. OBASA[1], M. STRAYHORN[2], M. RAY[1], M. CHATTERJEE[1], T. HAIGHT[3], ***S. L. JULIANO**[1];
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Abstract: TBI is a major cause of death and disability worldwide. Evidence is also accumulating that TBI may be a risk factor for developing dementia. To study this problem, we used a small mammal with a gyrencephalic cortex, the ferret. The brains of these animals have substantial sulci and gyri as well as a large amount of white matter, compared to lissencephalic animals.Our initial findings using ferrets revealed that after a blast (shock wave) injury, ferrets expressed abnormal forms of tau and changes in the ratio of the 3R and 4R tau isoforms at 4 weeks post injury (WPI), which increased expression at 12 WPI. The telecephalon of these animals also showed substantial increases in astrocytic reactivity at grey-white matter interfaces, the subpial plate, surrounding blood vessels, and generalized in the white matter. Although many of these findings were observable at 4 WPI, they did not show statistically significant differences until 12

WPI. These outcomes suggest that ferrets represent a good model to study TBI as a potential link to dementia given the increase and persistence in expression of tau related markers. Our current experiments implement a brain injury model that includes a strong rotational injury (e.g. similar to whiplash) in addition to repetitive blast injury. This injury model results in significant increases in astrogliosis and 3R expression more quickly than observed after blast injury alone especially in the prefrontal cortex and hippocampus. Four weeks post injury, the increases in tau isoforms and astrocytosis significantly differed from sham using immunohistochemistry and western blot. Several neocortical areas were assessed including occipital, lateral temporal (including hippocampus) medial frontal, and prefrontal. The area most affected was the prefrontal cortex. We also observed that several, but not all, markers persisted their significant increase in expression until 6 months post injury. This was especially true for 3R, which remained increased in neocortical locations. We also observed several behavioral alterations at 4 WPI including changes in parameters measured with Open Field, Socialization, Eye Contact, and Actigraphy; activity patterns measured with Actigraphy are highly correlated with sleep. At 4 weeks and up to 4 months post injury, control ferrets showed activity patterns that were clustered into distinct bouts during a 24 hour period, while the injured animals displayed significantly altered sleep/activity patterns that were fractured and less focused.These findings confirm the ferret as an important animal model to investigate the consequences of TBI and potential links to dementia.

Disclosures: S.C. Schwerin: None. **A. Obasa:** None. **M. Strayhorn:** None. **M. Ray:** None. **M. Chatterjee:** None. **T. Haight:** None. **S.L. Juliano:** None.

Digital Abstract Session

P132. Mechanisms of Neurodegeneration in Animal and Other Models of Disease and Injury

Program #/Poster #: P132.04

Topic: C.10. Brain Injury and Trauma

Support: U.S. Army Research Office

Title: Blast shockwaves from detonated explosives produce correlative indicators of dementia risk including tau pathology and NCAM breakdown products in hippocampus

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Abstract: Blast-mediated shockwaves account for many of the hundreds of thousands of veterans from 21st century wars with brain injuries, due to both military and improvised explosives. Studies linking TBI to an increased risk of Alzheimer's disease (AD) are numerous, and Barnes et al. (*JAMA Neurol* 2018 75:1055) studied >350,000 veterans and found even mild

TBI was associated with 2.51-, 2.36-, and 3.05-fold increases in dementia risk (with, without, or unknown loss of consciousness, respectively). Using a unique brain explant protocol (Smith et al. 2016 *Exp Neurol* 286:107), our new report tested if blasts from military-grade explosives (RDX) produce AD-type changes in the hippocampus (Almeida et al. *Brain Path*, revision submitted). While blast intensities from 1.7-g spherical RDX did not cause cell death or neuromorphological alterations when directed at hippocampal explants, the detonations did in fact cause reductions in synaptic markers that are known to be down-regulated in cognitive disorders (e.g. synaptophysin, GluA1, synapsin IIb). Interestingly, synapsin IIa labeling remained unaltered, while neuropilar staining of synapsin IIb and neural cell adhesion molecule (NCAM) decreased, along with evident breakdown products of NCAM (NBDPs). Across explant groups, the extent of synaptic marker decline correlated with measures of AT8-positive tau levels, and the pre-tangle tau pathology was found among stochastic patterns of CA1 pyramidals. We further evaluated adhesion components due to their link to AD (Murray et al. 2016 *Neuroscience* 330:359). Compared to their respective levels in control explants, blast-induced reduction in NCAM180 was found ($p<0.001$), while NCAM140 ($p=0.0283$) and NCAM120 ($p=0.565$) exhibited smaller or no reductions. While RDX shockwaves reduced neuropilar NCAM180, mechanical stretching or flexing stress applied to the culture insert did not affect the NCAM isoform. Synaptic marker immunoreactivity levels after mechanical stress were 98.4 ± 11.8% of control explants. Our findings reveal that explosives produce distinct synaptopathy in a brain region involved in learning, emotional-laden memories, and social behavior, thus likely contributing to episodes of depression, anxiety, and attention problems. Given the prevalence of blast waves in wars, terrorist attacks, and training exercises, it is vital to understand the invisible actions of explosives. The identified indicators of blast-mediated synaptopathy will advance biomarkers and detection methods to improve diagnoses, treatment strategies, and therapeutic monitoring, in order to maintain cognitive health and manage the risk of developing dementia later in life.

Disclosures: **M. Fernandes De Almeida:** None. **T. Piehler:** None. **M.C. Pait:** None. **C.J. Norton:** None. **M.H. Giang:** None. **K.L.G. Farizatto:** None. **B.A. Bahr:** None.

Digital Abstract Session

P132. Mechanisms of Neurodegeneration in Animal and Other Models of Disease and Injury

Program #/Poster #: P132.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant F31EY030739
NIH Grant R01EY027701
Research to Prevent Blindness
Gareth R. Howell is the Diana Davis Spencer Foundation Chair for Glaucoma Research

Title: Determining the cell-specific mechanisms by which the endothelin system causes glaucomatous retinal ganglion cell loss

Authors: *O. J. MAROLA[1,4,5], A. A. HEWES[6], S. B. SYC-MAZUREK[1,2], J. M. HARDER[6], R. T. LIBBY[1,5,3], G. R. HOWELL[6,7,8];
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Abstract: Glaucoma is a leading cause of blindness characterized by death of retinal ganglion cells (RGCs). There is growing evidence that the endothelin (EDN) system is an important mediator of glaucomatous neurodegeneration. Our previous gene profiling studies in DBA/2J mice, a widely used mouse model of glaucoma, showed the EDN system is activated early in glaucoma, prior to RGC death, and components of the system are produced by multiple cell types. While the ligands EDN1 and EDN2 are expressed by microglia and macroglia (astrocytes and Müller glia), the receptors appear to be expressed by vascular mural cells (EDNRA) and neurons and macroglia (EDNRB). Injection of EDN ligands causes RGC death, and pharmacological inhibition of the EDN system delayed optic nerve damage in DBA/2J mice. We are now taking a genetic approach to dissect the cell-specific roles of the EDN system. First, we determined the apoptotic pathways controlling EDN-induced RGC death. The transcription factor JUN was shown to regulate RGC death after glaucoma-relevant injury. Here, we tested whether JUN mediates EDN-induced death. EDN1 or vehicle was injected into the vitreous of 7-24 week old male and female C57BL/6J mice, and mice with *Jun* deleted from retinal neurons and macroglia with Six3-cre. EDN1 caused caspase-3 activation in RGCs 5 days post-EDN1 ($n\geq5$, $p<0.001$) and 26% RGC loss after 28 days ($n\geq7$, $p<0.001$). *Jun* deletion prevented nearly all caspase-3 activation 5 days after EDN1 ($n\geq7$, $p<0.001$), and nearly all RGC loss after 28 days ($n\geq12$, $p=0.012$). Therefore, JUN activation was required for the majority of RGC death after EDN1 insult. Second, we investigated whether retinal neurons and macroglia cause EDN-induced RGC death via EDNRB. Interestingly, *Ednrb* deletion from retinal neurons and macroglia with Six3-cre did not prevent caspase-3 activation 5 days after EDN1 ($n\geq8$, $p=0.487$) nor did it attenuate RGC loss after 28 days ($n\geq6$, $p=0.850$). Therefore, neuronal and macroglial EDNRB was not necessary for EDN1-induced RGC death. We are now testing the hypothesis that EDN ligands mediate their effect through vascular cells. In support of this, EDN ligands act through vascular EDNRA to elicit potent vasoconstriction. EDN1 injection caused retinal arterial thinning ($n=6$, $p<0.001$), loss of retinal vascular perfusion, and RGC hypoxia. Therefore, EDN1 ligand could cause RGC death by eliciting vasoconstriction via vascular EDNRA. Studies are underway to conditionally ablate EDN ligands specifically from microglia and macroglia in DBA/2J mice to critically test the cell-specific roles of EDN ligands in glaucoma. Ultimately, we aim to determine the potential for targeting the EDN system to treat glaucoma.

Disclosures: O.J. Marola: None. **S.B. Syc-Mazurek:** None. **J.M. Harder:** None. **G.R. Howell:** None. **A.A. Hewes:** None. **R.T. Libby:** None.

Digital Abstract Session

P132. Mechanisms of Neurodegeneration in Animal and Other Models of Disease and Injury

Program #/Poster #: P132.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NASA Research Grant NNX13AN34G
NIH Grant 2R25 GM060507

Title: Brain Organoids: A novel tool to study oxidative stress-induced central nervous system damage using ionizing radiation

Authors: *F. OYEFESO[1], G. GOLDBERG[3], M. VAZQUEZ[2], A. BERTUCCI[2], X. MAO[1], A. MUOTRI[4], M. J. PECAUT[1];
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Abstract: Oxidative stress (OS) and the associated increases in inflammatory markers are known to play major roles during neurodevelopment, aging, and progressive degenerative disease states including Alzheimer's disease, Parkinson's disease, and neurodevelopmental deficits. Among the many endogenous and exogenous sources of oxidative stress, ionizing radiation (IR) is well known to trigger OS by producing pro-oxidants such as reactive oxygen species (ROS) and other free radicals. Increased levels of ROS could overwhelm antioxidant defensesprotecting lipids, proteins, and DNA, from OS and cause lasting changes to cell and tissue physiology and morphology. Despite progress in understanding the roles of OS in central nervous system (CNS) pathophysiology, studies using 2D neural cell culture models have been limited for their translational impact. However, three-dimensional (3D) brain organoid cell culture systems have emerged as novel models to simulate the organization and cell diversity of human brain regions in vitro. This model could also be a novel alternative to study the effects of IR on normal neural tissue preserving the CNS' 3D cytoarchitecure and functional integrity. In this study, we aim to investigate OS-induced changes to brain organoid cytoarchitecture, cell state, and secreted factors induced by proton exposures. Proton radiation is part of the space radiation environment as well as a source of radiation totreat cancer. These studies will investigate the effects of low-doses of IR (<2 Gy) to characterize the acute responses of cortical brain organoids to IR. This research will first explore changes to cell morphology and expression of proliferation, apoptotic, and cell-type specific markers using immunofluorescence and confocal microscopy. Then, we will evaluatechanges to markers for lipid peroxidation and DNA damage. Results from this study will support the use of brain organoids as a translational tool to investigate OS-induced cellular and molecular changes potentially involved in progressive CNS neurodegenerative diseases as well as toxicity to the CNS.

Disclosures: F. Oyefeso: None. **G. Goldberg:** None. **M. Vazquez:** None. **A. Bertucci:** None. **X. Mao:** None. **A. Muotri:** E. Ownership Interest (stock, stock options, royalty, receipt of

intellectual property rights/patent holder, excluding diversified mutual funds); TISMOO. **M.J. Pecaut:** None.

Digital Abstract Session

P132. Mechanisms of Neurodegeneration in Animal and Other Models of Disease and Injury

Program #/Poster #: P132.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Multiple Sclerosis Society
NIH

Title: Oligodendrocyte differentiation from primary progressive multiple sclerosis patient hiPSCs show intrinsic differences contributing to disease pathology

Authors: *M. J. PLASTINI, H. L. DESU, M. SAPORTA, R. BRAMBILLA;
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Abstract: Multiple sclerosis (MS) is the most common neurological disorder in young adults. Hallmarks of MS are CNS demyelination, inflammation and axonal damage, which lead to neurological impairments. Primary progressive MS (PPMS), the rarest form of the disease, is characterized by progressive and irreversible loss of neurological function with no periods of recovery and a far less pronounced immune-inflammatory response as the more common relapsing remitting MS (RRMS). Effective treatments for PPMS are lacking, likely due to an incomplete understanding of the pathophysiological processes underlying this disease form. Accumulating evidence suggests that intrinsic oligodendrocyte (OL) dysfunction may be responsible for, or at least contribute to, PPMS etiopathology. A recent single-nucleus RNA-seq study of OLs isolated from white matter lesions of PPMS patients revealed significantly reduced levels of multiple myelin-associated genes and upregulation of immune-specific genes (Jakel et al, 2019). This suggests that OLs in PPMS patients have intrinsic alterations compared to OLs from healthy individuals. On this basis, our hypothesis is that intrinsic dysfunction in OLs triggers OL cell death and demyelination, which leads to immune activation and PPMS initiation. To test this hypothesis we obtained human induced pluripotent stem cells (hiPSCs) from PPMS patients and matching controls and differentiated them into OLs to investigate intrinsic differences. We found that, following differentiation, the percentage of mature $O4^+$ OLs was significantly lower in PPMS patient lines compared to controls. RNA-seq analysis showed that the transcriptional profile of PPMS-derived $O4^+$ OLs was significantly different from that of control-derived $O4^+$ OLs, with upregulation of immune-related genes such as NLRP2, and downregulation of adhesion genes such as HEPACAM. Together, these data suggest that cells from PPMS patients may have a lower ability to form new OLs, possibly due to a switch toward a more inflammatory and less reparative phenotype.Pinpointing the intrinsic alterations in OLs of PPMS patients will uncover pathways and cellular processes that could be targeted for therapeutic purposes in this form of the disease for which treatments are limited.

Disclosures: **M.J. Plastini:** None. **H.L. Desu:** None. **R. Brambilla:** None. **M. Saporta:** None.

Digital Abstract Session

P133. Neuroprotective Therapeutics: Understanding Mechanisms in Pre-Clinical Models of Disease and Injury

Program #/Poster #: P133.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NICHD, NIH intramural program

Title: Neurotrophic factor-α1, a novel tropin is critical for the prevention of stressinduced hippocampal CA3 cell death and cognitive dysfunction in mice:comparison to BDNF

Authors: L. XIAO, *V. SHARMA, L. TOULABI, Y. XUYU, A. PELTEKIAN, I. ARNAOUTOVA, D. ABEDE, H. LOU, Y. LOH;
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Abstract: Stress induces various pathophysiology in the brain such as impaired neuronal network and cognitive dysfunction that are associated with neurodegenerative diseases. Carboxypeptidase E (CPE), a proneuropeptide/prohormone processing enzyme, also named neurotrophic factor-α1(NFα1) is highly expressed in the stress-vulnerable hippocampal CA3 neurons which receive contacts from granule cell projections that release BDNF. Whether NF-α1-CPE and/or BDNF are critical in protecting these CA3 neurons against severe stress-induced cell death is not well known. Here we show that stress induced by maternal separation, ear tagging and tail snipping at weaning in 3-week-old NF-α1-CPE-KO mice led to complete hippocampal CA3 degeneration, despite having similar BDNF and active phosphorylated TrkB receptor levels in comparision with WT. Mice administered with TrkB inhibitor, ANA12 showed no degeneration of the CA3 neurons after the weaning. Transgenic knock-in mice expressing CPE-E342Q, an enzymatically inactive form, replacing NF-α1-CPE, showed no CA3 degeneration and exhibited normal learning and memory after the weaning stress, unlike NF-α1-CPE-KO mice. In addition, radiolabeled NF-α1-CPE bound HT22 hippocampal cells in a saturable manner and with high affinity (Kd=4.37nM). Furthemore, treatment with NF-α1-CPE or CPE-E342Q equivalently activated ERK and increased BCL2 expression to protect against H2O2- or glutamate-induced cytotoxicity in HT22 $^{cpe-/-}$ cells. Our findings suggest that NF-α1-CPE is more important compared to BDNF in protecting CA3 pyramidal neurons against stress-induced cell death and cognitive dysfunction, independent of its enzymatic activity.

Disclosures: **L. Xiao:** None. **V. Sharma:** None. **L. Toulabi:** None. **Y. xuyu:** None. **A. Peltekian:** None. **I. Arnaoutova:** None. **D. Abede:** None. **H. Lou:** None. **Y. Loh:** None.

Digital Abstract Session

P133. Neuroprotective Therapeutics: Understanding Mechanisms in Pre-Clinical Models of Disease and Injury

Program #/Poster #: P133.02

Topic: C.10. Brain Injury and Trauma

Support: Anonymous

Title: The neuroprotective role of myo/nog cells assessed in a rat model of focal brain injury

Authors: *M. BRACCIA[1], N. MORRISON[1], S. JOSEPH-PAULINE[1], A. PAYNE[1], L. GUGERTY[1], J. MOSTOLLER[1], P. LECKER[1], E.-J. TSAI[1], J. KIM[1], M. MARTIN[1], R. BRAHMBHATT[1], G. GORSKI[1], J. GERHART[1], M. GEORGE-WEINSTEIN[1], J. STONE[2], S. PURUSHOTHUMAN[3], A. BRAVO-NUEVO[1];
[1]Philadelphia Col. of Osteo. Med., Philadelphia, PA; [2]Sch. of Med. Sci. Univ. of Syndey, Sydney, Australia; [3]Brain and Mind Ctr. and Central Clin. Sch. Univ. of Sydney, Sydney, Australia

Abstract: Myo/Nog cells are found at low frequencies in a variety of different tissues in adults and during embryogenesis. They can be identified by the expression of the skeletal muscle-specific transcription factor MyoD, the bone morphogenetic protein inhibitor Noggin, and via binding of a monoclonal antibody against BAI1. Myo/Nog cells are critical in ontogeny and for the development of the central nervous system. There is increasing evidence that Myo/Nog cells are neuroprotective in the neuronal retina. In this study, we examined Myo/Nog cell response to neuronal cell death in the rat neocortex 24 hours following a needlestick injury. Additionally, we studied the effects of adding or depleting Myo/Nog cells at the site of the needlestick lesion. Exogenous Myo/Nog cells promoted neuronal survival and suppressed apoptosis at the site of injury. We found that the addition of exogenous Myo/Nog cells to the lesion reduced the number of apoptotic cells around the injury site (ANOVA: F = 22.948, R2 = 61.09%, p = 6.695 x 10-14). Additionally, the death of mature neurons caused by the injury was ameliorated by the exogenous addition of Myo/Nog cells (ANOVA: F=34.825, R2=69.92%, p=2.395X10-18). Additional studies are needed to both understand the precise molecular mechanisms of neuroprotection these cells employ and to determine if exogenous Myo/Nog cells can be adapted as a form of therapy for neuronal injuries.

Disclosures: **M. Braccia:** None. **N. Morrison:** None. **S. Joseph-Pauline:** None. **A. Payne:** None. **L. Gugerty:** None. **J. Mostoller:** None. **P. Lecker:** None. **E. Tsai:** None. **J. Kim:** None. **M. Martin:** None. **R. Brahmbhatt:** None. **G. Gorski:** None. **J. Gerhart:** None. **M. George-Weinstein:** None. **J. Stone:** None. **S. Purushothuman:** None. **A. Bravo-Nuevo:** None.

Digital Abstract Session

P133. Neuroprotective Therapeutics: Understanding Mechanisms in Pre-Clinical Models of Disease and Injury

Program #/Poster #: P133.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH 5U01NS058162-07

Title: Comparing the Antiseizure and Neuroprotective Efficacy Midazolam and LY293558-Caramiphen Combination Against Soman in a Rat Model Relevant to the Pediatric Population

Authors: J. P. APLAND[1], *M. D. FURTADO[2], V. ARONIADOU-ANDERJASKA[3], T. FIGUEIREDO[2], V. PIDOPLICHKO[2], K. ROSSETTI[2], M. BRAGA[2,3];
[1]Neurosci. Br., USAMRICD, Aberdeen Proving Ground, MD; [2]Anatomy, Physiol. and Genet., [3]Psychiatry, Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: The currently FDA-approved anticonvulsant for the treatment of status epilepticus (SE) induced by nerve agents is the benzodiazepine midazolam. However, midazolam does not appear to offer neuroprotective benefits. This is particularly concerning with respect to the protection of children, because in the developing brain, synaptic transmission mediated via GABAA receptors, the target of midazolam, is still weak. In the present study, we exposed 12-day-old male and female rats to 1.2xLD50 soman, and compared the antiseizure, anti-lethality, and neuroprotective efficacy of midazolam (3 mg/kg) and the combination of LY293558 (an AMPA/GluK1 receptor antagonist; 10 mg/kg) and caramiphen (an antimuscarinic with NMDA receptor-antagonistic properties; 50 mg/kg) administered 1 h post-exposure. Despite that there was no statistically significant difference between the two treatment groups in the time it took for the anticonvulsants to stop initial seizures or survival rate, the latency for re-occurrence of seizures after cessation of the initial SE was dramatically longer in the rats treated with LY293558+CRM, and the total duration of SE was significantly shorter in this group ($p<0.001$; t-test). The difference between the two treatment groups in the total duration of SE was accompanied by differences in neuropathology. Total neuronal loss and loss of interneurons in the CA1 hippocampal area and the basolateral amygdala (BLA) were significant in the MDZ-treated group in comparisons with control non-exposed rats and with the LY293558+CRM-treated rats, at 1, 3 and 6 months post-exposure. There is an apparent deterioration over time, and at the 6-month time point, there is also some neuronal and interneuronal loss in the BLA of the LY293558+CRM treated group. Hippocampal volume was significantly reduced in the MDZ-treated rats but not in the LY293558+CRM-treated rats, at 3 and 6 months post-exposure. Spontaneous, background inhibitory activity was significantly reduced in the MDZ-treated group but not in the LY293558+CRM group, at all three time points (1, 3 and 6 months post-exposure), and this was accompanied by increased anxiety only in the MDZ-treated group, as determined by the Open Field and the ASR tests. The appearance/incidence of spontaneous recurrent seizures was greater in the MDZ-treated group, and the difference from the LY293558+CRM group reached statistical significance at the 6-month time point. The combined administration of

LY293558 and caramiphen, by blocking mainly AMPA, GluK1, and NMDA receptors, is a very effective anticonvulsant and neuroprotective therapy against soman in immature rats.

Disclosures: J.P. Apland: None. **M.D. Furtado:** Other; BioSEaD, LLC. **V. Aroniadou-Anderjaska:** None. **T. Figueiredo:** None. **V. Pidoplichko:** None. **K. Rossetti:** None. **M. Braga:** None.

Digital Abstract Session

P133. Neuroprotective Therapeutics: Understanding Mechanisms in Pre-Clinical Models of Disease and Injury

Program #/Poster #: P133.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: (IO) R016333044

Title: Triterpenoid saponin attenuates mortality associated with AQP4 and astrogliosis in Plasmodium yoelli infection in mice

Authors: *P. SANGUANWONG[1], E. M. OO[1], C. TURBPAIBOON[1], P. UAWITHYA[2], S. CHOMPOOPONG[1];
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Abstract: The anti-inflammatory effect of Astragaloside IV triterpene saponin (TS) has been previously reported in many studies but not in *Plasmodium yoelli* 17XL (*Py*) infection. This study aimed to study the effect of TS on the attenuation of the BBB damages in *Py* infected mice after combined treatment with the standard antimalarial drug, Artesunate (ART). At day 3 post-infection, *Py* mice have received TS for 5 days with 25 mg/kg of TS. ART 2.4 mg/kg was used as a standard antiparasitic drug which also given from day 3 until day 7 post-infection. Survival time, percent parasitemia, and anemia conditions were daily determined. On day 7, leakage of BBB was investigated by Evans blue extravasation assay. Meanwhile, serum and the brain were collected to determine the inflammatory cytokine proteins by Enzyme-linked immunosorbent (ELISA) assay. The changes in AQP4 and reactive astrogliosis-associated GFAP expression were determined by immune-peroxidase staining. The neurological symptoms were daily assigned by the Rapid murine coma and behavior scale (RMCBS) assignment. The result is that TS was not shown non-antiparasitic activity. However, TS was extended survival rate and time both single and co-treatment. Interestingly, TNF-alpha, IL-1beta, and IL-10 were significantly decreased when compared to the Py-ART group in both serum and brain tissue. Importantly, the TS+ART group was significantly shown more integrity than Py and Py-ART group. The AQP4 and GFAP expression were significantly reduced in TS+ART. Neurological symptoms of the TS+ART group significantly decreased when compared to the Py and Py-ART groups. In conclusion, co-treatment of TS with the antimalarial drugs could improve the survival time of mice. Moreover, it could attenuate systemic inflammation-associated BBB disruption in Py-infected mice via reduced critical pro-inflammatory cytokines followed to maintain BBB

integrity, resulting in suppressed dysregulation of astrocytes BBB. It worth mention that TS could be used as a promising novel adjunctive therapy for severe malaria pathology or neuroinflammatory situation.

Disclosures: **P. Sanguanwong:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Siriraj Research Fund, Grant number (IO) R016333044, Faculty of Medicine Siriraj Hospital Mahidol University. **E.M. Oo:** None. **C. Turbpaiboon:** None. **P. Uawithya:** None. **S. Chompoopong:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Siriraj Research Fund, Grant number (IO) R016333044, Faculty of Medicine Siriraj Hospital Mahidol University.

Digital Abstract Session

P133. Neuroprotective Therapeutics: Understanding Mechanisms in Pre-Clinical Models of Disease and Injury

Program #/Poster #: P133.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: An Essential Oil Blend Modulates Neuronal Health Responses after Chemical Stress

Authors: *R. PRICE[1], N. STEVENS[2], C. BASCOUL[2], B. RIGGS[2], R. OSGUTHORPE[2], A. ESSEX[1];
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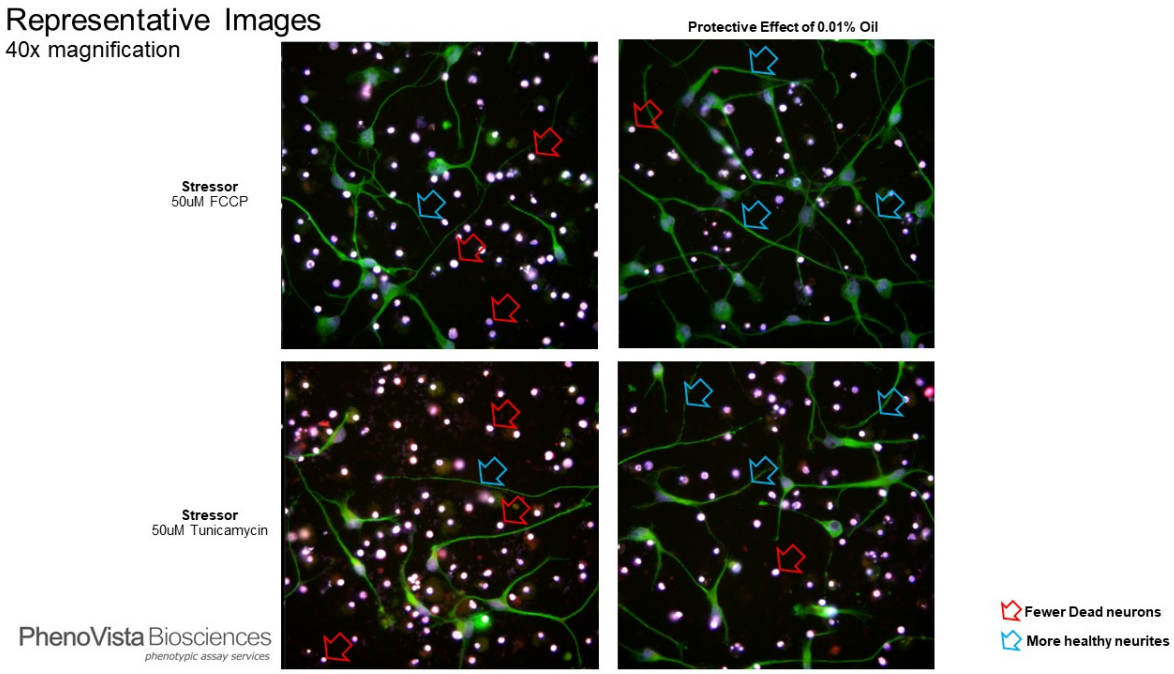
Abstract: Despite growing scientific evidence that essential oils possess important therapeutic benefits, research on their biological activities in complex human disease models is scarce. To enhance understanding in this regard, we analyzed the biological activities of an essential oil blend (EOB) in human iPSC-derived dopamine neurons. PhenoVista has established a range of high-content imaging assays for neuronal health, including neuronal viability, mitochondrial function, and neurite outgrowth. Briefly, iPSC-derived neurons are plated and cultured for 3 days, a neuroprotectant is added for 24 h, stressors for 24 h, and then cells are fixed, stained using Hoechst (nuclei), Tuj-1 (neurites), MitoTracker (mitochondrial function) and DRAQ7 (dead cell marker) for subsequent imaging and analysis. These models allow for measurement of changes in neuronal features impacted by stressors (here we used FCCP, a mitochondrial toxin, as well as tunicamycin and brefeldin-A) as well as improvements induced by EOB treatment. This EOB is primarily composed of essential oils and extracts from turmeric. As expected, the stresses impacted neuronal health in most measures, particularly viability levels and mitochondrial function. Interestingly, the EOB significantly reversed many (but not all) of these deficits. In conclusion, this turmeric EOB may potentially impact the overall health of human iPSC-derived neurons in response to environmental stress.



Disclosures: **R. Price:** A. Employment/Salary (full or part-time):; PhenoVista Biosciences. **A. Essex:** A. Employment/Salary (full or part-time):; PhenoVista Biosciences. **N. Stevens:** A. Employment/Salary (full or part-time):; doTERRA. **C. Bascoul:** A. Employment/Salary (full or part-time):; doTERRA. **R. Osguthorpe:** A. Employment/Salary (full or part-time):; doTERRA. **B. Riggs:** A. Employment/Salary (full or part-time):; doTERRA.

Digital Abstract Session

P133. Neuroprotective Therapeutics: Understanding Mechanisms in Pre-Clinical Models of Disease and Injury

Program #/Poster #: P133.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Protective effect of cobra venom NOX & hypothalamic peptide PRP on electrophysiological & behavioral characteristics of rats after the unilateral Labyrinthectomy

Authors: *N. BEHNAM DEHKORDI, V. H. SARKISIAN, L. V. DARBINYAN, L. E. HAMBARTSUMYAN, L. P. MANUKYAN, E. A. AVETISYAN, N. V. SARKISIAN; Natl. Acad. of Sci. Republic of Armenia, Yerevan, Armenia

Abstract: Protective effect of cobra venom *NOX* and hypothalamic peptide *PRP* on electrophysiological and behavioral characteristics of rats after the unilateral labyrinthectomy

Neda Behnam Dehkordi. Sarkisian V.H., Darbinyan L.V., Hambartsumyan L.E., Manukyan L.P. Avetisyan E.A, Sarkisian N.V. *L.Orbeli Institute of Physiology, NAS of Armenia, Yerevan*

ABSTRACT **Objectives:** Research is devoted to the study of systemic compensatory reactions

of the rat's brain developing in response to unilateral labyrinthectomy (UL) under the conditions of pharmacological intervention of PRP (Hypothalamic Proline-Rich Peptide) and NOX (Cobra Venom Naja Naja Oxiana).
Methods: The studies were carried out on 26 outbred white male rats weighting 250 ± 30 g. In order to study the dynamics of neurodegenerative changes in the hypothalamo-vestibular projections in UL conditions a comparative analysis of changes in the frequency of the of impulse activity stream of the lateral vestibular nucleus (LVN) deafferented neurons in response to high-frequency stimulation of paraventricular (PVN) and supraoptical (SON) hypothalamic nuclei was carried out. Changes in the behavior of rats in the "open field" caused by unilateral labyrinthectomy were also evaluated followed by administration of PRP and NOX.**Results:** 4, 9, and 17 days after UL, in the dynamics of neurodegenerative changes, high-frequency stimulation of PVN and SON leads to an increase in the imbalance of inhibitory (TD) and excitatory (TP) tetanic and post-tetanic potentiations (PTP, PTD) of Deiters nucleus neurons, the ratios of which after PRP injection remain almost at the same level as compared with the norm. On the damaged side, on day 4 after UL, TD and PTD prevail, and on day 9, reactions characterized by TD and TP proportional correlation and corresponding to the norm intensity. Vestibular disturbances in rats in an "open field" after UL are manifested primarily in changes in the indicators of vertical activity of the operated animals - the number and duration of rearing without support on the board of the installation decreases most significantly.**Conclusion:** In general the results obtained allow considering inhibitory processes mediated by GABA, as a protective effect of NOX and PRP against neurodegeneration.
Keywords: neurodegeneration. venom, hypothalamic peptide, unilateral labyrinthectomy, inhibition

Disclosures: N. Behnam Dehkordi: None. **V.H. Sarkisian:** None. **L.V. Darbinyan:** None. **L.E. Hambartsumyan:** None. **L.P. Manukyan:** None. **E.A. Avetisyan:** None. **N.V. Sarkisian:** None.

Digital Abstract Session

P133. Neuroprotective Therapeutics: Understanding Mechanisms in Pre-Clinical Models of Disease and Injury

Program #/Poster #: P133.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Oklahoma Health Research Program (HR 18-033)
Oklahoma State University Center for Health Sciences, Intramural funds (RLD)

Title: B-funaltrexamine inhibits lipopolysaccharide-induced chemokine expression and sickness behavior in mice.

Authors: *S. MYERS, D. BUCK, K. MCCRACKEN, J. T. CURTIS, R. L. DAVIS;
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Abstract: Neurological conditions including, infection, neurodegeneration, and psychiatric disorders involve neuroinflammation. Yet, most of the medications used to treat these disorders are not considered potently anti-inflammatory. We previously discovered that beta-funaltrexamine (β-FNA), a selective mu-opioid receptor (MOR) antagonist, inhibits inflammatory signaling *in vitro* in human astroglial cells. Notably, the mechanism of action does not seem to involve actions at the MOR. We also determined that β-FNA inhibited bacterial lipopolysaccharide (LPS)-induced sickness behavior and neuroinflammation in mice. In these initial *in vivo* experiments, mice were administered β-FNA, immediately followed by LPS administration, then assessed 24 h later. In the present study, we examined the extent to which β-FNA is protective when treatment occurs several hours after LPS administration. Also, we investigated the protective effects at an earlier time point (8 h) after LPS administration, when sickness behavior is more pronounced. Lastly, previous *in vitro* findings indicated that naltrexone (a non-selective opioid receptor antagonist from which β-FNA is derived) does not inhibit inflammatory signaling in astrocytes, thus we questioned if it would also be ineffective *in vivo*. In the current study, male C57BL/6J mice were administered LPS (0.83 mg/kg, i.p.) followed by treatment with β-FNA (50 mg/kg, i.p.) at 4 and/or 10 h post-LPS, depending on the experiment. At 8 or 24 h post-LPS, sickness behavior was assessed using a 10-min open-field test, followed by termination and collection of plasma and brain. Levels of inflammatory chemokines (interferon γ-induced protein, CXCL10; and monocyte chemotactic protein 1, CCL2) in tissues were measured using an enzyme-linked immunosorbant assay. β-FNA treatment 4 or 10 h after LPS administration inhibited CCL2 levels in plasma and brain (but did not significantly affect CXCL10 levels), and it failed to reduce sickness behavior. Conversely, β-FNA treatment within minutes of LPS treatment resulted in attenuation of both sickness behavior and chemokine expression. Delayed β-FNA treatment was not protective against LPS-induced behavioral deficits or inflammation 8 h post-LPS. Interestingly, naltrexone did not significantly reduce LPS-induced CCL2 and CXCL10 levels or sickness behavior. These results suggest that the timing of β-FNA treatment is critical for neuroprotection, and support earlier findings that the protective effects of β-FNA likely involve mechanisms beyond actions at MOR. However, further examination of the anti-inflammatory and neuroprotective effects of β-FNA is necessary.

Disclosures: S. Myers: None. **D. Buck:** None. **K. McCracken:** None. **J.T. Curtis:** None. **R.L. Davis:** None.

Digital Abstract Session

P134. Neuroinflammation-Microglia

Program #/Poster #: P134.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Transcriptional regulation of iNOS mediated by a novel long non-coding RNA in microglia

Authors: *N. W. MATHY, A. SHIBATA;
Creighton Univ., Omaha, NE

Abstract: Upon activation by inflammatory stimuli, microglia can produce a profoundly neurotoxic inflammatory response. Increased understanding of the regulatory factors controlling microglial activation may be critical to modulating inflammation in the CNS. Long non-coding RNAs (lncRNAs) are transcripts which lack protein coding potential, but have been shown to regulate gene expression via interactions with RNA-binding proteins, such as transcription factors.

Our previous work has shown that one lncRNA, referred to as lncRNA-25B, is highly induced in murine microglia in response LPS, TNF-α, and Ifn-γ. Knockdown of lncRNA-25B prior to LPS stimulation resulted in a significant decrease in the expression iNOS when compared to scrambled siRNA. Conversely, overexpression of lncRNA-25B prior to stimulation resulted in a significant increase in iNOS when compared to an empty vector control. This informed our hypothesis that lncRNA-25B is involved in the transcriptional regulation of iNOS.

We probed for an interaction between lncRNA-25B and NF-κB p65 by using RNA immunoprecipitation, as iNOS is an NF-κB target gene. Results showed that in a basal state, there was no significant enrichment of lncRNA-25B and NF-κB p65 when compared to control IgG. However, after LPS stimulation, there was a significant increase in the interaction between NF-κB p65 and lncRNA-25B compared to control IgG (11.2 fold). There was no significant enrichment in any condition using Actin as a control gene.

To test whether lncRNA-25B enhances NF-κB p65 binding to the promoter region of iNOS, chromatin immunoprecipitation was used. Microglia were transfected with either a scrambled control siRNA, or an siRNA to knockdown lncRNA-25B prior to stimulation with LPS. Knockdown of lncRNA-25B significantly reduced the enrichment of NF-κB p65 to the iNOS promoter at two distinct sites (53.7 fold and 2.8 fold) when compared to the scrambled siRNA control. No significant differences were present in the Actin control gene.

Further evidence revealing the binding of lncRNA-25B to the iNOS promoter region was demonstrated by chromatin isolation by RNA purification. Probes to lncRNA-25B were split into two pools, odd and even, while probes to LacZ were used as a negative control. Both odd and even probe pools revealed significant interaction between lncRNA-25B and two specific sites on the iNOS promoter after LPS stimulation when compared to unstimulated microglia. Taken together, these results suggest lncRNA-25B is involved in the transcriptional regulation of iNOS via NF-κB p65 in microglia in response to LPS stimulation. Future directions will investigate the role of lncRNA-25B *in vivo*.

Disclosures: **N.W. Mathy:** None. **A. Shibata:** None.

Digital Abstract Session

P134. Neuroinflammation-Microglia

Program #/Poster #: P134.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01NS088627
R01NS112144

Title: The emerging role of microglia in AQP4-IgG positive neuromyelitis optica

Authors: *T. CHEN, V. LENNON, L. WU;
Mayo Clin., Rochester, MN

Abstract: The emerging role of microglia in AQP4-IgG positive neuromyelitis optica Tingjun Chen,[1] Vanda A. Lennon,[1,2,3] and Long-Jun Wu[1,2,41]Department of Neurology, [2]Department of Immunology, and [3]Department of Laboratory Medicine/Pathology, Mayo Clinic, Rochester, Minnesota, USA.[4]Department of Neuroscience, Mayo Clinic, Jacksonville, Florida, USA.Neuromyelitis optica (NMO) is an autoantibody-triggered neuroinflammatory disease which preferentiallyattacks the spinal cord and optic nerve. Its defining autoantibody is specific for the water channel, proteinaquaporin-4 (AQP4), which primarily localized at astrocytic end-feet. Histopathology studies of NMOlesions demonstrated complement deposition and microglia activation in AQP4 lost area. Complementdependent cytotoxicity in NMO was well studied in previous researches. However, how microglia activityinvolved in NMO pathogenesis still remained unknown. We investigated early events in the evolvingpathophysiology of NMO in mice by continuously infusing IgG (NMO patient serum-derived or AQP4-specific mouse monoclonal), without exogenous complement, into the spinal subarachnoid space. Motorimpairment and sublytic NMO-compatible immunopathology were IgG dose dependent, AQP4 dependent,and, unexpectedly, microglia dependent. Astrocytes lost AQP4 but remained viable and upregulatedcomplement C3. In vivo spinal cord imaging revealed a striking physical interaction between microgliaand astrocytes, which is mediated by astrocytic C3 and microglial C3a receptor signaling. Moreover,activated microglia released complement C1q, indicating microglia activity induced early-activated CNS-intrinsic complement components and evolving NMO lesioned neuronal dysfunction. Our results indicatethat microglia merit consideration as a potential target for NMO therapeutic intervention.

Disclosures: T. Chen: None. **V. Lennon:** None. **L. Wu:** None.

Digital Abstract Session

P134. Neuroinflammation-Microglia

Program #/Poster #: P134.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NH/NINDS R01NS080844
NIH/MSCEPR (COBRE) P20GM121334
Mississippi INBRE Research Scholars Program
Newborn Medicine Funds from the Department of Pediatrics, University of Mississippi Medical Center

Title: Inflammation exacerbates reduced uterine perfusion pressure-induced spinal cord inflammation and hyperalgesia in rat dams

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Abstract: Emerging epidemiological and experimental studies suggest that systemic inflammation induced by preeclampsia during pregnancy may affect CNS functions including pain signal processing. Our previous studies in rats with reduced uterine perfusion pressure (RUPP) demonstrated that systemic inflammation during pregnancy induced CNS inflammation of rat dams, which has shown to increase pain sensitivity in other pathological conditions. This study was designed to further examine whether maternal inflammation via lipopolysaccharide (LPS) exposure enhances pain sensitivity associated with RUPP in dams. LPS (100 μg/kg) was administered intraperitoneally into pregnant rats on day 13 of gestation (G13) and RUPP surgery was performed on G14. Four groups of 8 dams per group were included in the following treatments: Saline+Sham, Saline+RUPP, LPS+Sham, and LPS+RUPP. The dams were subjected to tail flick testing via thermal stimuli and von Frey filament testing via mechanical stimuli in a double-blind manner. Spinal inflammation and unmyelinated c-fiber projections were examined on day 21 after delivery. To ensure scientific rigor the molecular assays and histological assessment were evaluated by triplicate, and the sample sizes were calculated to reach a statistical power of at least 0.85 for a $P<0.05$. All animals were from the same strain and same vendor. Data were analyzed by two-way ANOVA followed by the Student-Newman-Keuls test. All induced inflammation groups (Saline+RUPP, LPS+Sham, and LPS+RUPP) showed significant increases in thermal sensitivity across postnatal days but only the LPS+RUPP group showed significantly increased sensitivity to mechanical stimuli across postnatal days. Additional LPS exposure enhanced the RUPP-induced microglia and astrocyte activation and unmyelinated c-fiber projections in the lumbar spinal cords of dams on day 21 after delivery. Collectively, LPS-induced systemic inflammation during pregnancy exacerbates RUPP-induced nociceptive afferent plasticity which alters spinal pain signal processing contributing to the development of nociceptive hypersensitivity in rat dams.

Disclosures: L. Fan: None. **H.J. Lee:** None. **J. Chung:** None. **L. Tien:** None. **J.W. Lee:** None. **N. Dankhara:** None. **M.H. Lee:** None. **E. Chen:** None. **X. Dai:** None. **M.A. Tucci:** None. **Y. Pang:** None. **A.J. Bhatt:** None. **N.B. Ojeda:** None.

Digital Abstract Session

P134. Neuroinflammation-Microglia

Program #/Poster #: P134.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Natural Sciences and Engineering Research Council of Canada (RES0029350)
Brain Canada Foundation (RES0030547)
GlycoNET (RES0050801)

Title: Anti-inflammatory effect of ganglioside GM1 and modulatory effects of microglia functions by gangliosides

Authors: *D. GALLEGUILLOS[1,2], Q. WANG[1], N. STEINBERG[1,2], A. ZAIDI[1], G. SHRIVASTAVA[3], Z. DWORSKY-FRIED[1], K. DHAMI[4], C. POWER[3,2], K. TODD[4,2], A. TAYLOR[1,2], S. SIPIONE[1,2];
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Abstract: Microglia are the resident myeloid cells of the Central Nervous System (CNS). In addition to their role in CNS immunity, microglia participate in synapse development and plasticity and provide trophic support for brain cells. Their dynamic nature allows them to execute a variety of tasks under normal and pathological conditions. Gangliosides are sialic acid-containing glycosphingolipids enriched in the CNS. Their location in the outer leaflet of the plasma membrane positions them as mediators of cell-to-cell communication and, as a component of lipid rafts, they play important roles in cell signaling. The deletion of specific ganglioside biosynthetic enzymes in mouse leads to neurodegeneration as well as microgliosis and up-regulation of pro-inflammatory cytokines. On the other hand, the administration of exogenous gangliosides, GM1 in particular, results in neuroprotection in various models of neuronal injury and neurodegeneration. However, little is known about the function/s of gangliosides in microglia. Furthermore, it is unclear whether at least some of the neuroprotective effects of GM1 might be mediated by a direct effect on microglia and neuroinflammation. The goal of this study is to shed light on the role of gangliosides in microglia physiology and pathophysiology. We measured the effects of GM1 administration on the response of BV2 cells as well as mouse, rat and human primary microglia to inflammatory stimuli (LPS, IL-1beta and phagocytosis of latex beads). Exogenous administration of GM1 dramatically decreased inflammatory microglia responses, even when administered after microglia activation. The anti-inflammatory action of GM1 was confirmed *in vivo*, via intraperitoneal administration of LPS followed by intracerebroventricular administration of GM1 in mice. These anti-inflammatory effects were partially reproduced *in vitro* by increasing endogenous ganglioside levels with L-t-PDMP. On the contrary, inhibition of ganglioside synthesis with GENZ-123346 exacerbated microglial activation in response to LPS stimulation. Exogenous GM1, but not L-t-PDMP, also increased microglial phagocytic activity and chemotaxis, two important microglia functions in health and disease conditions, while inhibition of ganglioside synthesis decreased both. In summary, our data suggest that gangliosides are important modulators of microglia functions that are crucial for healthy brain homeostasis. Furthermore, we also demonstrated that the administration of ganglioside GM1 exerts an important anti-inflammatory activity and other beneficial effects on microglia that could open new avenues for therapeutical interventions.

Disclosures: **D. Galleguillos:** None. **Q. Wang:** None. **N. Steinberg:** None. **A. Zaidi:** None. **G. Shrivastava:** None. **Z. Dworsky-Fried:** None. **K. Dhami:** None. **C. Power:** None. **K. Todd:** None. **A. Taylor:** None. **S. Sipione:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Alberta.

Digital Abstract Session

P134. Neuroinflammation-Microglia

Program #/Poster #: P134.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS Grant 19H04279
JSPS Grant 17H06396

Title: Conditioned medium of ACR-exposed microglia induces neurite retraction of monoaminergic neurons

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Abstract: [Background] Acrylamide (ACR), is an environmental electrophile widely used in the production of polymers and gels and recently also reported to be formed when carbohydrate-rich foods are cooked at high temperature. Neurotoxicity of ACR has been reported in humans and experimental animals, while the underlying mechanism remains obscure. Our previous studies demonstrated that exposure to environmental electrophiles such as 1-bromopropane or acrylamide (ACR) causes degeneration of noradrenergic axons. In this study, 1C11 mouse neuroectodermal stem cell line and BV2 microglia cell line were used to investigate the mechanisms of ACR-induced degeneration of monoaminergic neurons. [Methods] 1C11 stem cells were differentiated into noradrenergic neural cells $1C11^{NE}$ or serotonergic neural cells $1C11^{5\text{-}HT}$ neural cells, and then exposed to ACR or to conditioned medium from ACR-treated BV2 microglial cells. Cell viability and loss of plasma membrane integrity were determined using MTS assay and LDH assay respectively, to evaluate the cytotoxicity of ACR exposure on $1C11^{NE}$ and $1C11^{5\text{-}HT}$ cells. Changes in neurite length of noradrenergic or serotonergic neurons were examined by optical microscope and quantified by NIH Image J. Transcriptomic RNA-seq analysis were performed to indentify differential expressed genes in $1C11^{NE}$ after exposure to ACR or conditioned medium from BV2. [Results] Results of MTS and LDH cell viability/cytotoxicity assay showed that exposure to ACR at lower than 1 mM didn't decrease $1C11^{NE}$ or $1C11^{5\text{-}HT}$ cell viability or increase LDH release, suggesting no induction of cell death in neural cells. Quantification results of neurite length showed that treatment with ACR at 1 mM did not decrease the neurite length of $1C11^{NE}$, but treatment with supernatant from BV2 microglial cells exposed to ACR at 1 mM significantly decreased neurite length of $1C11^{NE}$ or $1C11^{5\text{-}HT}$. The addition of 1mM ACR into supernatant of BV2 culture medium also significantly decreased the neurite length of $1C11^{NE}$ or $1C11^{5\text{-}HT}$. Moreover, transcriptomic RNA-seq analysis identified 2544 differential expressed genes in $1C11^{NE}$ after exposure to ACR or conditioned medium from BV2. In conclusion, the above results suggest that microglia play a critical role in ACR-induced retraction of monoaminergic neurons. Further analysis of RNA-seq data is ongoing to investigate changes of gene expression profile.

Disclosures: **C. Zong:** None. **H. Sato:** None. **B. Schneider:** None. **G. Ichihara:** None. **M. Urushitani:** None. **S. Iwama:** None.

Digital Abstract Session

P134. Neuroinflammation-Microglia

Program #/Poster #: P134.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R15 MH107007

Title: Status-dependent differences in acute stress-induced neuroinflammation in the hamster vmPFC

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Abstract: 90% of humans experience traumatic stress, however, only 10% develop psychopathologies like PTSD. Social status influences stress reactivity such that social dominance can promote stress resilience, representing an environmental factor that buffers individuals from maladaptive responses to stress. The negative consequences of severe and chronic stress include neuroinflammation, which is associated with neuronal degeneration and oxidative stress. We use a Syrian Hamster model of social defeat to investigate status-dependent differences in susceptibility and resiliency to acute social stress. We hypothesized that neuroinflammation mediates susceptibility in the status dependent pairs such that dominants show an increased protective neuroinflammatory response compared to subordinates, from a social defeat stressor. We paired hamsters for 14 days in daily 5-minute social encounters to allow them to form a social hierarchy with one another. After dominance relationships were established, the animals were socially defeated in a resident intruder model. Twenty-four hours later, the animals were tested for stress-related behavior using a conditioned defeat test and social interaction test. Brains were collected and analyzed for markers of neuro -inflammation and -degeneration in the ventral medial prefrontal cortex. Overall, we found that dominants were more resistant to stress-induced changes in neuroinflammation compared to subordinates. Dominant hamsters demonstrated a protection against morphological changes in neuroinflammatory markers that are indicative of a negative phagocytic response in the infralimbic cortex. While dominants did show increased markers of synaptic degeneration similar to subordinates, they showed protection from overall cellular degeneration in the vmPFC. This result, especially when compared with previous studies, suggests that synaptophysin may not be good marker for status dependent synaptic degradation (Thome et al., 2001; Grizzell et al., 2014a,b). After CD testing, we found that there was a significant increase in submissive like behavior among subordinates in comparison to dominants which suggests that the neuroinflammatory markers correspond to the alteration in social avoidance behavior. However,

the results from SIT testing were inconclusive and may suggest that it may be an inappropriate test for anxiety like behaviors in male Syrian hamsters, barring the implementation of social vigilance and or a larger scoring arena. Altogether, this study provides a neurobiological basis for the development of novel pharmacological interventions used to treat traumatic stress exposure.

Disclosures: R.M. Rodriguez: None. **J.A. Grizzell:** None. **T.T. Clarity:** None. **M.S. Jenkins:** None. **M.A. Cooper:** None.

Digital Abstract Session

P134. Neuroinflammation-Microglia

Program #/Poster #: P134.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Race to Erase MS Grant 90079114
Hilton Foundation MS Grant 90074168

Title: Bryostatin-1 inhibits CNS inflammation through modulation of the innate immune system

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Abstract: Multiple sclerosis (MS) is a neurodegenerative disease characterized by chronic inflammation and remyelination failure. Current therapies have anti-inflammatory effects on the peripheral immune system, aimed at alleviating the relapsing-remitting stage, but fail to target the compartmentalized innate immune-mediated inflammation seen during the progressive phase of disease. A growing body of evidence suggests that the phenotype of innate immune cells such as microglia/macrophages dictates the success or failure of remyelination and modulates neurodegeneration. We previously found that bryostatin-1 (bryo), a CNS-penetrant PKC modulator, has immunomodulatory properties that are most prominent in myeloid-lineage cells. We hypothesized that bryo acts directly on CNS macrophages/microglia to inhibit neurotoxic phenotypes and promote functions associated with remyelination and repair. Mixed glial cultures (MGC) derived from neonatal C57BL/6 mice were treated with vehicle or bryo (50 or 200 nM) +/- LPS (100 ng/ml), IFNγ (20ng/mL), or IL4 (20 ng/mL). Expression of iNOS (inflammatory) and Arg-1 (anti-inflammatory) were assessed by immunoblot and verified through immunofluorescence, and transcriptional profiling was performed with RNAseq. To evaluate the impact of bryo on innate immune phenotypes in vivo, we induced MOG_{35-55}/CFA experimental autoimmune encephalomyelitis (EAE) in 8-week-old female C57BL/6 mice and began treatment with vehicle or bryo (30 μg/kg) by daily intraperitoneal injection beginning on post-immunization day (PID) 28. This time-point corresponds to late-stage disease, when peripheral inflammation has subsided, but innate immune activation persists in the CNS. Flow cytometry was performed from the CNS at PID 42, and CD206 expression was assessed on CNS-resident

macrophages/microglia. In MGC, bryo prevented iNOS expression induced by LPS and dramatically potentiated Arg-1 expression in the presence of IL-4, at a dose as low as 1 nM. RNA sequencing demonstrated that bryo upregulated genes associated with repair and anti-inflammatory pathways. Following LPS/LPS-IFNγ treatment, bryo decreased expression of A1 reactive astrocyte markers like C3 and Gbp2 while augmenting expression of A2 markers. Systemic treatment with bryo led to a significant increase in CD206+ macrophages/microglia in the CNS at EAE PID 42 ($p < 0.05$, student t-test). Our results demonstrate that bryo acts directly on CNS macrophages/microglia, promoting regenerative functions while inhibiting neurotoxic phenotypes. Bryo has therapeutic potential in progressive MS.

Disclosures: E. Abramson: None. **M. Smith:** None. **S. Hwang:** None. **P.A. Calabresi:** None. **S.H. Snyder:** None. **P. Kim:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending. **M. Kornberg:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending.

Digital Abstract Session

P134. Neuroinflammation-Microglia

Program #/Poster #: P134.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Modeling neuroinflammatory diseases with iPSC-derived microglia

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Abstract: Microglia contribute to a range of neuroinflammatory and neurodegenerative disorders, but scientists have long suffered from a lack of quality in vitro models for these cell types. We have developed a robust and scalable method to produce highly pure, functionally validated, and ready-to-use microglia from human induced pluripotent stem cells (iPSC). These microglia show greater than 97% purity as measured by immunostaining for IBA1 and TMEM119. These cells can be cryopreserved, thawed, and cultured in defined maintenance media with or without astrocytes or neurons for prolonged culture. We have used high-content imaging for phenotypic characterization of these models, including observing a re-distribution of inflammasome proteins (NLRP3 and ASC) from diffuse and cytoplasmic to organized punctate structures. This inflammasome formation could be blocked by the inhibitor MCC950. These data show functionally relevant and measurable phenotypes in these human iPSC-derived microglia may be suitable for modeling neuroinflammatory diseases and in drug-screening assays.

Disclosures: L. Thal: A. Employment/Salary (full or part-time):; PhenoVista Biosciences. **C. Koehler:** A. Employment/Salary (full or part-time):; PhenoVista Biosciences. **R. Price:** A. Employment/Salary (full or part-time):; PhenoVista Biosciences. **K. Choi:** A.

Employment/Salary (full or part-time):; BrainXell, Inc. **M. Hendrickson:** A. Employment/Salary (full or part-time):; BrainXell, Inc. **Z. Du:** A. Employment/Salary (full or part-time):; BrainXell, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BrainXell, Inc..

Digital Abstract Session

P134. Neuroinflammation-Microglia

Program #/Poster #: P134.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Indiana CTSI UL1TR002529

Title: Characterizing microglial lipidome and metabolome with amyloid beta exposure and phagocytosis

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Abstract: Microglia are specialized immune cells in the brain that maintain tissue homeostasis by eliminating misfolded protein deposits and cellular debris by the process of phagocytosis. In Alzheimer's disease (AD), microglia are exposed to amyloid β (Aβ) aggregates but are unable to clear them from the environment. There has been an increasing interest in using Aβ-targeting therapies for AD, however, the Aβ-associated cellular and molecular changes in glia remain poorly understood. Lipids and metabolites orchestrate critical regulatory events within the cells or by their secretion into the cellular microenvironment. How does Aβ treatment affect microglial lipid and metabolite response? To answer this question, we used Multiple-Reaction Monitoring (MRM)-profiling, a sensitive mass spectrometry technique, to characterize the changes in cellular and secretory lipids and metabolites that were differentially regulated in Aβ-treated primary mouse microglia and in their conditioned medium. Microglia were isolated from male and female C57B/6 adult mice and cultured in highly defined media with reduced serum condition. The cells were treated with aggregated human $A\beta_{1-42}$ for 1, 12, or 24 hours. We screened around 1500 total lipid species categorized into 10 main classes and around 700 metabolites to identify the Aβ-associated molecular changes in microglia. We found that inflammation-associated long-chain saturated free fatty acids (C20:0, C22:0, etc.) increased at initial timepoints but reduced with longer Aβ treatment. Triacylglycerides and phosphatidylglycerols were the most abundant lipid classes at 24 hours indicating a metabolic shift in microglial molecular repertoire with prolonged Aβ exposure. Further, we identified 42 significant metabolites in Aβ-treated microglia that are associated with alanine, aspartate and glutamate metabolism, arginine biosynthesis, citrate cycle, etc., including L-2-aminobutyric acid, hydroxybutyric acid, etc. Selected metabolites identified in the conditioned medium, for example, stearic acid, palmitic acid, etc. have also been shown to affect the phagocytic properties of peripheral macrophages and may also play a role in the microglial phagocytic response. Ongoing work involves evaluating the lipid and metabolite changes in different phagocytic and

non-phagocytic glial cell subsets in Alzheimer's mouse models such as 5xFAD mice to identify functional targets for phagocytosis. We thus conclude that identifying key functional molecules involved in Aβ phagocytosis, metabolism, and signaling is critical for elucidating microglia's role in health and disease.

Disclosures: P. Prakash: None. **P.K. Wijewardhane:** None. **G. Chopra:** None.

Digital Abstract Session

P134. Neuroinflammation-Microglia

Program #/Poster #: P134.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH EY015537
NIH EY013434
NIH EY001583
NIH EY007035
TR001880

Title: Morphologic and molecular activation of microglial cells by stimulation of the P2X7 receptor follows mechanical strain

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Abstract: The initial processes that link mechanical strain to neuroinflammation provide critical targets for intervention in disorders like traumatic brain injury and glaucoma. We hypothesize that stimulation of P2X7 receptors after mechanosensitive release of agonist ATP can activate microglial cells and increase neuroinflammation. *In vivo* retinal activation of P2X7 with BzATP led to reduction of overall size of microglia and elevated expression of Iba1, suggestive of activation of microglia. Primary cultures of retinal microglia expressed P2X7 receptors, with BzATP triggering a rapid rise in intracellular Ca^{2+} that was blocked by antagonist A839977. BzATP also led to process retraction, cell body enlargement and increased expression of *NOS2* and *ARG1*. Expression of mRNA for microglial marker *TMEM119* decreased *in vitro* after exposure to BzATP or LPS and *in vivo* after elevation of pressure; immunoblots confirmed the decline in TMEM119 protein with ATP. Early responses of microglial cells to transient elevation of intraocular pressure were examined. Microglial morphology was altered by pressure elevation, with cell bodies enlarging and processes retracting. Expression of genes associated with non-M0 microglial states (*NOS2*, *TNFα*, *ARG1*, *YM1*) were also detected in the retina. Pressure elevation increased levels of extracellular ATP, while morphological and molecular changes were reduced in retina from $P2RX7^{-/-}$ mice. In summary, the P2X7 receptor plays a key role in the rapid activation of microglial cells following mechanical strain.

Disclosures: **K.E. Campagno:** None. **W. Lu:** None. **H. Tso:** None. **A. Cenaj:** None. **P. Sripinun:** None. **F. Albalawi:** None. **N. Mas-Gomez:** None. **C.H. Mitchell:** None.

Digital Abstract Session

P135. Neuroinflammation: Beyond Microglia

Program #/Poster #: P135.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Medical Research Council UK (SNCF G1002055)
ERA-NET NEURON (MR/R005532/1)

Title: Immunomodulatory effects of chondroitin sulfate proteoglycans after spinal cord injury are mediated via a TLR4-dependent mechanism

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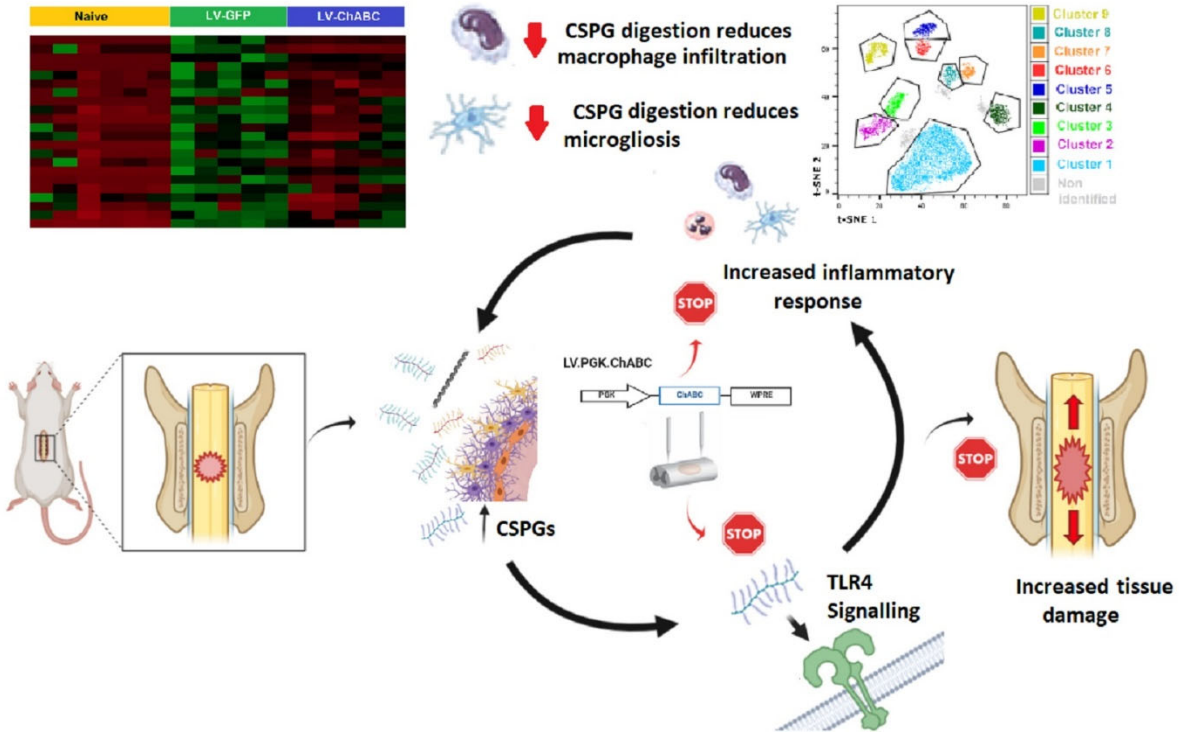
Abstract: Chondroitin sulfate proteoglycans (CSPGs) are highly expressed in spinal injury scar tissue and are well established to be potent inhibitors of axonal growth and neuroplasticity. Here we report a role for CSPGs that goes far beyond inhibition of neuronal sprouting and demonstrate their critical role in mediating pathological chronification of the inflammatory response. CSPG digestion by Chondroitinase ABC enzyme (ChABC) demonstrated a remarkable immunomodulatory role of CSPGs, which was predominant in the resolution phase of the inflammatory response. By the use of flow cytometry and protein expression luminex analysis we demonstrated that CSPG digestion enhances immune cell clearance, modulates macrophage and microglial cell phenotype and reduces pro-inflammatory cytokine expression in the lesion site. Moreover, in vitro mechanistic studies reveal that TLR4 signalling is critical in driving CSPG-mediated inflammatory pathology, being essential in the development of an inflammatory phenotype of immune cells and delaying the resolution phase of inflammation.
Overall, our findings demonstrate that CSPGs modulate immune cell responses and this ECM-immune cell interaction suggests a potential new avenue of treatment not only for SCI, but also other neurological diseases with a marked inflammatory component.



Disclosures: **I. Francos-Quijorna:** None. **M. Sanchez-Petidier:** None. **E.R. Burnside:** None. **A. Torres Espín:** None. **L. Marshall:** None. **J. Verhaagen:** None. **V. Moreno-Manzano:** None. **E.J. Bradbury:** None.

Digital Abstract Session

P135. Neuroinflammation: Beyond Microglia

Program #/Poster #: P135.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Alzheimer's Foundation of America (AFA) - ZEN-16-362441
NIH grant - 1R01 AG058467-01 (NIA)
NIH grant - 1R21 NS091593-01 (NINDS)

Title: Dissecting cell-type specific gene expression patterns temporally in the brain during systemic inflammatory response syndrome

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Abstract: Sepsis-associated encephalopathy (SAE) is an acutely progressing brain dysfunction that develops during systemic inflammatory response syndrome. The mechanism of initiation of neuroinflammation during SAE, which ultimately leads to delirium and cognitive dysfunction,

remains elusive. To understand the succession of events and to get a cell-type specific snapshot of transient as well as sustained gene expression changes, it is essential to study the acute inflammatory responses in the central nervous system (CNS) in a time course manner. We thus aimed to study the molecular events of SAE to capture its onset and progression into the CNS, and further identify the cellular players involved in mediating acute inflammatory signaling. Gene expression profiling on the cerebral vessels isolated from the brains of the mice treated with peripheral lipopolysaccharide (LPS) revealed that the cerebral vasculature responds within minutes to acute systemic inflammation by upregulating the expression of immediate early response genes, later followed gradually by inflammatory cytokine genes and simultaneous activation of the NF-κB pathway. Intriguingly, this early response was detected only in the cerebral vessels but not in the rest of the brain. To identify the earliest responding cell type, we used fluorescence-activated cell sorting (FACS) to sort the glial and vascular cells from the brains of the mice treated with LPS at different time points. RNA-seq was then performed on microglia and cerebral endothelial cells (CECs). Bioinformatic analysis followed by further validation in all the cell types revealed that panendothelitis i.e. the activation of CECs is the earliest event in the CNS during the inception of acute neuroinflammation. Microglial activation occurs later than that of CECs suggesting that CECs are the most likely initial source of pro-inflammatory mediators which could further initiate glial cell activation. This is then followed by the activation of apoptotic signaling in the CECs which is known to lead to the blood-brain barrier disruption and allow peripheral cytokines to leak into the CNS, exacerbate the gliosis, and result in the vicious neuroinflammatory cascade. Our results reveal the chronological expression of acute inflammatory signaling in each of the gliovascular cells and portray the temporal kinetics of gene expression in a cell-type specific manner during the induction of SAE. Together, these results model the earliest sequential events during the advancement of systemic inflammation into the CNS and facilitate to understand the interplay between the vascular and glial cells in initiating and driving acute neuroinflammation.

Disclosures: **M. Kodali:** None. **H. Chen:** None. **F. Liao:** None.

Digital Abstract Session

P135. Neuroinflammation: Beyond Microglia

Program #/Poster #: P135.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DGE -1644869
R03 NS099920

Title: Endothelial caspase-9 plays an inflammatory role in neurovascular injury

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Abstract: Neurovascular injuries can lead to neuroinflammation and to the development of neurodegenerative diseases. However, the molecular link between the neurovasculature, glial cells and their contribution to neurodegeneration remains to be understood. The mouse model of retinal vein occlusion (RVO) provides an avenue to investigate the endothelial-glial signaling mechanisms and its contribution to neuroinflammation. Using the RVO mouse model, previous studies in the laboratory revealed that neurovascular injury triggered activation of endothelial caspase-9 (EC-Casp9) in a non-apoptotic manner. Further, inhibition of caspase-9 caused a decrease in neuronal death, retinal edema and astrocytic caspase-6. But this intervention did not change the total number nor the activated state of microglia after injury. In this study we aimed to test the hypothesis that non-apoptotic activation of EC-Casp9 does not lead to microgliosis but to an increase in astroglial caspase-6 which will result in upregualted levels of GFAP and decrease of aquaporin-4 (AQP4) post RVO. To test this hypothesis, we used a tamoxifen-inducible endothelial caspase-9 knockout (iEC Casp9KO) mouse line. After tamoxifen administration, we induced RVO blinded to genotypes in two months old, male endothelial caspase-9 knockouts (iEC Casp9KO, n= 7) and endothelial caspase-9 littermate controls (Casp9 WT, n=11) by laser photocoagulation of major retinal veins (n=2-3). Uninjured Casp9 WT (n=3) and iEC Casp9KO (n=4) were used as controls. After collecting the retinas, we examined microglia and astroglia using different immunohistochemical markers to test for glial activation 1 and 2 days post-RVO. Confocal images were blindly quantified with Image J using threshold and color threshold analysis. Welch's t test, one-way ANOVA and Dunnett's multiple comparisons were performed for statistical analysis. Ablation of endothelial caspase-9 did not affect the total number of microglia (Iba-1^{+}) cells in injured animals, but caused a significant decline in the area of the macrophagic microglial marker, CD68. Retinas from injured iEC Casp9KO showed a significant decrease of GFAP expression in astrocytes 1 day post-RVO and a downward trend in AQP-4 2 days post-RVO. Moreover, astrocytes from iEC Casp9KO had a significant decrease in the expression of cl-caspase-6 1 and 2 days after injury. Overall, these data suggest that EC-Casp9 leads to increased microglial phagocytic activity and astroglial caspase-6 in neurovascular injury. Future studies include assessing the contribution of astroglial caspase-6 signaling to neurodegeneration.

Disclosures: C. Colón Ortiz: None. **M. Avrutsky:** None. **C.M. Troy:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; R03 NS099920. **J. Lawson:** None. **J. Smart:** None.

Digital Abstract Session

P135. Neuroinflammation: Beyond Microglia

Program #/Poster #: P135.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: FWO grant

Title: Inflammation as a driving force for optic nerve regeneration: comprehensive characterization of the cellular and molecular players

Authors: *L. ANDRIES[1], L. DE GROEF[1], L. MASIN[1], E. LEFEVERE[1], M. SALINAS-NAVARRO[1], I. SCHEYLTJENS[2], K. MOVAHEDI[2], L. MOONS[1];
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Abstract: Despite intensive research, induction of long-distance axonal regeneration and functional recovery of the damaged central nervous system (CNS) remain a challenge. The ever-innovating insights into the dichotomous role of neuroinflammation sprouted the idea that, instead of suppressing the inflammatory machinery, directing and instructing it may be a better therapeutic objective to trigger axonal regrowth. The overarching goal of this project is to unravel the underlying cellular and molecular players that link inflammation to axonal regeneration using optic nerve crush (ONC) (degeneration model) and ONC combined with inflammatory stimulation (ONC+IS) (regeneration model). The responses of resident glia and invading inflammatory cells during neuroregenerative processes are still controversial and insufficiently described. Therefore, we investigated the kinetics of myeloid cell influx in the retina and optic nerve, at different time points after ONC and ONC+IS, in wild-type, *Cx3Cr1.CreERT2xR26.STOP.YFP* and *LysMGFP* mice combining flow cytometry and immunohistochemical stainings. A large influx of monocytes, monocyte-derived macrophages (MDMs) and neutrophils was noticed in both tissues after ONC+IS from 2dpi onwards, which was not observed after ONC only. In both experimental conditions we detected a significant increase in the number of microglia at 6 and 8dpi, but microglia became larger and more internally complex after ONC+IS, a sign of augmented activation. Next, the immune cell heterogeneity in the retina was analysed using a single-cell RNA sequencing (scRNAseq) and subsequent bioinformatics analysis on $CD11b^+$ retinal cells to identify transcriptomic changes after ONC(+IS). In the regenerating retina, an increased heterogeneity and a higher number of the infiltrating myeloid cells, mainly macrophages, were detected. Depleting these macrophages, using *Ccr2*$^{-/-}$ mice, resulted in a lower number of MDMs but more neutrophils and in a decreased axonal regeneration upon ONC+IS, suggesting these macrophages and their transcriptome are important during axonal regeneration. With our research, we created a single-cell atlas of the healthy, injured and regenerating retina. We also initiated experiments in mice treated with plexxicon to further define a role for the different cell population involved in inflammatory-induced axonal regeneration.
Taken together, these data combined with an even more comprehensive bioinformatic analysis of the transcriptomics approach will enable us to pinpoint the inflammatory cell populations and processes that are specific to a pro-regenerative response.

Disclosures: L. Andries: None. **L. De Groef:** None. **L. Masin:** None. **M. Salinas-Navarro:** None. **E. Lefevere:** None. **I. Scheyltjens:** None. **K. Movahedi:** None. **L. Moons:** None.

Digital Abstract Session

P135. Neuroinflammation: Beyond Microglia

Program #/Poster #: P135.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Endogenous neurosteroids increase anti-inflammatory mediators in immune cells and brain

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Abstract: Peripheral and brain inflammation are increasingly recognized as critical mediators in the initiation and progression of neurologic and psychiatric disorders (Cervellati et al., 2020, PMID: 32143546; Morrow & Price, 2019, PMID: 31637451). We have previously shown that endogenous neurosteroids, including pregnenolone and allopregnanolone (3α,5α-THP) inhibit inflammatory toll-like receptor 4 (TLR4) signal activation in mouse RAW264.7 macrophages and the brain of alcohol-preferring (P) rats (Balan et al., 2019, PMID: 30718548). Our recent data indicate that 3α,5α-THP inhibits activation of MyD88- but not TRIF-dependent TLR pro-inflammatory signals and the production of pro-inflammatory mediators (TNFα, MCP1, IL-1β, IL-6) through its ability to block TLR-MyD88 binding in P rat brain and mouse and human macrophages (unpublished data). As TLR4 activation can also increase anti-inflammatory signals (Siegemund & Sauer, 2012, PMID: 23080196), we examined whether endogenous neurosteroids have effects on anti-inflammatory TLR4 signals in peripheral immune cells (mouse and human macrophages) and P rat brain. Aurelian and Balan (2019, PMID: 31030249) previously showed that P rats exhibit innate suppression of the TLR4-associated anti-inflammatory chemokine CX3CL1 in the nucleus accumbens (NAc) compared to non-alcohol preferring (NP) counterparts. Using immunoblotting assays, we report that IP administration of 3α,5α-THP (15 mg/kg), pregnenolone (75 mg/kg) or 3α,5α-tetrahydrodeoxycorticosterone (3α,5α-THDOC; 15 mg/kg) significantly increases the NAc levels of CX3CL1 and anti-inflammatory cytokine IL-10 in male P rats. Using ELISA, we confirm that 3α,5α-THP significantly increases CX3CL1, in both male (30%, $p<0.05$) and female (45%, $p<0.05$) P rats NAc. Furthermore, 3α,5α-THP (1 μM) and 3α,5α-THDOC (1 μM) increased the level of IL-10 (~30%) in LPS-activated RAW264.7 macrophage cells. Finally, we studied these effects in human macrophages that were differentiated from PBMCs by GM-CSF (15 ng/ml; 1 week). Cells were treated with TLR4 agonist LPS (1 μg/ml; 24h) with/without 3α,5α-THP (1 μM) or 3α,5α-THDOC (1 μM). Both 3α,5α-THP and 3α,5α-THDOC increased (~35%) the level of CX3CL1. Collectively, the data indicate that the therapeutic potential of these neurosteroids involves modulation of TLR signaling pathways resulting in diminished pro-inflammatory signal activation and enhancement of anti-inflammatory signaling. Ongoing studies are designed to evaluate the mechanisms of the neurosteroid enhancement of anti-inflammatory CX3CL1 and IL-10 signaling both in immune cells and the brain.

Disclosures: **I.S. Balan:** None. **L. Aurelian:** None. **R.B. Meeker:** None. **T.K. O'Buckley:** None. **G. Boero:** None. **A. Morrow:** None.

Digital Abstract Session

P135. Neuroinflammation: Beyond Microglia

Program #/Poster #: P135.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Human Frontier Science Program
HHMI
Klarman Cell Observatory
NIH Grants MH105960, EY028625, EY021526, NS029169, NS104248, EY029360, EY028448, EY030204, EY026939, EY028625, P30EY012196, and AG056636
Wings for Life Spinal Cord Research Foundation
SRA from Biogen Inc.

Title: Tissue dynamics in CNS injury: exploring mechanisms underlying selective neuronal resilience

Authors: *I. BENHAR[1], J. DING[1], K. SHEKHAR[1], I. E. WHITNEY[2], M. SUD[1], G. BURGIN[1], A. JACOBI[3], N. M. TRAN[2], W. YAN[2], C. WANG[3], Z. HE[3], J. R. SANES[2], A. REGEV[1]; [1]Broad Inst. of MIT and Harvard, Cambridge, MA; [2]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA; [3]F.M. Kirby Neurobio. Ctr., Boston Children's Hospital, Harvard Med. Sch., Boston, MA

Abstract: The inability of neurons in the adult mammalian central nervous system (CNS) to regenerate after injury or in disease leads to devastating physiological outcomes. Both neuron-intrinsic and extrinsic mechanisms play a role in this deficit. A key challenge in analyzing responses to CNS insult is the diversity of cell types involved - neuronal and non-neuronal - and dynamic changes in their composition and interactions over time. We addressed this complexity using the mouse optic nerve crush (ONC) injury model, which results in extensive death of retinal ganglion cells (RGCs), and is a tractable CNS injury model system for studying processes underlying neuronal degeneration and regeneration. Curiously, although ~80% of RGCs die within 2 weeks of ONC, death is not uniform across different RGC types. We set out to chart the tissue response following ONC, and gain insight into mechanisms underlying selective neuronal resilience. Using single-cell RNA-sequencing, we first generated a comprehensive molecular atlas of 46 RGC types in adult (P56) mouse retina, which we tracked after ONC, identifying resilient and susceptible types and their gene expression profiles. Manipulating some of these genes in vivo improved RGC survival and regeneration (Tran et al., 2019). Broadening our view to the tissue level, we additionally profiled >130,000 immune, glial and epithelial cells from retina at baseline and at six time points after ONC. By analyzing changes in gene expression throughout the time course, we identified distinct subsets of these cells, and temporal changes in

their composition and transcriptional states. We charted the immune cell cascade in the retina, highlighting changes in resident microglia, as well as blood-derived infiltrating leukocytes, and used immunohistochemistry and in situ hybridization to visualize these cells in the tissue. Combining computational analysis and experimental validation, we are inferring the cellular circuitry, spatial relationships and molecular interactions governing post-injury tissue dynamics. These findings could help identify potential targets for promoting neuroprotection and regeneration in the CNS.

Disclosures: I. Benhar: None. **J. Ding:** None. **K. Shekhar:** None. **I.E. Whitney:** None. **M. Sud:** None. **G. Burgin:** None. **A. Jacobi:** None. **N.M. Tran:** None. **W. Yan:** None. **C. Wang:** None. **Z. He:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rugen. F. Consulting Fees (e.g., advisory boards); SpineX. **J.R. Sanes:** F. Consulting Fees (e.g., advisory boards); Biogen. **A. Regev:** A. Employment/Salary (full or part-time):; From August 1, 2020, A.R. is an employee of Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Celsius Therapeutics, Immunitas. F. Consulting Fees (e.g., advisory boards); ThermoFisher Scientific (until July 31, 2020), Syros Pharmaceuticals (until July 31, 2020), Neogene Therapeutics (until July 31, 2020), Asimov (until July 31, 2020).

Digital Abstract Session

P135. Neuroinflammation: Beyond Microglia

Program #/Poster #: P135.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Cleon C. Arrington Research Initiation Grant Program (RIG-93)
National Ataxia Foundation

Title: Rnf216 as a regulator of neuroinflammatory pathways

Authors: *D. GROSSMAN, A. GEORGE, A. MABB;
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Abstract: Ubiquitin E3 ligases are enzymes that mark substrates with ubiquitin proteins which leads to different cellular fates. Ring finger protein 216 (RNF216) is a ubiquitin E3 ligase in the nervous system that is involved in synaptic plasticity, cellular autophagy, and the immune response. Previous literature shows that RNF216 participates in inflammation by regulating ubiquitination and subsequent proteasomal degradation of receptor-interacting serine-threonine kinase 1 (RIPK1), toll-interleukin 1 receptor domain containing adaptor protein (TIRAP), and TIR-domain-containing adapter-inducing interferon-β (TRIF), targeting TNF receptor associated factor 3 (TRAF3) for degradation. Toll-like receptors (TLRs) initiate signal transduction pathways which can lead to cytokine production, proliferation, survival, adaptive immunity, phagocytosis, and apoptosis. One of the RNF216 isoforms, TRIAD3A, controls the intensity and

duration of TLR signaling, specifically TLR4 and TLR9, through ubiquitination and subsequent proteolytic degradation. The role of RNF216 in regulation of the factors that contribute to inflammation is not well studied in the central nervous system (CNS). Using a mouse hypothalamic cell line (GT1-7) in which *Rnf216* was knocked down using the Crispr-Cas9 system, we sought to determine whether RNF216 might regulate TLR signaling pathways in the CNS. The findings of this exploratory project elucidate how disruptions in RNF216 may alter neuroinflammation. This is significant because elucidating potential mechanisms of neuroinflammation could lead to novel therapeutic targets for neuroinflammatory disorders.

Disclosures: D. Grossman: None. **A. George:** None. **A. Mabb:** None.

Digital Abstract Session

P135. Neuroinflammation: Beyond Microglia

Program #/Poster #: P135.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01MH52716
F32HD097816

Title: Evidence for mast cell proliferation in the developing brain

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Abstract: Perinatal brain development is characterized by distinctive phases of neuronal proliferation and synaptic pruning to establish appropriate connections required for normal brain function. Disturbances in this tightly orchestrated process pre- and post-natally lead to both developmental disorders including autism and neurological conditions in adulthood including seizures and schizophrenia. The peripheral immune system can be a source of both homeostatic regulation of neurodevelopment and chronic pro-inflammatory damage. Recent studies implicate mast cells, a population of innate immune cells, in maintaining proper sexual differentiation in the rat preoptic area through interactions with resident microglia. However, factors recruiting mast cells to other regions of the brain, including the mast cell-dense choroid fissure, and their brain-specific activities are poorly understood. Here, we sought novel insights into the origin and regulation of this large population of granule-rich immune cells positioned adjacent to the hippocampus throughout development. Establishment of a flow cytometric protocol to isolate mast cells from the postnatal rat brain enabled characterization of their phenotype, replication status, and migration patterns in the postnatal (PN) period. Through immunohistochemical analysis, we discovered that mast cells in the lateral ventricles of the neonatal brain replicate during the first week of life – an activity previously thought to be restricted to bone marrow. This replicative activity of mast cells exhibited both brain region specificity and tissue specificity across the first postnatal week. Mast cells replicated at higher rates in the choroid fissure than in the skin at both PN1 and PN7. Our findings suggest brain mast cell (MC_B) replication is

differentially regulated compared to peripheral mast cells in the highly replicative bone marrow (MC_{BM}) and more quiescent skin (MC_s). Continued exploration of this immunogenic niche and its potential contribution to nearby neurogenic niches will illuminate the homoeostatic role of the innate immune system in shaping brain development in both males and females.

Disclosures: A.C. Blanchard: None. **L.A. Pickett:** None. **E.L. Reinl:** None. **M.M. McCarthy:** None.

Digital Abstract Session

P135. Neuroinflammation: Beyond Microglia

Program #/Poster #: P135.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Abnormal expression of neuroinflammation-related genes is associated with the C9orf72 mutation in a human stem cell model of ALS and FTD

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Abstract: Neurodegenerative diseases affect millions of adults world-wide and pose a massive socio-economic burden. Amyotrophic Lateral Sclerosis (ALS) is a fatal and incurable neurodegenerative disease marked by the loss of motor neuron function leaving the patient unable to use their muscles. Frontotemporal Dementia (FTD) presents with the loss of cortical neurons in the frontal lobes of the brain leaving FTD patients confused and with behavioural issues. On the surface, ALS and FTD seem like very different diseases. However, a hexanucleotide repeat expansion at C9ORF72 has been determined to be the most common mutation in both diseases. For the past thirty years, most research on neurodegenerative diseases have focused on the cell autonomous aspect of the disease- how a dysfunction inside the neuron causes its malfunction and subsequent death. It has now become evident that non-cell autonomous events, mainly neuroinflammation imposed by the glia in the disease niche, is a hallmark of all neurodegenerative diseases. The work described here uses human induced pluripotent stem cells derived astrocytes from healthy patients and patients suffering from C9ORF72 ALS and FTD to model the inflammatory cascade present in neurodegeneration. Using reverse transcription-polymerase chain reaction to quantify RNA transcription for genes involved in different aspects of the astrocyte inflammatory response, data suggests that C9ORF72 astrocytes have an aberrant reaction to inflammatory signals compared to healthy patient derived astrocytes. Additionally, C9ORF72 astrocytes show an abnormal cellular morphology. This data suggests that an atypical inflammatory response may contribute to C9ORF72 ALS and FTD.

Disclosures: C.P. Chossis: None. **S.I. Van Deursen:** None. **A.L. O'Neil:** None.

Digital Abstract Session

P136. Neuroinflammation: Animal Models

Program #/Poster #: P136.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Cigarette smoke dose dependently exacerbates clinical symptoms and pathology in a mouse model of multiple sclerosis

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Abstract: Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system. The etiology of the disease is complex and involves an interplay between genetic and environmental factors. Cigarette smoking has emerged as a major risk factor associated with the onset of MS. To further address this topic, we studied the effect of chronic cigarette smoke (CS) exposure in an experimental autoimmune encephalomyelitis (EAE) mouse model. Among several models of human MS, EAE is the most commonly employed animal model for investigating the pathogenesis of and therapeutic interventions for MS. In our study, we chose a myelin oligodendrocyte glycoprotein amino acid 35-55 (MOG35-35)-induced EAE mouse model to investigate the effect of CS exposure on EAE prevalence, onset, progression, severity, and spinal cord pathology. Our results show that CS can affect clinical symptoms and spinal cord lesions in a dose-dependent manner.
Conflict of interests: The research described in this abstract is sponsored by Philip Morris International. All authors are PMI employees.

Disclosures: **K. Koshibu:** A. Employment/Salary (full or part-time):; PMI. **J. Ho:** A. Employment/Salary (full or part-time):; Philip Morris International Research Laboratories Pte. Ltd. **W. Xia:** A. Employment/Salary (full or part-time):; Philip Morris International Research Laboratories Pte. Ltd. **L. Garcia:** A. Employment/Salary (full or part-time):; PMI. **A. Kondylis:** A. Employment/Salary (full or part-time):; PMI. **B. Phillips:** A. Employment/Salary (full or part-time):; Philip Morris International Research Laboratories Pte. Ltd. **K. Luettich:** A. Employment/Salary (full or part-time):; PMI. **J. Hoeng:** A. Employment/Salary (full or part-time):; PMI. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PMI. **M. Peitsch:** A. Employment/Salary (full or part-time):; PMI. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PMI.

Digital Abstract Session

P136. Neuroinflammation: Animal Models

Program #/Poster #: P136.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Chellgren Endowed Professor fund (RLC)

Title: The effect of bacterial endotoxin LPS on synaptic transmission in various models

Authors: *N. T. MARGUERITE[1], *J. BERNARD[1], A. GREENHALGH[1], O. ISTAS[1], C. R. BALLINGER-BOONE[1], S. M. BIERBOWER[4], E. E. DUPONT-VERSTEEGDEN[2], A. GHOWERI[3], D. A. HARRISON[1], M. C. MCNABB[1], C. M. SAELINGER[1], O. THIBAULT[3], R. L. COOPER[1];
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Abstract: The release of the endotoxin lipopolysaccharides (LPS) from gram-negative bacteria is key in the induction of the downstream cytokine release from cells targeting other cells throughout the body. However, LPS itself has direct effects on cellular activity and can alter synaptic transmission. In investigating the direct actions of LPS on synaptic transmission various animal models were investigated and differences in responses were observed. Since antidepressants related to the serotonergic system have been shown to have a positive outcome for septicemic conditions impacting the central nervous system, the actions of serotonin (5HT) on neurons also exposed to LPS were investigated. At the model glutamatergic synapse of the crayfish neuromuscular junction (NMJ), 5HT primarily acts through a 5-HT2A receptor subtype to enhance transmission to the motor neurons. LPS from *Serratia marcescens* also enhances transmission at the crayfish NMJ but by a currently unknown mechanism; however, LPS depresses glutamatergic transmission at the Drosophila NMJ, but this NMJ is non-responsive to 5-HT. The serotonergic response remains intact in the presence of LPS at low and high concentrations. LPS blocked transmission at the cholinergic frog NMJ and glutamatergic rodent CNS but can be reversed with wash out.

Disclosures: N.T. Marguerite: None. **J. Bernard:** None. **A. Greenhalgh:** None. **O. Istas:** None. **C.R. Ballinger-Boone:** None. **S.M. Bierbower:** None. **E.E. Dupont-Versteegden:** None. **A. Ghoweri:** None. **D.A. Harrison:** None. **M.C. McNabb:** None. **C.M. Saelinger:** None. **O. Thibault:** None. **R.L. Cooper:** None.

Digital Abstract Session

P136. Neuroinflammation: Animal Models

Program #/Poster #: P136.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant F30 MH120974
NIH Grant R01 NS114480
NIH Grant R21 NS099973
NSF Grant NSF 1557971

Title: In vivo imaging of the microglial landscape after whole brain radiation therapy

Authors: *B. S. WHITELAW, S. TANNY, E. HERNADY, C. JOHNSTON, D. ROSENZWEIG, K. O'BANION, B. MARPLES, A. MAJEWSKA;
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Abstract: WBRT (whole brain radiation therapy) is a standard of care treatment for primary brain tumors and patients with multiple brain metastases, but can lead to cognitive dysfunction, dementia, and even death. Radiation affects many cell types in the brain, causing complex pathological responses that are incompletely understood. Microglia, the resident immune cells of the brain, promote a pro-inflammatory environment following WBRT, with wide-ranging effects. Pharmacological depletion of microglia ameliorates WBRT-induced cognitive dysfunction in mice, suggesting a causative role for microglia consistent with findings in other neurodegenerative diseases. However, little is known about the dynamics of the microglial reaction to WBRT *in vivo*. In this study, we investigated the effects of WBRT (single dose 10 Gy using two 5Gy AP/PA beams) on the microglial landscape using longitudinal 2-photon imaging *in vivo*. First, using CT-image-guided WBRT, we validated radiation dosimetry in the brain of a mouse with a cranial window implant. Within 1-week after WBRT, we saw significant changes in the microglial landscape, characterized by apparent loss of microglial cells (~20%) and re-arrangements of microglial location. Despite these large-scale changes, microglial motility and surveillance at the cellular level were largely unaffected. We are currently investigating the longer-term effects of WBRT, as well as microglia-dendrite interactions. Overall, this study provides new insights into how microglia react to WBRT by combining image-guided WBRT with longitudinal *in vivo* imaging.

Disclosures: B.S. Whitelaw: None. **S. Tanny:** None. **E. Hernady:** None. **C. Johnston:** None. **D. Rosenzweig:** None. **K. O'Banion:** None. **B. Marples:** None. **A. Majewska:** None.

Digital Abstract Session

P136. Neuroinflammation: Animal Models

Program #/Poster #: P136.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DA013137
HD043680
MH106392
NS100624

Title: SARS-CoV-2 of olfactory epithelial and pyriform cortical cells in moderate and recovering infections in non-human primates

Authors: *H. LI[1], C. MACTUTUS[1], K. MCLAURIN[1], J. RAPPAPORT[2], R. BOOZE[1]; [1]Univ. of South Carolina, Columbia, SC; [2]Tulane Natl. Primate Res. Ctr., Covington, LA

Abstract: As the major reason for COVID-19, the pandemic of coronal virus SARS-CoV-2 potentially induces olfactory dysfunction w/ anosmia. However, the mechanism(s) of SARS-CoV-2 associated anosmia and viral presence in the brain is unknown. ***Methods:*** To address the SARS-CoV-2 viral distribution and potential reservoir in the brain, four aged African Green monkeys and four Indian rhesus macaques were exposed via small particle aerosol or multiple mucosal routes to SARS-CoV-2 isolated from USA-WA1/2020. The infected primates were followed up to four weeks post-infection with clinical assessments and viral titers via mucus swab (buccal, nasal, and pharyngeal). RNAscope *in situ* hybridization was used to identify the distribution of SARS-CoV-2, ACE2 and TMPRSS2 expression in olfactory epithelium and pyriform cortex. Additionally, we performed dual-labeling of PDGFR-β, a cell marker for pericytes in the pyriform cortex, and SARS-CoV-2 probes to clarify the major cell type for viral infection in the brain. ***Results:*** Our data revealed abundant SARS-CoV-2 mRNA expression in the olfactory epithelium, especially in the sustentacular and basal stem cell layers, which co-localized with expression of ACE2 and TMPRSS2. SARS-CoV-2 mRNA and DNA, ACE2, and TMPRSS2 were notable in the pyriform cortex. The SARS-CoV-2 viral expression co-localized with the PDGFR-β positive cells in pyriform cortex. However, there was no relationship between viral loads (from buccal, nasal, pharyngeal, bronchial brush, etc.), clinical assessment or lung histopathologic score and SARS-CoV-2 expression in pyriform cortex. ***Conclusions:*** 1) SARS-CoV-2 mRNA and DNA in the olfactory epithelium were located in the sustentacular layer, basal stem cell layer, and Bowman's gland cells; 2) The pyriform cortex is a viral reservoir in the brain, evidenced by harboring SARS-CoV-2 mRNA and DNA; 3) Pericytes were the predominant cell type to express SARS-CoV-2 in brain; 4) Independent of viral loads (from buccal, nasal, pharyngeal, bronchial brush), clinical assessment, or lung histopathologic scores, SARS-CoV-2 expression was present in the pericytes of the pyriform cortex. Collectively, the present study has significant implications for our understanding of SARS-CoV-2 infection and reservoirs in the olfactory system, and the persistence of CNS infection of the pericytes in the pyriform cortex.

Disclosures: H. Li: None. **C. Mactutus:** None. **K. McLaurin:** None. **J. Rappaport:** None. **R. Booze:** None.

Digital Abstract Session

P136. Neuroinflammation: Animal Models

Program #/Poster #: P136.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Chellgren Endowed Professor fund (RLC)

Title: Temperature dependence on the passive effects of K+ on membrane potential and synaptic transmission

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Abstract: In modifying a typical physiological laboratory protocol that measures resting membrane potential in relation to the concentration of extracellular potassium ions $[K^+]_o$, the additional effects of temperature were addressed. These temperature effects are of interest because they are not commonly addressed in experimental physiology in relation to the effect of altered $[K^+]_o$. One aim focuses on the effects of environmental change using heterothermic animals as the model organism. A second aim focuses on clinical therapies related to the use of varied temperatures in mammals. A third aim addresses a unified issue with all organisms regarding the effects of temperature changes on cellular metabolism. Experimentally, the skeletal muscle of a crayfish and larval Drosophila serve as motor unit models to obtain data. The crayfish model is robust for long term survival in minimal physiological saline, is easily obtainable, and allows for a relative ease in dissection, whereas the larval Drosophila is small, but serves as a comparison in generalities to the effects. Both neuromuscular junctions are glutamatergic, and have graded excitatory junction potentials. Graphing membrane potential in relation to both $[K^+]_o$ and temperature, along with theoretical curves for the Nernst and Goldman-Hodgkin-Katz (G-H-K) equations, provides important distinctions and understanding of the relationship of temperature in these equations. Freely available online software is used in addressing the theoretical values one would expect. Discussion of other factors impacted by temperature for biological membranes will also be covered. It appears that the permeability to K+ may be altered with temperature changes.

Disclosures: D. Buendia Castillo: None. **P. Naidugari:** None. **E.E. Dupont-Versteegden:** None. **R.L. Cooper:** None.

Digital Abstract Session

P136. Neuroinflammation: Animal Models

Program #/Poster #: P136.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH-NICHD
Defense Advanced Research Projects Agency

Title: Socializing reduces distress-associated behavior in mice

Authors: H. WEI, A. ADEWAKUN, ***R. FROEMKE**;
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Abstract: Lipopolysaccharide (LPS) induced neuroinflammation is a shared symptom for most neurodegenerative diseases. One of the harmful outcomes of presence of LPS in the brain is the cognitive impairment which is a result of a downstream inflammatory responses that had been initiated in the gut.In animal models these cognitive impairments include lethargy, arched or curled body posture, inactivity, and reduced consumption. Socialization in animals is positively correlated with physical and mental, learning, and reduced mortality. The main goal of this study is to investigate if socialization through co-housing has a direct therapeutic effect on LPS induced neuroinflammation.We utilized an LPS-induced endotoxemia model for our experiments. A total of 24 mice C57/BL6 mice were divided into two groups: socially isolated mice or co-housed mice.Continuous seventy-two hours recording sessions were performed and videos were analyzed and scored. We found that co-housing mice not only reduced the recovery time but also decreased the duration of exhibition of the sickness behavior. Analysis showed that the single-housed mice displays sickness behavior, including extended hunched posture, for about thirty-five minutes following the LPS injection, while the co-house mice only spent twelve minutes displaying sickness behavior. In addition, there was a significant weigh loss in the LPS injected mice. Motion tracking analysis revealed that socially isolated LPS injected mice remained stationary for hours compared to co-housed mice. In conclusion, our findings show that co-housing mice post endotoxemia endotoxemia demonstrate therapeutic which could be further exploited for translational studies.

Disclosures: R. Froemke: A. Employment/Salary (full or part-time):; NYU School of Medicine. **A. Adewakun:** A. Employment/Salary (full or part-time):; NYU School of Medicine. **H. Wei:** A. Employment/Salary (full or part-time):; NYU School of Medicine.

Digital Abstract Session

P137. Neuroinflammation: HIV and Infections

Program #/Poster #: P137.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DA013137
HD043680
MH106392
NS100624

Title: Remodeling Neuronal Circuitry via a SERBA Modifies the Progression of HIV-1 Associated Neurocognitive Disorders

Authors: *K. A. MCLAURIN, H. LI, R. M. BOOZE, C. F. MACTUTUS;
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Abstract: Given the prevalence of neurodegenerative diseases (e.g., Alzheimer's disease, HIV-1 associated neurocognitive disorders (HAND), Parkinson's disease), there is a critical need to develop therapeutics to modify disease progression. Here, we demonstrate that treatment with S-

Equol (SE), a selective estrogen receptor beta agonist (SERBA), modifies the progression of preattentive processes, a neurobehavioral mechanism mediating alterations in higher-order cognitive processes, in the HIV-1 transgenic (Tg) rat by remodeling neuronal circuitry at the synaptic level. HIV-1 Tg and F344/N control rats were treated with either a daily oral dose of 0.2 mg SE (HIV-1 Tg: male, *n*=17, female, *n*=18; Control: male, *n*=18, female, *n*=17) or placebo (HIV-1 Tg: male, *n*=16, female, *n*=16; Control: male, *n*=20, female, *n*=17) from postnatal day (PD) 28 to PD 90. First, a longitudinal experimental design was utilized to critically test the efficacy of SE for modifying the progression of preattentive processes from PD 60 to PD 210. HIV-1 Tg rats treated with placebo exhibited prominent alterations in the progression of preattentive processes relative to control animals; deficits which occurred independent of biological sex. Treatment with SE, however, enhanced the progression of preattentive processes in HIV-1 Tg rats, resulting in a development trajectory that approximated control animals. Second, morphological characteristics of dendritic spines, a proxy for synapse function, were assessed in pyramidal neurons from layers II-III of the medial prefrontal cortex to evaluate the mechanism by which SE exerts its therapeutic effects. In animals treated with placebo, presence of the HIV-1 transgene decreased the efficacy of synapses by shifting the morphological parameters of dendritic spines towards an immature phenotype and reducing the complexity of pyramidal neurons. Profound long-term modifications in dendritic spines and neuronal morphology, consistent with increased synaptic efficacy, were observed in HIV-1 Tg rats treated with SE; results which support a mechanistic basis for enhancements in preattentive processing. Collectively, results support SERBAs as an innovative therapeutic approach for modifying the progression of HAND; an approach that exerts its effects by enhancing synaptic efficacy. Furthermore, understanding the mechanism by which SERBAs enhance cognitive function affords an opportunity to evaluate its broader therapeutic utility for other neurodegenerative diseases resulting from aberrations in synapse structure and function.

Disclosures: K.A. McLaurin: None. **H. Li:** None. **R.M. Booze:** None. **C.F. Mactutus:** None.

Digital Abstract Session

P137. Neuroinflammation: HIV and Infections

Program #/Poster #: P137.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant DA013137
NIH Grant HD043680
NIH Grant MH106392
NIH Grant NS100624

Title: Progressive dendritic spine alterations in the nucleus accumbens from HIV-1 viral protein exposure: a mechanism for apathy and treatment with S-Equol

Authors: *V. A. MADORMO, K. A. MCLAURIN, H. LI, C. F. MACTUTUS, R. M. BOOZE; Univ. of South Carolina, Columbia, SC

Abstract: As combination antiretroviral therapy (cART) significantly increases life expectancy of HIV-1 seropositive individuals, understanding the progression of HIV-1 associated neuropsychiatric complications is a critical need. Apathy, operationally defined as reduced self-initiated cognitive, emotional, and behavioral activity, is a frequent neuropsychiatric sequelae of HIV-1, with estimates suggesting a frequency as high as 65% in HIV-1 seropositive individuals. Prior work has demonstrated that the HIV-1 transgenic (Tg) rat shows a dysregulation of self-generated voluntary and goal-directed behaviors in various behavioral tests involving rewards, supporting the utility of the HIV-1 Tg rat as a biological model for motivational impairments. Motivational impairments have been experimentally linked to dysfunction with medium spiny neurons (MSNs) in the nucleus accumbens (NAc) and are readily correlated with decreases in spine number, degradation of synaptic proteins, and dendritic pruning. Further, S-Equol (SE) has been implicated as a potential adjunctive therapeutic for the aberrations in synapse structure and function from HAND. Thus, in the current study, a longitudinal experimental design was used to investigate progressive synaptodendritic alterations in the NAc from long-term HIV-1 viral protein exposure in HIV-1 Tg rats (n = 10 male, 10 female per age group) compared to controls (F344/N, n = 10 male, 10 female per age group). DiOlistic labeling was used to explore alterations in spine morphology of MSNs in the NAc at successive 30 day intervals to observe spine profile shifts in vivo due to HIV-1 from postnatal day (PD) 30 to PD 180 and after treatment with SE from PD 30 to PD 90. Relative to F344/N controls, HIV-1 Tg rats spines displayed a population shift towards a greater size in all measures (i.e. volume, backbone length, head diameter, and neck diameter) from PD 30 through 90, followed by a drastic population shift to decreased sizes at PD 120 through PD 180. The shift at later ages suggests HIV-Tg animals have a decreased synaptic connectivity as they age. Further, on all measures, HIV-Tg animals treated with SE displayed morphological similarities to control MSNs than to HIV-Tg animals. Dendritic spine alterations support a potential underlying neural mechanism for HIV-1 associated apathy and demonstrate the progression of the alterations; a progression which may be altered by SE. These findings provide insight into the underlying pathophysiological neural mechanism of motivational alterations in HIV-1 patients and a potential structural target for the development of therapeutics and cure strategies.

Disclosures: **V.A. Madormo:** None. **K.A. McLaurin:** None. **H. Li:** None. **C.F. Mactutus:** None. **R.M. Booze:** None.

Digital Abstract Session

P137. Neuroinflammation: HIV and Infections

Program #/Poster #: P137.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NIDA R21DA047936
R03DA043428
NIH CA223956
R01DA035714

NIH CA223956

Title: Drugs repurposing for the treatment of HIV-Associated Neurocognitive Disorder by AI-based literature mining

Authors: *B. C. CUI[1], M. AKSENOVA[1], J. SYBRAND[4], D. ODHIAMBO[1], M. LUCIUS[1], H. JI[1], J. TURNER[5], E. PENA[2], S. B. LIZARRAGA[3], J. ZHU[1], M. D. WYATT[1], I. SAFRO[4], M. SHTUTMAN[1];
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Abstract: HIV-1 Associated Neurocognitive Disorder (HAND) is a common and clinically detrimental complication of HIV infection. Viral proteins including Tat, released from infected cells, cause neuronal toxicity. Substance abuse in HIV-infected patients greatly exacerbates the severity of neuronal damage. To repurpose small molecule inhibitors for anti-HAND therapy, we employed MOLIERE, an AI-based literature mining system that we developed. All human genes were analyzed and prioritized by MOLIERE to find previously unknown targets connected to HAND. The list was narrowed to those with known small molecule inhibitors developed for other applications and lacking systemic toxicity in animal models. We tested the activity of small molecules targeted against the proteins of five prioritized genes to protect against the combined neurotoxicity of HIV-Tat and cocaine in primary neuronal cultures. Four prevented Tat and cocaine toxicity. The compounds are: the FDA approved drug Amlexanox; Tazemetostat (EPZ-6438), a potent selective EZH2 inhibitor that is in Phase II clinical trials; DEAD-Box RNA helicase 3 inhibitor RK-33, and DNA-Dependent Protein Kinase inhibitor NU7441. Both RK-33 and NU7441 were shown to selectively kill tumors without any observed systemic toxicity in animal models. Despite the disparate molecular targets of these drugs, analysis revealed a common mechanism of neuroprotection; namely that modulation of astrocyte and microglia status prevents the toxicity of Tat and cocaine. These findings show that MOLIERE literature mining provides a novel way to identify new mechanisms of neurotoxicity of HIV and drugs of abuse, and also accelerates the possibility of repurposing drugs for novel HAND treatments.

Disclosures: B.C. Cui: None. **M. Aksenova:** None. **J. Sybrand:** None. **D. Odhiambo:** None. **M. Lucius:** None. **H. Ji:** None. **J. Turner:** None. **E. Pena:** None. **S.B. Lizarraga:** None. **J. Zhu:** None. **M.D. Wyatt:** None. **I. Safro:** None. **M. Shtutman:** None.

Digital Abstract Session

P137. Neuroinflammation: HIV and Infections

Program #/Poster #: P137.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R21NS104603

Title: Long-term changes in the adult human brain linked to Zika virus infection

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Abstract: Systemic viral infections pose a global health challenge. In contrast to the currently pandemic-causing SARS-CoV-2 virus, the Zika virus (ZIKV) is highly neurophilic, as apparent in congenital syndromes (e.g. microcephaly) and increased neurological complications in some adults, such as Guillain-Barré syndrome (GBS). However, despite the observation that such adult neurological manifestations extend to functions of the central nervous system and may result in long-term sequalae, little is known about the impact of ZIKV on the adult brain. In particular, the majority of neuroimaging studies on adult ZIKV patients are case reports. Therefore, the present study performed extensive structural neuroimaging in rare adult ZIKV patients with both peripheral and central neurological manifestations (ZIKV-CNS-GBS group). We focused on the chronic phase (1 year after the onset of neurological symptoms) to understand the long-term structural effects of ZIKV infection. Importantly, the study included a GBS patient group with non-ZIKV etiology (NZIKV-GBS) and healthy controls to specifically investigate the neuroimaging evidence of the ZIKV-associated CNS complications. We examined voxel-wise fractional anisotropy (FA) and mean diffusivity (MD) differences in white matter (WM) tracts, whole-brain analysis of volumetric differences in gray matter (GM), and WM lesions using FLAIR hyperintensities. Both FA and MD revealed compromised WM integrity in the corticoreticular pathway (CRP) and the posterolateral superior longitudinal fasciculus (SLF II) in ZIKV-CNS-GBS compared to NZIKV-GBS. Given the functional role of the CRP in motor control, the diminished WM integrity may underlie the more severe and atypical GBS manifestation that we observed in ZIKV-CNS-GBS patients. Conversely, reduced WM integrity in the SLF may additionally contribute to the CNS manifestations; E.g. the role of SLF II in visuospatial processing may be compromised in ZIKV-CNS-GBS patients with vertigo and diplopia. Furthermore, we observed increased GM volume in the inferior temporal gyrus and the inferior orbitofrontal region in both patient groups compared to controls, which may support the WM results given their structural link to the CRP. To the best of our knowledge, this is the first case-control study to provide preliminary neuroimaging evidence for the long-term impact of ZIKV infection on adult brain structure in the context of CNS manifestations, and to report the impact of GBS etiology on GM and WM changes.

Disclosures: S. Jun: None. **R.O. Bido-Medina:** None. **J. Oviedo:** None. **I. Miches:** None. **L. Tusen:** None. **P. Stoeter:** None. **M. Rodriguez:** None. **S. Sadaghiani:** None.

Digital Abstract Session

P137. Neuroinflammation: HIV and Infections

Program #/Poster #: P137.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DA013137
HD043680
MH106392
NS100624

Title: HIV-1 Tg Rat: Telomere Length Assessment following SSRI or phytoestrogen treatment

Authors: *A. DENTON, H. LI, K. MCLAURIN, C. MACTUTUS, R. BOOZE;
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Abstract: Affective alterations, including motivational dysregulation and depression, significantly impact the quality of life in HIV-1 seropositive individuals; the neural mechanisms underlying these alterations, however, have not yet been fully elucidated. Telomere attrition, which is associated with inflammation in adults with affective alterations, may underlie affective alterations in HIV-1. Thus, three complementary aims were utilized to evaluate telomere attrition, and the potential utility of two proposed therapeutics (i.e., escitalopram, S-Equol (SE)), in the HIV-1 transgenic (Tg) rat. First, using a longitudinal experimental design the impact of genotype and/or biological sex on the progression of telomere length was evaluated in skin tissue of HIV-1 Tg (*n=7* per group) and F344/N control (*n*=7 per group) animals every thirty days from postnatal day (PD) 30 to PD 180. Both HIV-1 Tg and control groups exhibited an initial increase, followed by a prominent decrease, in telomere length; albeit significant genotypic differences in age of peak telomere length was observed. Specifically, HIV-1 Tg animals displayed a prominent rightward shift in age of peak telomere length relative to control animals (i.e., PD 90 versus PD 60, respectively). HIV-1 viral proteins, therefore, alter telomere development. Second, telomere length was evaluated in blood collected from HIV-1 Tg (*n*=31) and F344/N control (*n*=42) animals following chronic (i.e., 6 weeks) treatment with either escitalopram (4 mg/kg) or placebo. Presence of HIV-1 viral proteins, biological sex, and/or escitalopram treatment had no statistically significant effect ($p>0.05$) on telomere length. Finally, long-term modifications in telomere length were examined in the skin tissue of HIV-1 Tg (*n*=7 per group) and F344/N control (*n*=7 per group) animals following daily, oral SE (0.2 mg) treatment during the formative period (PD 28 to PD 90). Independent of treatment or biological sex, HIV-1 Tg animals exhibited increased telomere length relative to control animals (Main Effect of Genotype: $F(1,38)=5.4$, $p\leq0.03$). Furthermore, independent of genotype, treatment with SE increased telomere length in female, but not male, animals (Sex x Treatment Interaction: $F(1,38)=10.3$, $p\leq0.003$). Taken together, HIV-1 viral proteins have the most profound impact on early telomere development, supporting a potentially early inflammatory environment in the HIV-1 Tg rat; inflammation which dissipates across time and is not present at advanced ages. Furthermore, SE precludes the shortening of telomere length in female animals, supporting a potential efficacious therapeutic for diseases linked to telomere attrition.

Disclosures: A. Denton: None. **H. Li:** None. **K. McLaurin:** None. **C. Mactutus:** None. **R. Booze:** None.

Digital Abstract Session

P137. Neuroinflammation: HIV and Infections

Program #/Poster #: P137.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIGMS RISE R25GM082406
RCMI MD007579

Title: Lentiviral delivery provides improved HIV-1 Nef expression in astrocytes in vivo

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Abstract: HIV has remained a global epidemic infecting over 76 million people globally. Current therapy can prevent death due to AIDS and reduce transmission by reducing viral replication to low levels. With this advance, new challenges have emerged, including the increased prevalence of HIV associated neurocognitive disorders (HAND) in people living with HIV. Even with the presence of cART, the virus quickly invades the brain, establishing a reservoir. HAND is thought to be caused by the ongoing production of HIV neurotoxins in the brain, even when replication is suppressed. This damage is particularly relevant in astrocytes because they serve as a reservoir for HIV and produce HIV neurotoxic proteins, with Nef being the most abundant. In the past, we have expressed HIV-1 Nef by infusing ex-vivo transfected astrocytes into rats' brains to study loss of spatial learning. While that model had the advantage of cell-specific expression, it suffered the limitations of infusing new cells into the brain and transient expression of Nef. To overcome these limitations, we compared the effectiveness of infusing a lentiviral vector that expresses HIV-1 Nef using the astrocyte-specific GFAP promoter into the brain with the infusion of exogenous cells. We hypothesized that even if the Nef gene is delivered by a lentivirus that infects multiple cell types, this GFAP construct will limit expression to astrocytes, giving the best combination of duration and exposure, with less tissue disruption. To test this, 60-day old Sprague Dawley rats underwent bilateral stereotaxic surgery to infuse ex-vivo transfected astrocytes or a Lenti-GFAP-SF2Nef-IRES-mCherry viral vector into the rats' brains. Later, immunofluorescence for astrocytic markers was done to verify the weekly expression of both methods. Our data shows that after one week, ex-vivo transfected astrocytes were expressed. However, after three weeks, coronal slices of the brain showed longevity of expression of the vector and faded expression of the exogenous cells. The integrity of the expression was also verified in culture as a control for the infused materials. Altogether, these data suggest that the infusion of the lentiviral vector provides a superior approach due to steadier expression and duration of exposure, despite requiring a longer time to reach full expression. Moreover, as previously observed in other studies, comorbid use of illicit drugs, such as cocaine, represents an additional potential contributor to worsened HAND as cocaine accelerates the damage to the CNS by HIV neurotoxins. Future studies will address the specificity of the vector's expression in astrocytes to study HIV-1 Nef modulation of drug seeking.

Disclosures: **J.G. Pla-Tenorio:** None. **M. Cruz-Rentas:** None. **R. Noel:** None.

Digital Abstract Session

P137. Neuroinflammation: HIV and Infections

Program #/Poster #: P137.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant DA035714
NIH Grant DA041932

Title: Effects of HIV-1 Tat protein on the extracellular dopamine dynamics in inducible Tat transgenic mice using fast scan cyclic voltammetry

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Abstract: Effects of HIV-1 Tat protein on the extracellular dopamine dynamics in inducible Tat transgenic mice using fast scan cyclic voltammetry**S.E. Davis**[1], M. Ferris[2], and J. Zhu[1]**1**Department of Drug Discovery and Biomedical Sciences, College of Pharmacy, University of South Carolina, Columbia, SC**2**Department of Physiology and Pharmacology, School of Medicine, Wake Forest University, Winston-Salem, NC
Disclosures**S.E. Davis:** None. M. Ferris: None. J. Zhu: None.
AbstractInhibition of the dopamine (DA) transporter (DAT) by the HIV-1 protein transactivator of transcription (Tat) has been implicated as a mediating factor of HIV-1 associated neurocognitive disorders (HAND). Our laboratory has demonstrated that DA uptake through DAT is reduced in an inducible Tat transgenic (iTat-tg) mouse model. This study determined the extracellular DA dynamics in iTat-tg mice using slice voltammetry. DA release was stimulated using either tonic (1 Hz, 1 pulse) or phasic (20 Hz, 5 pulses) parameters in the presence of nomifensine (1 nM, 10 nM, 100 nM, 1 μM, and 10 μM) in the caudate putamen of iTat-tg mice following 7-day administration of saline or doxycycline (Tat expressing). Compared to saline control mice, the change in the peak extracellular DA vs. baseline was significantly increased in response to nomifensine in iTat-tg mice [$F(1,10) = 8.18$, $p < 0.017$]. The increase in the change in peak DA vs. baseline was in a concentration-dependent manner [$F(4,40) = 44.5$, $p < 0.001$]. Furthermore, there was a significant interaction of nomifensine and Tat expression [$F(4,40) = 4.62$, $p < 0.01$]. There was no significant difference in DA peak levels vs. baseline, T20, or T80, with either tonic or phasic stimulation. No significant effect of doxycycline for the change in peak DA vs. baseline using tonic stimulated DA release was found. These findings suggest that increased extracellular DA in response to nomifensine may be mediated by Tat induced inhibition of DAT. Future work focused on attenuation of Tat induced inhibition of DAT may serve as a potential treatment option for HAND.

Disclosures: **S.E. Davis:** None. **M. Ferris:** None. **J. Zhu:** None.

Digital Abstract Session

P137. Neuroinflammation: HIV and Infections

Program #/Poster #: P137.08

Topic: B.11. Glial Mechanisms

Support: 5R01AI124677-03

Title: Dynamic neural-glia interactions mediate the loss of perisomatic inhibitory synapses in Toxoplasma gondii infection

Authors: *G. L. CARRILLO[1], V. BALLARD[3], T. GLAUSEN[6], Z. BOONE[4], J. TEAMER[2], C. HINKSON[7], E. WOHLFERT[6], I. BLADER[6], M. A. FOX[5];
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Abstract: Prolonged infection and inflammation within the brain can alter the connectivity and function of neuronal circuits. The intracellular protozoan parasite, *Toxoplasma gondii*, is one pathogen that can chronically infect the brain and lead to encephalitis and seizures. Currently, over 30% of the human population worldwide is infected with *Toxoplasma gondii,* and these infections are associated with behavioral alterations and an increased risk for developing psychiatric illness, including schizophrenia. Current evidence from studies in humans and mouse models suggest that both seizures and schizophrenia result from a loss or dysfunction of inhibitory synapses. In line with this, we recently reported that persistent *Toxoplasma gondii* infection alters the distribution of glutamic acid decarboxylase 67 (GAD67), an enzyme that catalyzes GABA synthesis in inhibitory synapses. These changes could reflect a redistribution of presynaptic machinery in inhibitory neurons or a loss of inhibitory nerve terminals. To directly assess the latter possibility, we employed serial block face scanning electron microscopy (SBFSEM) and quantified inhibitory perisomatic synapses in neocortex and hippocampus following parasitic infection. These ultrastructural analyses (coupled with genetic and immunohistochemical analyses) revealed that persistent infection not only led to a significant loss of perisomatic synapses, it induced the ensheathment of neuronal somata and perisomatic nerve terminals by myeloid-derived cells (including microglia), suggesting they may actively displace or phagocytose synaptic elements. This neural-glia interaction coincides with increased expression of classical complement cascade components, an immune pathway that has been shown to play a critical role in microglia-induced synapse loss during neurodegeneration or disease by opsonization. Our recent studies exploit various visualization techniques, including 2-photon *in vivo* imaging to explore the innate complement pathway as a mechanism by which microglia phagocytose inhibitory nerve terminals in long-term parasitic infection.

Disclosures: G.L. Carrillo: None. **V. Ballard:** None. **T. Glausen:** None. **Z. Boone:** None. **J. Teamer:** None. **C. Hinkson:** None. **E. Wohlfert:** None. **I. Blader:** None. **M.A. Fox:** None.

Digital Abstract Session

P138. Ischemia: Cellular and Molecular Mechanisms

Program #/Poster #: P138.01

Topic: C.08. Ischemia

Support: Leducq 15CVD02

Title: Rab7a regulates endothelial tight junction protein trafficking and paracellular permeability of the blood-brain barrier after ischemic stroke

Authors: *A. COTTARELLI[1], M. HSU[2], S. SHAHRIAR[1], M. GLENDINNING[1], G. PROCHILO[1], J. R. SMITH[3], J. NEAL[1], A. L. EDINGER[4], A. ARAC[5], D. AGALLIU[6]; [1]Columbia Univ., New York, NY; [2]Univ. of Wisconsin - Madison, Madison, WI; [3]Univ. of Washington, Seattle, WA; [4]Univ. of California Irvine, Irvine, CA; [5]Neurol., UCLA, Los Angeles, CA; [6]Neurol., Columbia Univ. Med. Ctr., New York, NY

Abstract: Brain endothelial cells (ECs) form a paracellular and transcellular barrier to blood-borne solutes via tight junctions (TJs) and scarce endocytotic vesicles. The blood-brain barrier (BBB) plays a pivotal role in the healthy and diseased CNS. BBB damage after ischemic stroke contributes to increased mortality; yet the roles of paracellular versus transcellular mechanisms in this process are not well-understood. We have previously shown by intravital two-photon microscopy, using a transgenic strain in which endothelial TJs are labeled with eGFP, that stepwise impairment of transcellular followed by paracellular barrier mechanisms accounts for BBB deficits in stroke. Moreover, Caveolin-1 deficient mice, which have reduced endothelial transcellular permeability, display a normal increase in paracellular permeability after transient MCAO, suggesting that these two mechanisms are independent.Here, we address the role of TJ remodeling in regulation of endothelial paracellular permeability following stroke. The small GTPase Rab7a plays an essential role in regulation of trafficking inside the cell as proteins move from the late endosome to the lysosome for degradation. By generating mice lacking Rab7a in ECs (Rab7a iEC-KO), we demonstrate that the absence of endothelial Rab7a prevents junctional dismantling occurring 48 hours after ischemic stroke, thus reducing paracellular BBB permeability. As a consequence, Rab7a iEC-KO mice display reduced neuronal death in the cortex and an improved neurological outcome 48 hours after stroke induction. Moreover, we demonstrate in murine brain ECs (mBECs) that the active, GTP-bound, form of Rab7a is increased after 48 hours of treatment with TNFα and IL-1β, indicating that the proinflammatory environment in the ischemic brain is responsible for Rab7a-mediated BBB disruption. Consistent with these results, we find that cytokine treatment increases expression of Ccz1, a GTP exchange factor (GEF) known to promote Rab7a activation. Silencing Rab7a in mBECs rescues the cytokine-induced impairment in paracellular barrier properties without interfering with the endocytic pathwayThese findings suggest that Rab7a regulates TJ protein trafficking and degradation at the late phase of stroke, which is responsible for the enhancement of paracellular permeability of the barrier, and that inhibition of Rab7a activation in ECs may protect CNS damage after ischemic stroke.

Disclosures: **A. Cottarelli:** None. **M. Hsu:** None. **S. Shahriar:** None. **M. Glendinning:** None. **G. Prochilo:** None. **J.R. Smith:** None. **J. Neal:** None. **A.L. Edinger:** None. **A. Arac:** None. **D. Agalliu:** None.

Digital Abstract Session

P138. Ischemia: Cellular and Molecular Mechanisms

Program #/Poster #: P138.02

Topic: C.08. Ischemia

Title: Evaluation of enzymes associated with the glutathione system in the cerebral ischemic process in rats

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Abstract: Cerebral ischemia is a neurodegenerative disease, considered the second cause of death in the world, developing countries being the most affected. It is known that the initiating factors of oxidative stress during the cerebral ischemic process are the production of nitric oxide (NO) and peroxynitrite ($ONOO^-$), which trigger the mechanisms of cell damage. Under extreme conditions of oxidative stress, antioxidant systems such as the glutathione system are activated, the enzymes Glutathione reductase (GR), Glutathione peroxidase (GPx) and Glutathione transferase (GST) make up this system and have been associated with cell survival and good biological functioning. The aim of this work is to evaluate the enzymes of the glutathione system, by varying their enzymatic activity during a cerebral ischemic process in rats. 45 male rats of the Wistar strain with a weight of 190-230 g were used, kept under animal storage conditions. They were randomly divided into 3 groups: Control group (no treatment) with n = 3; Ischemia group, who underwent surgery for obilateration of the left primitive carotid artery (OAPC) for 10 min and subsequent reperfusion of blood flow, tissue was obtained at 2, 4, 6, 8, 12, 24 and 36 hours after reperfusion with n = 3 for each time evaluated; Group L-NAME + Ischemia, rats administered the inhibitor intraperitonally, one hour before OACP; then surgery and damage follow-up were performed in the same way as in the second group with n = 3 for each time evaluated. In this research L-NG-Nitroarginine Methyl Ester (L-NAME) was used as a potent inhibitor of neuronal and endothelial nitric oxide synthesis, in order to prevent the toxic effects of NO and $ONOO^-$. Subsequently, the activity of the enzymes GR, GPx and GST was evaluated, as well as the total lipoperoxidation process and its main components: malonyldialdehyde (MDA) and 4-hydroxyalkenals (4-HDA) using spectrophotometric techniques. The results show that in the early stage of the cerebral ischemic process, oxidized glutathione is generated which is then reduced by the GR enzyme and an increase in the levels of MDA and 4-HDA. In the late phase, GR and GST increase their activity, however, the GPx enzyme decreases its activity, while the products of lipoperoxidation increase with time. In the

group treated with L-NAME, a decrease was observed both in the activity of the three enzymes analyzed and in the levels of the lipoperoxidation products compared to the group without inhibitor. It is concluded that the enzymes GR, GPx and GST modify their activity in response to the cellular detoxification needs in each post-reperfusion stage of a cerebral ischemic process.

Disclosures: I. Cesar Arteaga: None. **D. Juárez Serrano:** None. **C. Garcia-Olvera:** None. **P. Aguilar-Alonso:** None.

Digital Abstract Session

P138. Ischemia: Cellular and Molecular Mechanisms

Program #/Poster #: P138.03

Topic: C.08. Ischemia

Support: Florida Department of Health 20K09

Title: Concurrent use of nicotine and oral contraceptives in female rats alters brain metabolism and exacerbates ischemic injury

Authors: *J. SIEGEL, F. DIAZ, A. P. RAVAL;
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Abstract: Smoking-derived nicotine (N) and oral contraceptives (OC) synergistically induce mitochondrial complex IV dysfunction and cause severe ischemic brain damage in female rats (1, 2). The goal of the current study is to understand the impact of N+OC on brain metabolism, we investigated the global metabolomic profile of adolescent and adult female rat brains exposed to N +/-OC. Adolescent (6 weeks old) and adult (12 weeks old) Sprague-Dawley female rats were randomly (n=8/group) exposed to either saline, N +/- OC for 16-21 days. The metabolic analysis of brain tissue shows significant changes in glycolysis, TCA cycle, lipid metabolism, and neurotransmitters in both adult and adolescent rats exposed to N +/- OC in relation to saline treatment, with changes more pronounced in adolescent rats. Since glucose and lipid metabolisms are critical for neuronal function, alterations in these energy producing pathways can deteriorate and further exacerbate ischemic brain damage. References: 1. Raval AP et al., J Neurochemistry 2012; 121(1):157-67.2. Diaz F and Raval AP. J Cereb Blood Flow Metab. 2020 Jun 14:271678X20925164

Disclosures: J. Siegel: None. **F. Diaz:** None. **A.P. Raval:** None.

Digital Abstract Session

P138. Ischemia: Cellular and Molecular Mechanisms

Program #/Poster #: P138.04

Topic: C.09.Stroke

Support: NIH grant R01-NS37570
AHA 15PRE25500022

Title: Lymphangiogenesis near the cribriform plate after cerebral ischemia

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Abstract: Conventional lymphatic vessels typically reside within the tissue parenchyma and facilitate drainage of fluid, lipid, and immune cells to local lymph nodes. However, the central nervous system (CNS) has traditionally been described as "immune privileged" because there are no conventional lymphatic vessels or peripheral immune cells within the CNS parenchyma. Recently, it has been hypothesized that antigens, antigen-presenting cells (APCs), and cerebrospinal fluid (CSF) may drain from the CNS into lymphatics near the cribriform plate or meningeal lymphatics in order to provide antigen drainage and fluid homeostasis. This recent re-discovery of lymphatic vessels surrounding the CNS under steady-state conditions ignited an intense debate about mechanisms of how antigens or immune cells drain from the CNS parenchyma to lymphatic organs. Additionally, the role of lymphatic vessels in the CNS during neuroinflammation is not well understood yet. Here, we study how lymphangiogenesis near the cribriform plate contributes to recovery of brain tissues following transient ischemic stroke. We employed the transient middle cerebral artery occlusion (tMCAO) model of ischemic stroke to 10-12 weeks old C57BL/6J male mice by placing a filament through internal carotid artery to occlude MCA for 60 minutes, followed by reperfusion after removing the filament. We showed that lymphangiogenesis occurs near the cribriform plate, which peaked at day 7 and decreases after 14 days following tMCAO. The lymphangiogenesis occurred through interaction of vascular endothelial growth factor receptor (VEGFR)-3 and vascular endothelial growth factor (VEGF)-C which are produced by $CD11b^{+}$, $CD11c^{+}$ dendritic cells. These lymphangiogenic vessels could transport CSF and immune cells which were confirmed by injection of Evans Blue dye through cisterna magna and flow cytometry, respectively. Flow cytometry analysis showed robust leukocyte accumulation, which include macrophages ($CD45^{hi}$, $CD11b^{+}$, $CD11c^{low}$), dendritic cells ($CD45^{hi}$, $CD11b^{+}$, $CD11c^{hi}$), CD8 T cells ($CD45^{hi}$, $CD11b^{-}$, $CD11c^{-}$, $CD8^{+}$), and CD4 T cells ($CD45^{hi}$, $CD11b^{-}$, $CD11c^{-}$, $CD4^{+}$) near the cribriform plate after 7 days of tMCAO, which also correlated with lymphangiogenesis ($CD45^{-}$, $Podoplanin^{+}$, $CD31^{+}$). Inhibition of VEGFR-3 reduced lymphangiogenesis near the cribriform plate and improved motor ability at earlier time points following tMCAO. Understanding the biology and mechanisms of CNS lymphatic drainages may lead to novel therapeutics for neuroinflammatory diseases.

Disclosures: Y. Choi: None. **M. Hsu:** None. **C. Laaker:** None. **M. Sandor:** None. **Z. Fabry:** None.

Digital Abstract Session

P138. Ischemia: Cellular and Molecular Mechanisms

Program #/Poster #: P138.05

Topic: C.08. Ischemia

Support: EU grant WF7 no 304884

Title: Sema3a antibody for neurodegenerative diseases of the optic nerve

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Abstract: Abstract
Glaucoma and other ischemic or neurodegenerative diseases of the optic nerve are associated with a process of progressive apoptosis of the retinal ganglion cells (**RGC**) that, once initiated, is not stopped by current treatments. Dying RGC send apoptotic signals to neighboring healthy RGC. This leads to further loss of RGC which induces further loss of RGC which induces substantial loss of visual function leading to blindness .Glaucoma is one such condition,but the same phenomenon of self-reinforcing degenerative process is also apparent in other central nervous system(**CNS**) disease. Semaphorin3A (**Sema3A**) is a protein involved in the signal cascade leading to apoptosis of neural cells in the CNS. Sema3A inhibitors interfere with this signal cascade and preserve RGCs.Our goal is to create an antibody to block the apoptotic program of Sema3A.
We created animal models of optic nerve assaults analog to diseases of optic nerve in human. The antibodies created were evaluated in vitro in neural cells culture and the most potent one, 3H4, was loaded in implants which were implanted in the right eye of rats or rabbits . Four models of optic nerve and retina were used: complete transaction of optic nerve and retinal detachment in rats, acute glaucoma and ischemic optic nerve (**NAION**) in rabbits. Two weeks following injury the retinae were stained retrograde with Di-Asp 10. Viable RGC were counted on flat mounted retina. We succeeded to save 46% -52% of the RGC in the implanted eyes with 3H4. Control injured optic nerves, treated with implants and loaded with DMEM presented 10% - 12% RGC of the normal count. The antibody 3H4 was found to be a potent substance that blocks the apoptotic program of Sema3A.

Disclosures: **A.S. Solomon:** None. **I. Benhar:** None. **A. Barzilai:** None.

Digital Abstract Session

P139. Mechanisms and Biomarkers of Perinatal Ischemia

Program #/Poster #: P139.01

Topic: C.08. Ischemia

Support: NIH Grant K08NS101122

Title: Neuronal activity mapping during exploration of a novel vs. familiar environment in young adult mice following neonatal hypoxia ischemia.

Authors: M. MAZURKIEWICZ, P. WAGLEY, A. KAMBHAM, B. PACE, D. SKWARZYNSKA, *J. BURNSED;
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Abstract: Background: Hypoxic ischemic encephalopathy (HIE) is a major cause of neonatal seizures and later cognitive and behavioral problems. Early-life seizures may result in permanent changes to neuronal circuitry which likely have consequences on cognitive and memory outcome. Use of transgenic mice, tissue clearing and advanced microscopy allow mapping of neuronal activity involved in memory and learning in young adult mice following neonatal HI.Objective: Map neuronal activity in HI and uninjured mice during novel and familiar environment exploration.Methods: HI is created using unilateral carotid ligation+45 min of 8% O2 in Cre-tamoxifen transgenic mice (TRAP2) on postnatal day (p)10. Sham mice receive incision+anesthesia without hypoxia/ligation. Mice are exposed to novel environment on p30 and again to this environment (familiar) on p31. Injection of 4-hydroxytamoxifen 1hr after experiment on p30 or p31 allows expression of fluorescent protein (TdTomato) in neurons active (expressing immediate early gene, cfos) during the prior 90 minutes of behavior. Mice are perfused and 200µm thick tissue is processed using tissue clearing methods, stained with DAPI and imaged on a confocal microscope. Zeiss Zen is utilized to obtain and process images, Imaris 9.2 and Fiji are used for analysis.Results: Mice in both groups explored the environment significantly less on the second day (familiar environment) compared to the first day (novel environment) ($p=0.00001$). Increased neuronal activity was observed during novel environment exploration in the somatosensory cortex, motor cortex, hippocampus (dentate gyrus, CA1 and CA3), amygdala and lateral thalamus compared to familiar.Conclusions: Young adult mice exhibit increased exploratory activity in a novel environment vs. familiar. Unbiased, whole brain mapping of neuronal activity during both conditions revealed that the exploratory circuit, learning and memory circuit and anxiety circuit are highly active during exploration of a novel environment, compared to a familiar environment. Ongoing studies are quantifying neuronal activity in exploratory circuit, learning and memory circuit and anxiety circuit and exploring the impact of HI on this behavior and the neuronal activity map.

Disclosures: M. Mazurkiewicz: None. **P. Wagley:** None. **A. Kambham:** None. **B. Pace:** None. **J. Burnsed:** None. **D. Skwarzynska:** None.

Digital Abstract Session

P139. Mechanisms and Biomarkers of Perinatal Ischemia

Program #/Poster #: P139.02

Topic: C.08. Ischemia

Support: NIH Loan Repayment Program

Title: Neurochemically identified cortical interneuron subtypes are differentially vulnerable to hypoxic-ischemic brain injury in neonatal piglet

Authors: *C. E. O'BRIEN[1], E. KULIKOWICZ[1], R. C. KOEHLER[1], L. J. MARTIN[2], J. K. LEE[1];
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Abstract: Introduction: Post-cardiac arrest seizures are common and are associated with worse neurologic outcomes in children. The etiology of post-cardiac arrest seizures is unknown. We hypothesized that neocortical inhibitory GABAergic interneurons (INs), cells known to regulate cortical neuron excitability, degenerate after cardiac arrest. We localized and counted two subtypes of INs that differentially express the calcium-binding proteins parvalbumin and calretinin in neonatal piglet cerebral cortex at 4 days after hypoxic-ischemic injury. Methods: Male piglets (3–5 days old) were randomized to sham surgery or hypoxic-ischemic (HI) injury with 45 minutes of hypoxia, 8 minutes of asphyxia, and cardiopulmonary resuscitation. Pigs were recovered for three hours, extubated, and survived 4 d. Neuronal injury was evaluated on hematoxylin and eosin staining. Immunohistochemistry was used to identify parvalbumin (PV) and calretinin (CR) in forebrain sections. Comparisons between the two groups were made with the Mann-Whitney test. Results: The brains from 16 pigs (n=8 for both sham and HI groups) were studied. The HI insult was confirmed by neuronal injury in putamen (a vulnerable subcortical region in this model). The HI group had significantly fewer normal neurons as compared to the sham group (29.5 [19.2, 38.6] versus 57.0 [50.3, 80.7]; $p<0.001$). There were significantly more ischemic-necrotic neurons in the somatosensory cortex of HI pigs (8.9 [2.3, 31.9] versus 1.4 [0.9, 2.4]; $p=0.008$); however, the number of total normal neurons in layers 2 through 6 were similar. The parvalbumin positive IN numbers in putamen ($p=0.705$) and somatosensory cortex ($p=0.747$) were not different in HI compared to sham. However, calretinin positive INs in layer 2 of the somatosensory cortex were significantly reduced in HI piglets compared in sham piglets (44 [38, 57) versus 102 [80, 114]; $p=0.003$). Conclusions: The neocortical calretinin positive subtype of IN, but not the parvalbumin positive IN class, is vulnerable to HI brain injury when overall cortical damage is mild. Selective damage to local inhibitory IN networks that target distal dendrites of pyramidal neurons, such as calretinin INs, could be involved in initiation and propagation of post-cardiac arrest seizures at early stages in the evolution of cortical damage.

Disclosures: C.E. O'Brien: None. **E. Kulikowicz:** None. **R.C. Koehler:** None. **L.J. Martin:** None. **J.K. Lee:** None.

Digital Abstract Session

P139. Mechanisms and Biomarkers of Perinatal Ischemia

Program #/Poster #: P139.03

Topic: C.08. Ischemia

Support: NIH Grant NS114972

Title: A biomarker of poor outcome for neonatal encephalopathy using MRI diffusion-weighted imaging connectome and fixel-based analysis

Authors: Z. SHI[1], J.-W. JEONG[2], M.-H. LEE[1], N. FERNANDES[1], S. DEOL[3], S. MODY[1], S. ARSLANTURK[4], R. B. CHINNAM[5], *S. TAN[6,1];
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Abstract: Better biomarkers of eventual outcome are needed for neonatal encephalopathy. To identify the most potent neonatal imaging maker associated with 2-year outcomes, we retrospectively performed diffusion-weighted imaging connectome (DWIC) and fixel-based analysis (FBA) on MRI obtained in the first four weeks of life in term neonatal encephalopathy newborns. Diffusion tractography was available in 15 out of 24 babies with MRI, 5 each with normal, abnormal motor outcome, or death. All fifteen except one underwent hypothermia as initial treatment. In abnormal motor and death groups, DWIC found 19 white matter pathways with severely disrupted fiber orientation distributions. Using random forest classification, these disruptions predicted the follow-up outcomes with 89%-99% accuracy. These pathways showed reduced integrity and myelination in abnormal motor and death vs. normal tone groups ($p < 10-6$). FBA identified three regions, thalamus, internal capsule, and cerebellar peduncle, of which fiber densities were reduced in abnormal motor and death vs. normal tone groups ($p < 0.016$). This study suggests that early DWIC and FBA may be helpful in distinguishing normal from abnormal follow-up outcomes, based on the severity of white matter injury. These neonatal markers could facilitate improved prognostication, better elucidate plasticity and repair, and evaluate treatments for neonatal encephalopathy.

Disclosures: S. Tan: None. **Z. Shi:** None. **J. Jeong:** None. **M. Lee:** None. **N. Fernandes:** None. **S. Deol:** None. **S. Mody:** None. **S. Arslanturk:** None. **R.B. Chinnam:** None.

Digital Abstract Session

P139. Mechanisms and Biomarkers of Perinatal Ischemia

Program #/Poster #: P139.04

Topic: C.08. Ischemia

Support: NIH Grant NS114972

Title: Studying Cerebral Palsy Using the Transition From Partial to Total Placental Insufficiency in a Rabbit Model

Authors: *N. VENKATESH;
Wayne State Univ. Sch. of Med..

Abstract: Studying Cerebral Palsy Using the Transition From Partial to Total Placental Insufficiency in a Rabbit Model Neha Venkatesh[2], Zhongjie Shi[1], Kehuan Luo[1], Sanket Jani[1], Melissa February[1], Nithi Fernandez[1], Nadiya Sharif[2], Sidhartha Tan[1]
Objective: All placental abruptions have their beginnings from a partial abruption. We developed a new rabbit model mimicking the partial-to-total placental insufficiency and compared it with the previous model of only complete uterine ischemia.**Methods**: Global fetal

hypoxia ischemia (H-I) was induced by uterine ischemia at E22 or E25 (70% or 79% term gestation, respectively), in pregnant New Zealand white rabbits. Full H-I was complete uterine ischemia for 40 min, and the new Partial+Full H-I added a 30-min partial ischemia before the complete 40-min ischemia. Fetuses were delivered either at E31.5 (full term) vaginally for neurobehavior test, or by C-section at E25 for *ex-vivo* brain cell-viability evaluation at 24, 48 and 72 hours thereafter. **Results**: Onset of fetal bradycardia was within the first 2 minutes of either H-I protocol. There was no difference between the Full H-I (n=435) and Partial+Full H-I (n=122) groups in death or severely affected kits (79% vs 80%), or normal kits (14% vs 11%), or any of the individual newborn neurobehavioral tests at E22, nor at E25 (n=312 in Full H-I group, and n=80 in Partial+Full H-I group; death or severely affected: 62% vs 64%; normal kits: 27% vs 29%). There was no difference in cell viability between the Full H-I (n=8) and Partial+Full (n=6) groups ($p>0.05$).**Conclusions**: The E22 or E25 Partial+Full H-I model produced similar neurobehavioral CP phenotype as our previous Full H-I model, and may be more suitable for testing of potential neuroprotectants after the onset of fetal bradycardia.

Disclosures:

Digital Abstract Session

P139. Mechanisms and Biomarkers of Perinatal Ischemia

Program #/Poster #: P139.05

Topic: C.08. Ischemia

Title: Effect of prenatal hypoxia-ischemia on cognitive testing in newborn rabbits using conditioning

Authors: *N. SHARIF[1], Z. SHI[2], K. LUO[2], S. TAN[2];
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Abstract: Prenatal fetuses surviving hypoxic-ischemia (H-I) typically show postpartum symptoms of intellectual disability and cerebral palsy. Also, some children who show no signs of motor deficits remain at risk for cognitive deficits, while others may show no signs of cognitive deficits but live with motor deficits. In this study, a rabbit model was used to reflect acute placental insufficiency in humans, which results in global fetal brain H-I. Surviving rabbit kits after birth were conditioned with human feeding and underwent cognitive tests to evaluate their capabilities to identify a human face, and therefore, the prospect of classical and operant conditioning on minimizing cognitive deficits. H-I in fetuses was induced via uterine ischemia at 79% term gestation in pregnant New Zealand White Dams. Postpartum, half of the randomly selected post H-I and naïve surviving newborns (motor non-affected determined by neurobehavioral tests) were conditioned to recognize a human face (i.e. the original feeder) via consistent exposure to the individual for 9 days. A series of tests were then conducted using a six-arm maze to identify if the fetuses were able to recognize the original feeder, as well as isolate the source of cognitive recognition. Statistical significance was determined by Fischer's Exact tests. In unconditioned kits, both the H-I and naïve testing groups demonstrated a response

to both the negative and positive stimuli, showing no preference to go to the feeder. In conditioned kits, both naïve and H-I newborns showed a preference for the feeder, ($p = 0.0142$ and 0.0001, respectively). Since conditioning had been done with feeder in a lab coat, conditioned kits still showed a preference for the feeder without a lab coat. There was a difference in performance between the conditioned and unconditioned kits within the H-I ($P= 0.0001$) and Naïve ($P= 0.0215$) groups. Test 3 demonstrates that the conditioned kits, naïve ($P = 0.0037$) and H-I ($P= 0.035$) still preferred the original feeder even when she was wearing a mask of the bystander over the bystander wearing a mask of the original feeder. The ability to identify the discriminative stimulus amongst other stimuli, suggests that cognitive deficiencies could have been alleviated. Early-stage conditioning of fetuses affected by intellectual disabilities associated with H-I creates a possibility to minimize cognitive damage while the brain is still in early development. It is speculated that the conditioned kits relied more on olfactory systems rather than visual perception.

Disclosures: **N. Sharif:** None. **Z. Shi:** None. **K. Luo:** None. **S. Tan:** None.

Digital Abstract Session

P140. Biomarkers of Hemorrhagic Stroke

Program #/Poster #: P140.01

Topic: C.09.Stroke

Support: JSPS KAKENHI Grant JP17H02117
JSPS KAKENHI Grant JP19J23508
JSPS KAKENHI Grant JP20H04048

Title: Bdnf mrna expression level in the ipsilateral motor cortex correlates with motor impairment of paralyzed forelimb after intracerebral hemorrhage in rats

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Abstract: Intracerebral hemorrhage (ICH) is a subtype of stroke that causes major motor impairments. It is well-recognized that functional recovery after stroke is attributed to both brain remodeling and neural plasticity. Brain-derived neurotrophic factor (BDNF) is known to have important roles for neural plasticity, so that it is expected that BDNF could be a potential biomarker for stroke recovery. However, BDNF expression after ICH remained poorly understood, because most of the stroke studies addressed the ischemic stroke. This study aimed to characterize BDNF expressions in the motor cortex after ICH and investigate the relationship between behavioral impairments and BDNF expression using an ICH rat model. 7-weeks-old male Wistar rats were divided into 2 groups: a SHAM group ($n = 7$) and an ICH group ($n = 8$). ICH was induced by the injection of collagenase in the left striatum near the internal capsule (AP: -2.0 mm, ML: 3.7 mm, DV: -6.0 mm). For behavioral assessments, the cylinder test and the

open field test were performed before surgery and 3 days, 1 week, 2 weeks, and 4 weeks after surgery. Following these behavioral assessments for 4 weeks, BDNF mRNA and protein expressions in the ipsilateral and contralateral motor cortex were assayed using RT-PCR and ELISA. All study procedures were approved by the ethics committee for animal research of Hokkaido University in Japan and conducted according to the guidelines of the committee. Forelimb motor impairment and decrease in locomotor activity were observed in the ICH group, whereas ICH rats did not present anxiety-related behavior. There was no significant difference in cortical BDNF expression between the SHAM and ICH group at 4 weeks after stroke. However, the asymmetry index of BDNF mRNA expression showed that the interhemispheric balance of BDNF mRNA expression shifted to the ipsilateral hemisphere after ICH. Furthermore, BDNF mRNA expression in the ipsilateral cortex positively correlated with paralyzed forelimb motor function only in the ICH group. There was no significant correlation between cortical BDNF expression and the other behavioral outcome such as locomotor activity and anxiety-related behavior. This study found for the first time that BDNF mRNA expression in the ipsilateral motor cortex could be related to post ICH motor impairment. These results might help to develop a novel treatment strategy for neurotrophin-dependent recovery after stroke.

Disclosures: T. Inoue: None. **Y. Takamatsu:** None. **H. Maejima:** None. **M. Okamura:** None.

Digital Abstract Session

P141. Pharmacological Treatments For Stroke in Humans and Non-Human Primates

Program #/Poster #: P141.01

Topic: C.08. Ischemia

Support: NIH Grant R01NS104117
Brazilian CAPES (88881.311939/2018-01)

Title: Synergistic effect of an PAF-receptor antagonist with an inflammatory response modulator on cerebral ischemia reperfusion injury in rats: a dose-response study

Authors: *L. S. BELAYEV[1], L. KHOUTOROVA[1], M. M. REID[1], A. OBENAUS[2], C. R. ROQUE[3], P. K. MUKHERJEE[1], N. A. PETASIS[4], R. B. ORIA[3], N. G. BAZAN[1];
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Abstract: Ischemic stroke is characterized by the sudden loss of blood circulation to an area of the brain, resulting in a corresponding neurologic function loss. Despite significant signs of progress achieved, effective stroke treatment remains a formidable challenge due to the complexity of the disease. Using a model of transient focal cerebral ischemia, the present study tested the hypothesis that blocking pro-inflammatory platelet-activating factor receptor (PAF-R) plus selected docosanoid after middle cerebral artery occlusion (MCAo) would lead to neurological recovery. The following small molecules were investigated: LAU-0901 (LAU), a

PAF-R antagonist that blocks pro-inflammatory signaling, Aspirin-triggered NPD1 (AT-NPD1), which activates cell-survival pathways, and their combination are exerting potent anti-inflammatory activity in the brain. Male Sprague-Dawley rats subjected to 2h of MCAo and were randomly assigned to seven treatment groups: LAU (45 and 60mg/kg, IP, at 3h), AT-NPD1 (111, 222, and 333µg/kg, IV, at 3.15 h after onset of stroke), LAU+AT-NPD1 or vehicle (n=5-6 per group). The behavioral function was evaluated on days 1, 3, and 7; a grading scale of 0-12 was employed. *Ex vivo* MRI of the brains was conducted using 11.7 T MRI on day 7. Physiological variables showed no significant differences among groups. Treatments with LAU-0901 (45 and 60 mg/kg) alone improved total neurological scores on days1-7 by 34-36%; AT-NPD1 (222 and 333 µg/kg) by 23-29% compared to vehicle groups. AT-NPD1 in a low dose (111 µg/kg) was not different from the vehicle group. The neuroprotective effect was enhanced using the LAU-0901+AT-NPD1, which resulted in improved total neurological scores up to 32-38% on days 1-7. Total lesion volumes, which were computed using T2WI, were significantly reduced with LAU-0901 (45 and 60 mg/kg) by 73-90% and AT-NPD1 (222 and 333 µg/kg) alone by 83-85% compared to the vehicle group. Ischemic core, penumbra, and total lesion volumes were dramatically reduced with LAU-0901+AT-NPD1 by 94, 92, and 93% compared to the vehicle. In conclusion, these data suggest that LAU-0901 and AT-NPD1 alone in moderate doses provide high-grade neuroprotection in a model of focal cerebral ischemia. Combination therapy with LAU-0901 and AT-NPD1 is more effective than the single therapy, affording synergistic neuroprotection with improved neurological recovery. Altogether, our findings support combinatory therapy as the basis for future therapeutics for ischemic stroke.

Disclosures: L.S. Belayev: None. **L. Khoutorova:** None. **M.M. Reid:** None. **A. Obenaus:** None. **C.R. Roque:** None. **P.K. Mukherjee:** None. **N.A. Petasis:** None. **R.B. Oria:** None. **N.G. Bazan:** None.

Digital Abstract Session

P141. Pharmacological Treatments For Stroke in Humans and Non-Human Primates

Program #/Poster #: P141.02

Topic: C.08. Ischemia

Title: On the effect of ganglioplegic Gangleron (Gangleronum[@]) on the sensitivity of arterial baroreceptor reflex in rats. Prospects for expanding the area of clinical application

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Abstract: On the effect of ganglioplegic Gangleron (Gangleronum[@]) on the sensitivity of arterial baroreceptor reflex in rats. Prospects for expanding the area of clinical application
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of Organic and Pharmaceutical Chemistry, NAS of Armenia, Yerevan**ABSTRACTObjectives**: Gangleron has hypotensive, antianginal, antiarrhythmic actions. Unlike other ganglioplegics, it has a moderately hypotensive effect and does not have a pronounced orthostatic hypotension. It predominantly blocks the N-cholinergic receptors of the parasympathetic ganglia, moderately depresses the respiratory center due to the inhibiting of pCO2- sensitive carotid glomus chemoreceptors. The impact of Gangleron on the sensitivity of depressive function of arterial baroreceptor reflex (ABR) was studied.**Methods:** The studies were carried out on 2 series (experimental 6 and control 7 animals) anesthetized white outbred rats of both sexes weighing 220-270 g. ABR testing was performed by the Oxford method, an increase in BP was caused by phenylephrine (15 μg / kg, i.v.) 10" after the administration of Gangleron (3 mg / kg, i.v.). The index of the sensitivity of the ABR depressor function was the regression coefficient linking the changes in BP (blood pressure) and heart rate (HR) during the first 15" testing of the ABR.**Results:** Gangleron by 15" significantly reduced the hypertensive effect of phenylephrine by 22.8% and reduced the time of development of the maximum cardiochronotropic response, shifting it from 15" to 10", did not change BP_{max} value achieved in both groups to 25" and did not significantly affect HR (heart rate) values. The sensitivity of the depressor ABR was significantly higher in the experimental group (-3.2 ± 0.7 mm Hg / bp/m) than in the control group (-2.1 ± 0.48 mm Hg / bp/m), the correlation coefficient r = 0.9 in both groups.**Conclusion:** A single use of Gangleron (3 mg / kg, i.v.) increased the sensitivity of ABR in rats. This fact may serve as the basis for starting studies of the use of Gangleron in complex antihypertensive therapy, where stimulation of carotid ABR with the help of implantable devices is a rapidly developing direction in the treatment of resistant hypertension. The property of Gangleron to improve depressive ABR along with depression of the respiratory center and moderately hypotension can be used in psychogenic cardiology, and to prevent panic attacks.
Keywords: Gangleron, arterial baroreceptor reflex, hypertension, ganglioplegic, carotid glomus.

Disclosures: V.H. Sarkisian: None. **N.D. Behnam:** None. **M.E. Shirinyan:** None. **A.A. Harutyunyan:** None. **V. Topuzyan:** None.

Digital Abstract Session

P141. Pharmacological Treatments For Stroke in Humans and Non-Human Primates

Program #/Poster #: P141.03

Topic: C.09.Stroke

Support: Eye Ear Nose Throat Foundation of New Orleans

Title: Higher Plasma levels of NPD1 correlate with better outcome and faster recovery in patients with spontaneous Intracerebral Hemorrhage.

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Abstract: Background: Intracerebral hemorrhage (ICH) results in increased intracranial pressure, blood-brain barrier damage, and cerebral edema. Pro-oxidant and pro-inflammatory milieu formed as a result of cell damage and cell lysis contributes to further injury. NPD1 is a docosahexaenoic acid (DHA) derived mediator with potent anti-inflammatory and anti-apoptotic properties and is highly enriched in the brain. NPD1 is made in response to tissue injury and counteracts the deleterious effects of inflammation and cell death. We hypothesized that ICH patients with better recovery/outcome will have increased levels of NPD1 in the blood. **Methods:** We have analyzed blood samples from sixteen patients with spontaneous ICH. RNA was extracted from PAXgene blood RNA tubes for 15-LOX-1 gene expression, critical in the endogenous synthesis of NPD1. Additionally, plasma was also isolated for Lipid analysis via Mass Spectrometry. Following a liquid-liquid lipid extraction, samples were analyzed using liquid chromatography-mass spectrometry (LC-MS/MS). The results were compared to controls (normal patients) and normalized to internal standards. Outcome measures included 90-day modified ranking score (MRS). **Results:** This is the first study to show the identification of NPD1 in ICH patients. The results show significantly higher NPD1 levels in ICH patients with 90-day MRS 0-3 (49.63pg/mL SD 43.78) vs. MRS 4-6 (1.88pg/mL SD 1.7) p=0.0012. We have also measured the gene expression of 15-LOX-1, a critical enzyme in the synthesis of NPD1. 15-Lox-1 is almost non-detectable in samples from patients with MRS 4-6, while patients with better outcome had higher 15-Lox-1 gene expression. NPD1 levels were higher in patients <65 yrs, ICH volume <30ml, and non-white, while PDX, was higher in patients >65 years, though changes in PDX were not significant. The amounts of DHA, EPA, and Arachidonic acid, as well as other lipid mediators such as resolvins and prostaglandins, did not show any significant variations. **Conclusions:** NPD1 and its stereoisomer PDX from the plasma of patients with spontaneous ICH were at very substantial levels. Furthermore, NPD1 levels and the expression of its critical enzyme 15-LOX-1 were elevated in patients who had better outcome and faster recovery. The results prompt further studies to comprehend the full scope of the effects of NPD1 in ICH. NPD1 plasma levels can also be used as an outcome predictor and a potential therapeutic option to enhance neuro-repair and neuro-recovery.

Disclosures: A. Asatryan: None. **D. Nguyen:** None. **A. Shirazian:** None. **B. Jun:** None. **I. Iwuchukwu:** None. **N. Bazan:** None.

Digital Abstract Session

P142. Stroke: Assessment of Injuries

Program #/Poster #: P142.01

Topic: C.09.Stroke

Support: CONACYT

Title: Changes in occupational and instrumental activities after a cerebrovascular event in a Mexican sample

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Abstract: Objective: To identify the changes in occupational and instrumental activities (functional independence), and the presence of depressive symptoms after a cerebrovascular event (CVE) in a sample of Mexicans patients. Reasons to attend cognitive rehabilitation were also assessed. Participants and Method: A sample of 17 Mexicans adults with 22 through 92 years of age, who had suffered any type of CVE were included in the study. Participants included 6 women and 11 men, with a mean age of 60 years old (SD=20) and 12 average years of education (SD=6). Participants were assessed through a clinical interview to identify attendance characteristics to rehabilitation process and to record changes in occupational and instrumental activities after a CVE. The Barthel index was applied to assess functional independence and the Yesavage scale (for elderly adults) or the Beck Depression Inventory (for young adults) were used to examine depressive symptoms. Results: Before the CVE most of men were employed (e.g. gardener, driver, salesman) and most of women were housewives. Main changes after the CVE, included that 53% of participants (2 women and 7 men) quit their employment or any previous activities. Four participants didn´t reported any data related to their income sources, 5 of them increased their financial support and in six of them it was decreased after the CVE. In 41% of the sample, the functional independence disappeared and 23.5% reported depressive symptoms. Meanwhile 71% (n=12)) attended to a rehabilitation program: 4 received neuropsychological therapy, 7 received physical rehabilitation and 1 participant had both types of treatment. Five patients (29%) did not attend a rehabilitation process, 4 had no obvious physical or cognitive sequelae, so this service was not indicated, and one of the participants did not provide this information. Conclusions: These results reflect a change in occupational and functional state in patients after a CVE, which results in a reduction of economic incomes. Also, more than half of the sample attended some type of rehabilitation, with the 29% participating in a neuropsychological therapy. In general, these results suggest the importance of rehabilitation services in patients with brain damage sequelae, particularly of a neuropsychological type which may support their possible functional and occupational reincorporation.

Disclosures: G. Ramírez-Alvarado: None. **B. Palacios-Hernandez:** None. **B. Tellez-Alanis:** None.

Digital Abstract Session

P142. Stroke: Assessment of Injuries

Program #/Poster #: P142.02

Topic: C.09.Stroke

Support: Funding through the Hotchkiss Brain Institute, Calgary, Alberta, Canada.

Title: Kinarm robotic assessment of stroke and traumatic brain injury across severities at sub-acute and 6 month post event timepoints

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Abstract: Objective: This exploratory study examined the performance of individuals with traumatic brain injury (TBI) and stroke on various KINARM robotic tests and their potential associations with clinical scales of severity at both subacute (T1) and 6-month post event (T2) timepoints. Methods: Twenty-seven individuals (age 18-78 years; 74% male) with stroke (6 minor, 6 moderate) or TBI (6 mild, 4 moderate, 5 severe) were recruited within 3 weeks of injury (T1) and at a 6 month follow-up (T2). Injury severity was determined using the NIH Stroke Scale (NIHSS) and the Glasgow Coma Scale (GCS) for stroke and TBI, respectively. Neurological impairment was quantified using the KINARM robotic device. Five standard KINARM tasks [Visually Guided Reaching (VGR), Arm Position Matching (APM), Kinesthesia (KIN), Object Hit (OH), and Object Hit and Avoid (OHA)] evaluated aspects of sensorimotor, visuospatial, proprioceptive, and cognitive function. Overall Task Scores for each injury group were calculated on each task to evaluate performance compared to normative values. Spearman correlations were used to assess the relationship between robotic assessments vs injury severity at each time point. Results: Most stroke participants performed outside the 95% range of normative data on every robotic task (90.0% on VGR, 80.0% on APM, 77.8% on KIN, 88.8% on OH, and 70.0% on OHA) at T1. In comparison, fewer individuals with TBI performed outside the range of normative data (33.3% on VGR, 13.3% on APM, 40.0% on KIN, 33.3% on OH, and 33.3% on OHA). At the second time point, severity of stroke was more strongly correlated with performance on some measures (KIN ρ=-0.731) than others (APM ρ=0.240). We did not observe similar relationships between TBI severity and the robotic scores (KIN ρ=0.014, APM ρ=-0.445). Robotic scores improved by T2, on average, in both stroke and TBI. Conclusions: Robotic assessment successfully detected neurological impairment after stroke at both time points and individuals with stroke more commonly showed impairments than those with TBI. Interestingly, the robotic measures most likely to detect impairment in TBI were tasks requiring the recruitment of multiple brain regions and increased cognitive demand, perhaps due to the injury mechanisms of TBI being more diffuse in nature. While preliminary, these findings reveal the strengths and weaknesses of the KINARM to evaluate neurological impairment in different severities of acquired brain injury.

Disclosures: J.B. Tabor: None. **M. Piitz:** None. **C. Ho:** None. **P. Whelan:** None. **S.P. Dukelow:** None. **C.T. Debert:** None.

Digital Abstract Session

P142. Stroke: Assessment of Injuries

Program #/Poster #: P142.03

Topic: C.09.Stroke

Support: NICHHD-NIH Grant K12HD073945
University of Washington Research Foundation Royalty Research Fund
NIH P51 OD010425
NSF Engineering Research Center Grant EEC-1028725
NSF Graduate Research Fellowship
Weill Neurohub
University of Washington Mary Gates Research Scholarship

Title: Acute neurophysiological dynamics during focal ischemic lesioning in non-human primate sensorimotor cortex

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Abstract: The primate cerebral cortex is a complex structure responsible for a variety of sophisticated tasks and behaviors including somatosensation and movement, which can be severely impaired following stroke. However, the development of effective treatments for sensorimotor dysfunctions arising from stroke is hindered by a lack of understanding of the underlying neural dynamics following injury. To drive the development of novel stimulation-based therapies, it is critical that these phenomena are investigated in a model sharing a high degree of complexity and evolutionary history with human cortex: the non-human primate (NHP) cortex. Here, we employ our previously developed lesion-based toolbox to investigate neurophysiological dynamics during injury of the NHP sensorimotor cortex. We induced focal ischemic lesions using the photothrombotic technique in the NHP sensorimotor cortex. Lesion induction was validated *in vivo* with optical coherence tomography angiography imaging and histologically post-mortem. We recorded neurophysiological dynamics as unilateral lesions developed over the course of three hours using implanted semi-transparent electrocorticographic (ECoG) arrays. To assess the changes in neural activity during the formation of a lesion, we calculated changes in local field potential power across different frequency bands (high gamma: 60-150 Hz, gamma: 30-60 Hz, beta: 12-30 Hz, theta: 4-7 Hz). As expected, recording sites in the lesioned area and the surrounding region exhibited decreases in power with some adjacent regions increasing in power. Interestingly, we observed global decreases in power across the network in both beta and theta bands. We also assessed functional connectivity changes across the network using signal coherence between electrode pairs. We observed an average decrease in functional connectivity across the network in all frequency bands following lesioning. Surprisingly, channels within the lesioned area and between the lesioned area and nearby regions exhibited an increase in connectivity in the higher frequency bands. In this study, we address the short-term changes in neural activity and connectivity during the induction of a lesion. While neural activity decreased in the lesioned region, increased activity and functional connectivity in the nearby regions may be evidence of compensatory activity following stroke that can be

exploited to promote functional recovery. The results of this study have great implications for the development of neurorehabilitation strategies for stroke and other neurological disorders.

Disclosures: **K. Khateeb:** None. **S. Chavan:** None. **K. Coubrough:** None. **J. Bloch:** None. **M. Rahimi:** None. **D.J. Griggs:** None. **T. Liu:** None. **M. Le:** None. **R. Wang:** None. **A. Yazdan-Shahmorad:** None.

Digital Abstract Session

P142. Stroke: Assessment of Injuries

Program #/Poster #: P142.04

Topic: C.09.Stroke

Support: This project was supported by the National Institute of Health National Center for Neuromodulation grant to PJP

Title: Alterations in the fronto-parietal brain network for balance control in chronic stroke patients

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Abstract: Impaired balance control following stroke contributes to falls among patients, thus limiting their mobility and independence in activities of daily living. Designing effective interventions require mechanistic framework. However, mechanisms by which stroke impairs balance control remain poorly understood. Remarkably, the contribution of cortical reorganization following stroke to impaired balance control remains unknown. We aim to address this knowledge gap by investigating cortical mechanisms associated with balance control in chronic stroke patients. Our recent work in healthy individuals identified a network of fronto-parietal brain regions that adjusts its activity based on the difficulty of the balance control task. We hypothesize that stroke disrupts the response of the identified fronto-parietal network, leading to impaired balance control among patients. This ongoing study is intended to study stroke survivors with mild-to-moderate severity and healthy adults age- and gender-matched to stroke patients. Participants are instructed to maintain balance in response to balance perturbations with varying difficulty levels on a computerized balance platform. We use 64-channel electroencephalography (EEG)-based neuroimaging analysis to assess the activation within the frontal and parietal brain regions while participants perform the balance task. Our preliminary findings (planned sample: n = 20, recruited: n = 5, 2 females) showed increased modulation in parietal areas as patients with stroke performed the balance control task. Consistent with previous studies, stroke patients' performance on the clinical tests reflected impaired balance and mobility when compared with healthy controls. Findings from this study

will be critical to determine how stroke influences the cortical network essential for balance control, as well as inform the design of interventions aimed at reducing falls among patients.

Disclosures: **N. Rao:** None. **H. Rabieh:** None. **M. Mutnick:** None. **S. Shah:** None. **S. Chaudhari:** None. **N. Mehta:** None. **E. Todd:** None. **M. Gale:** None. **J.L. Contreras-Vidal:** None. **P.J. Parikh:** None.

Digital Abstract Session

P142. Stroke: Assessment of Injuries

Program #/Poster #: P142.05

Topic: C.09.Stroke

Support: F31 NS120500-01

Title: Transcutaneous electrical trigeminal nerve stimulation in chronic hemiparetic stroke

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Abstract: Accumulating evidence suggests that chronic stroke induced motor impairments exhibit a dependence on heighted brainstem mediated monoaminergic drive to the spinal cord. Explicitly, a relative increase in descending monoamines is believed to increase spinal motoneuron excitability and thus (1) give rise to spasticity and (2) amplify the diffuse neural commands postulated as responsible for flexion synergy expression. This yields a potential investigational avenue, whereby altering monoaminergic drive may causally manipulate stroke induced motor impairments while also providing insight into their pathophysiological generation. Thus, we hypothesize that modulation of monoaminergic brainstem areas, such as the locus coeruleus, through non-invasive transcutaneous trigeminal nerve stimulation (TNS) will alter motoneuron excitability in individuals with chronic hemiparetic stroke and change the expression of motor impairments.
To investigate this potential, we recruited 8 individuals with moderate to severe chronic hemiparetic stroke (> 1 year post stroke) and quantified spinal motoneuron excitability before, during, and after one hour of both real and sham TNS. Real TNS was accomplished via biphasic electrical stimulation applied bilaterally to the supraorbital nerve at an individual's perceptual threshold (TNS; 100 Hz, 300 μs, avg: 4.0 mA). Spinal motoneuron excitability was estimated via the tonic vibration reflex of the paretic biceps brachii. Specifically, participants were instructed to generate and maintain 15% of maximum voluntary elbow flexion torque for 10s, after which a tonic vibratory stimulus (128 Hz) was applied to the paretic biceps brachii muscle for 5 s at a constant force of application. During this 5 s vibratory stimulus, visual torque feedback was removed and individuals were instructed to maintain their perceived level of force. The reflexively generated elbow flexion torque during this vibratory stimulus was then used as an

estimate of spinal motoneuron excitability.
When compared to an active sham stimulus, real TNS elicited greater decreases in reflexively generated elbow flexion torque during stimulus. This was observed as a significant decrease in the reflexively generated elbow flexion torque evoked by tonic vibration of the biceps brachii during real TNS and not sham conditions. This suggests that greater monoaminergic drive may increase spinal motoneuron excitability in this population and that TNS may be used as a method of probing this drive. Further work to understand the effects of TNS on estimates of intrinsic spinal motoneuron excitability with high-density surface electromyographic arrays is ongoing.

Disclosures: J.A. Beauchamp: None. **C.J. Heckman:** None. **J. Yao:** None. **J.P. Dewald:** None.

Digital Abstract Session

P142. Stroke: Assessment of Injuries

Program #/Poster #: P142.06

Topic: C.09.Stroke

Support: AHA 20PRE35210339

Title: Quantifying stretch reflex modulation during volitional reaching

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Abstract: The stretch reflex is an important monosynaptic pathway, involved in maintaining posture, joint stabilization, and perturbation rejection. During movements, these reflexes are modulated by supraspinal structures via corticofugal pathways to achieve high movement accuracy even in unpredictable environments at low energy costs. By measuring reflex excitability during voluntary movement, the modulation can be used as a proxy measure for neural activity in supraspinal structures. This allows for studying how these structures control movement, and how neurological injuries like stroke may affect stretch reflex modulation.
We have developed a novel protocol to quantify stretch reflex excitability during a ballistic reaching task and assessed the protocol on a group of control participants (n = 2) and participants with chronic hemiparetic stroke (n = 4). This novel method relies on a newly developed haptic device (NACT3D) capable of combining unconstrained 3D motion with 270°/s elbow extension perturbations. Participants moved ballistically from a near to a far target, with perturbations occurring in random catch trials. Reflexive muscle activity was defined as a change in EMG (normalized to Maximal Voluntary Contraction or MVC) 25-150 ms following the perturbation, relative to unperturbed movements.
Preliminary results show that reflexive activity following a perturbation in controls is minimal (+0% to +10% MVC) across all muscles and motion phases. In hemiparetic stroke, perturbations prior to voluntary movement cause an increase in reflexive muscle activity in all muscles (+16 to +84% MVC). While reaching, perturbations cause an increase in reflexive muscle activity in the

biceps and pectoralis major (+17% to +45% MVC) but a decrease in muscle activity in the triceps and deltoid muscles (-9% to -27% MVC). Post-hoc analysis showed significant differences in muscle activity prior to motion vs. in motion ($p < 0.0001$), stroke vs. control ($p < 0.0001$), paretic vs. non-paretic limb within the stroke cohort ($p < 0.0001$), paretic vs. non-dominant across cohorts ($p < 0.0001$), and short- vs. long-latency reflexes in the stroke cohort ($p < 0.0001$). These results indicate that reflex modulation is affected by hemiparetic stroke, and for the first time show altered reflex modulation during movement following hemiparetic stroke. The developed protocol and device allow for studying reflex modulation during a variety of motion tasks and increase our understanding of the complex role of supraspinal structures in modulating stretch reflexes.

Disclosures: T.A.M. Plaisier: None. **A. Acosta:** None. **J.P.A. Dewald:** None.

Digital Abstract Session

P142. Stroke: Assessment of Injuries

Program #/Poster #: P142.07

Topic: C.09.Stroke

Support: CIHR

Title: Resting state connectivity associated with skilled motor practice in individuals with stroke: dissociable networks for learning and recovery

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Abstract: Activity patterns across brain regions that can be characterised at rest (i.e., resting state functional connectivity; rsFC) are disrupted after stroke, and linked to impairments in motor function. While changes in rsFC are associated with motor recovery, it is not clear how functional connectivity is modulated by skilled motor practice. The current study examined how rsFC is modulated by skilled motor practice after stroke, and whether or not changes in rsFC are linked to both motor learning and recovery. Two groups of participants (individuals presenting with chronic stroke, $n = 32$; and age-matched controls, $n = 31$) engaged in four weeks of skilled motor practice of a complex, gamified reaching task designed to prevent early plateaus in performance. Clinical assessments of motor function and impairment, and brain activity (via functional magnetic resonance imaging) were obtained before and after training. We employed an ROI-to-ROI approach to examine changes (post vs. pre) in functional connectivity for each group. Further, graph theory metrics were computed to quantify network characteristics and interconnectedness. While no differences in rsFC were observed in the healthy control group, increased connectivity was observed in the sensorimotor network (linked to learning; e.g., between motor and posterior parietal cortices) and circuitry underlying higher cognitive

processes (linked to recovery; e.g., between the thalamus and frontal regions) in the stroke group. Relative to healthy controls, a decrease in network efficiency was observed following training. Findings show that the dissociable rsFC patterns related to learning and recovery reflect distinct offline processing that occurs over the course of training. Ultimately, we suggest that patterns of rsFC changes observed after stroke reflect a shift towards a compensatory network configuration characterised by decreased network efficiency.

Disclosures: S.N. Kraeutner: None. **C. Rubino:** None. **S. Rinat:** None. **B. Lakhani:** None. **M.R. Borich:** None. **K.P. Wadden:** None. **L.A. Boyd:** None.

Digital Abstract Session

P143. Structural Changes Following Stroke

Program #/Poster #: P143.01

Topic: C.09.Stroke

Support: NIH/NIDCD Grant DC014664
NIH/NIDCD Grant DC014021
AHA Grant 15SFDRN26030003
NIH/NIGMS Grant P20GM135007

Title: Structural connectivity correlates of upper and lower limb coordination in individuals with chronic stroke

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Abstract: Preservation of white matter (structural) fibers connecting brain regions critical for motor control may directly enhance functional recruitment of these regions modulated by neurorehabilitation to improve motor recovery post-stroke. We have shown that reduced structural connectivity of the ipsilesional primary motor cortex is an independent predictor of gross upper extremity motor performance/strength and walking speed impairment in chronic stroke, even after statistically controlling for lesion volume. Little is known, however, about structural connectivity damage and intra- or interlimb coordination deficits post-stroke. The purpose of our study was to examine the relationship between structural connectivity among key sensorimotor brain regions and upper/lower extremity (UE/LE) coordination in chronic stroke. Data were collected from 55 participants (20 female; mean age 60.0 ± 10.2 years; time post-stroke 60.7 ± 56.0 months) and retrospectively analyzed. Participants underwent MRI and a comprehensive behavioral assessment that included UE/LE dexterity tasks (unilateral and bilateral tapping paradigms). Interrater reliability of tapping counts was assessed using intraclass correlation coefficients (ICCs), and relationships between structural connectivity amid a predefined sensorimotor subnetwork and UE/LE coordination tasks were examined using

Spearman's correlations. Interrater reliability for tapping counts was excellent, with ICC values ranging from 0.97-1.00. Correlations between structural connectivity and UE/LE coordination tasks were strongest involving ipsilesional cortico-subcortical connectivity between the primary motor cortex (M1)/supplementary motor area (SMA) and the cerebral peduncle/thalamus, and between the primary sensory and anterior cingulate cortex and the thalamus ($r=0.46$-0.79, $p\leq0.002$). Ipsilesional M1-SMA connectivity was specific to LE interlimb coordination ($r=0.44$, $p\leq0.002$). Structural connectivity among key sensorimotor regions, especially cortico-subcortical connectivity, is positively related to UE/LE coordination tasks in chronic stroke. There is a large degree of overlap between gross motor neural connections and those related to coordination, suggesting these tasks share similar motor networks. Greater insight regarding structural disconnection after stroke may inform targeted rehabilitation interventions aimed at improving motor coordination in this patient population.

Disclosures: D.M. Peters: None. **M. Wingood:** None. **J.C. Stewart:** None. **L. Bonilha:** None. **S.L. Fritz:** None. **J. Fridriksson:** None.

Digital Abstract Session

P143. Structural Changes Following Stroke

Program #/Poster #: P143.02

Topic: C.09.Stroke

Support: Stroke Association, UK
MNTRF, UHB

Title: Hippocampal health and its impact on post-stroke cognition

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Abstract: Background: One third of stroke patients are at risk of developing post-stroke dementia[1]. Alzheimer dementia is associated with pathology of the Hippocampus[2]. **The aim** of this study was to investigate the impact of hippocampus health on post-stroke cognition. **Methods:** 42 stroke patients (mean=63±12, 32 males) were recruited. Inclusion criteria: within three months of ischaemic stroke, MoCA[3] less 25, ability to consent, high chances of survival. Exclusion criteria: stroke affecting middle temporal structures, MR counter-indication, inability to concentrate for 30 minutes. Cognition was assessed using the BCoS[4]. Hippocampus[5] health

was examined using; T1-weighted imaging (volumetric, CAT12[6]) and magnetic resonance spectroscopy (NAA metabolite). Inter-measurement correlations were used to assess the validity of MR measurements. Multiple regression analyses was used estimate the relation between hippocampus health and cognition, while controlling for demographic and crude measures of brain health (lesion size, Framingham Risk Score[7], cortical atrophy[8], and small vessel disease[8].
Results: Hippocampus grey matter volume correlated negatively with age and Framingham Vascular risk ($r < -.49$) and positively with education and NAA ($r > .43$). Cognition was reliably predicted by hippocampus volume (standardised $\beta = .40$) and NAA ($s\beta = .48$), even after controlling for confounding factors.
Conclusion. This study highlight the role of hippocampus health in post-stroke cognitive recovery, offering a potential mechanism for diagnosing a risk for post-stroke dementia.
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Disclosures: R. Laverick: None. **M. Wilson:** A. Employment/Salary (full or part-time):; University of Birmingham. **K. Nader:** None. **A. Northhall:** None. **J. Esteves:** None. **D. Sims:** None. **S. Ispoglou:** None. **T. Hayton:** None. **P. Rotshtein:** None. **V. Sawlani:** None. **R. Evans:** None. **A. Hosseini:** None. **D. Auer:** None.

Digital Abstract Session

P143. Structural Changes Following Stroke

Program #/Poster #: P143.03

Topic: C.09.Stroke

Support: NIH R01HD084009
NIH R03HD094615
predoctoral fellowship T32EB025766

Title: Custom DTI probabilistic tractography techniques to estimate muscle fascicle morphology in the biceps brachii muscle for chronic hemiparetic stroke

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Abstract: Introduction: Motor pathways are thought to be reorganized after the onset of hemiparetic stroke, resulting in altered neural input to the paretic limb's skeletal muscles. This change in neural drive gives rise to disuse and secondary adaptations in muscle architecture, such as shorter muscle fascicles and decreased muscle volume, which can critically impact already existing functional impairments. Here, we implement custom diffusion tensor imaging (DTI) probabilistic tractography techniques to non-invasively measure fascicle lengths in arm muscles following a hemiparetic stroke. Methods: Diffusion-weighted imaging (spin-echo echo planar imaging sequence, voxel resolution=1.25x1.25x5 mm3) and T1 anatomical (voxel resolution=0.78x0.78x3 mm3) scans were taken of the biceps brachii (BIC) muscles in one participant with chronic hemiparetic stroke (M, 73 yo) and one control participant (M, 63 yo). Anatomical scans were used to select seed regions within the muscle, from which probabilistic tractography analyses were performed. Tractography results represented muscle fascicle bundles and were fitted to 3D polynomial curves to estimate fascicle lengths. Results: Fascicle lengths in the individual with stroke exhibited interlimb differences, with the median fascicle length of the paretic BIC being 43% shorter than the median of the non-paretic BIC. Meanwhile, fascicle lengths in the control individual did not exhibit interlimb differences. These results are consistent with previously published ultrasound measurements (Adkins et al., 2020) and indicate that the DTI method is similarly sensitive to the structural changes that are present in skeletal muscles in chronic stroke. Most importantly, our new results indicate that DTI techniques can capture a broader distribution of fascicles in comparison to the superficial measurements of ultrasound, which ultimately allows for the capability to analyze morphology across all muscles in individuals with stroke-induced motor impairments. Conclusion: The stroke-induced disuse of the paretic elbow results in shorter muscle fascicles in the paretic BIC, which causes a reduced elbow range of motion and further exacerbates functional impairments after stroke. The broad range of fascicle lengths captured in the current study suggests that there is a complex organization of fascicles, which is consistent with previous ex vivo cadaveric studies (Loeb et al., 1987), but has yet to be shown using in vivo techniques. Future work will apply these new DTI probabilistic tractography techniques to better understand how the inherent organization of muscle fascicles may be altered following a neuromuscular injury.

Disclosures: D. Joshi: None. **W.M. Murray:** None. **J.P.A. Dewald:** None. **C. Ingo:** None.

Digital Abstract Session

P144. Therapies For Stroke: Animal Models

Program #/Poster #: P144.01

Topic: C.09.Stroke

Support: NIH/NIA R56AG059693

Title: The efficacy of curcumin to facilitate recovery of function in a rhesus monkey model of cortical injury.

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Abstract: Curcumin is a primary component of the spice turmeric and is a potent anti-inflammatory and anti-oxidant compound. In rodent models, curcumin acts primarily on microglia and astrocytes to inhibit pro-inflammatory signaling pathways and in rodent models of stroke, ischemia, and traumatic brain injury it reduces inflammation and levels of reactive oxygen species. Further, rats with cortical injury treated with curcumin have smaller lesions and fewer neurological impairments than those treated with vehicle. However, the promising effects of curcumin have not yet been extensively tested in rhesus monkeys so it is not clear whether curcumin exerts the same biological effect in primate brains as in rodent brains. However, our prior cognitive studies recently demonstrated that curcumin enhances spatial working memory and motor function in aging monkeys receiving daily oral doses of dietary curcumin. Building on these findings, we administered oral doses of curcumin to adult, male rhesus monkeys following induced cortical injury to the hand-representation of primary motor cortex (M1). Treated monkeys demonstrated significantly enhanced recovery of function in terms of time to retrieve a food reward. In addition, treated monkeys returned to pre-injury grasp patterns, while monkeys that received vehicle developed a compensatory grasp pattern and did not return to pre-injury grasp patterns. Post-mortem assessment of brain tissue from these monkeys showed that within the perilesional grey matter of curcumin-treated monkeys the total density of microglia increased, with a greater proportion of antigen-presenting MHCII+ microglia. Further, in vitro whole-cell patch-clamp recording in acute slices of perilesional cortex showed that curcumin reduced injury-related hyperexcitability in perilesional layer 3 pyramidal neurons. These findings suggest that while curcumin treatment promoted microglial proliferation and activation, it also ameliorated neuronal excitotoxicity. While further work is needed to establish the link between these two processes, it is possible that curcumin promoted neuroprotection after injury through microglial proliferation that facilitated clearance of damaged neurons. Our findings show that curcumin is an effective treatment for enhancing recovery of function after cortical injury in our rhesus monkey model, but the precise cellular and molecular mechanisms remain unclear and will be further investigated.

Disclosures: **M.A. Pessina:** None. **Y. Zhou:** None. **M. Sakharkar:** None. **B.G.E. Bowley:** None. **D.L. Rosene:** None. **M. Medalla:** None. **T.L. Moore:** None.

Digital Abstract Session

P144. Therapies For Stroke: Animal Models

Program #/Poster #: P144.02

Topic: C.09.Stroke

Support: NIH Grant R01NS109221
Brazilian CAPES (88881.311939/2018-01)

Title: Pro-homeostatic docosanoids are highly neuroprotective in focal cerebral ischemia in rats

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Abstract: Ischemic stroke triggers a pattern of cellular and molecular disturbances that include lipid peroxidation, uncompensated oxidative stress, and neuronal injury. Although neuroprotective strategies have shown promise, no treatment has demonstrated efficacy after stroke. This study focuses on the neuroprotective bioactivity of docosanoid (DOC) mediators: Neuroprotectin D1 (NPD1), Resolvin D1 (RvD1), and their combination (NPD1+RvD1) against experimental ischemic stroke. These lipid mediators are biosynthesized "on-demand" in response to the onset of stroke to resolve neuroinflammation and restore homeostasis. Male Sprague-Dawley rats were anesthetized with isoflurane/nitrous oxide and underwent 2h of middle cerebral artery occlusion (MCAo). Behavior was evaluated on days 1, 3, and 7; a grading scale of 0-12 was employed. Animals were randomly assigned to eight treatment groups: NPD1 (111, 222, and 333µg/kg), RvD1 (111, 222, and 333µg/kg), NPD1 + RvD1 or vehicle (n=4-5 per group). All treatments were administered IV at 3h after the onset of MCAo. *Ex vivo* MRI was conducted on day 7. Physiological variables showed no significant differences among groups. Treatments with NPD1 (111, 222, and 333µg/kg) alone greatly improved neurological scores (by 32, 28, 35%) and RvD1 (111, 222, and 333µg/kg) alone by (by 35, 44, 31%) compared to the vehicle group. The neuroprotective effect was enhanced using the NPD1+ RvD1, which resulted in improved total neurological scores on days 1, 2, 3, and 7 by 25, 28, 28, 40% compared to the vehicle group. Ischemic core and penumbra volumes (computed from T2WI) were significantly reduced compared to the vehicle-treated group. We have shown that treatment with NPD1, RvD1 alone, and combination therapy provides high-grade neurobehavioral recovery and decreases ischemic core and penumbra volumes. Thus, these findings support the potential clinical feasibility of administering novel docosanoids therapy to patients with acute ischemic stroke.

Disclosures: N.G. Bazan: None. **M.M. Reid:** None. **A. Obenaus:** None. **L. Khoutorova:** None. **P.K. Mukherjee:** None. **C.R. Roque:** None. **N.A. Petasis:** None. **R.B. Oria:** None. **L. Belayev:** None.

Digital Abstract Session

P144. Therapies For Stroke: Animal Models

Program #/Poster #: P144.03

Topic: C.09.Stroke

Support: NIH Grant R01NS109221

Title: Intranasal delivery of precursors of N-32 and N-34 elovanoids, novel homeostatic mediators, improves behavior and protect ischemic penumbra in rats after ischemic stroke

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Abstract: Ischemic stroke alters the neurovascular unit, neuroinflammatory responses, and synaptic activity causing tissue death. Recently, we discovered and characterized a novel class of homeostatic lipid mediators, termed elovanoids (ELVs), which are derivatives from very long-chain polyunsaturated fatty acids (VLC-PUFAs,n-3). ELVs displays neuroprotective bioactivities, both *in vitro* and *in vivo* experimental ischemic stroke. We also found that intravenous administration of ELVs is neuroprotective in middle cerebral artery occlusion in rats. The aim of this study was to examine whether intranasal ELV is equally effective as intravenous ELVs in a rat model of MCAo. Male Sprague-Dawley rats (280-300g) received 2h MCAo by intraluminal suture. Treatments with VLC-PUFAs (C-32:6 or C-34:6) and vehicle were administered at 1h, 24h, and 48h after 2h of MCAo by intranasal delivery. The composite neurological battery was performed on days 1, 2, 3, and 7 after stroke; a grading scale of 0-12 was employed (normal score=0, maximal deficit=12). *Ex vivo* imaging of the brains was conducted on 11.7T MRI on day 7 for lesion volumes and edema (T2WI), followed by immunohistochemistry. No adverse physiological effects were observed in any treatment group. Behavioral scores were improved with C-32:6, C-34:6, on day three by (42% and 21%) and on day 7 (45% and 23% respectively) compared to the vehicle group. Total lesion volumes computed from T2WI were reduced with C-32:6, C-34:6, compared to the vehicle by 88% and 57%, respectively, on day seven. In addition, ischemic core and penumbra volumes were significantly reduced by all ELV treatments. We conclude that the intranasal administration of precursors of N-32 or N-34 ELVs provides high-grade neuroprotection. Our study opens avenues of exploration of ELVs as a possible therapeutic approach for ischemic stroke.

Disclosures: **M.M. Reid:** None. **L. Khoutorova:** None. **B.K. Park:** None. **N. Desai:** None. **P.K. Mukherjee:** None. **B. Jun:** None. **A. Obenaus:** None. **N.A. Petasis:** None. **L. Belayev:** None. **N.G. Bazan:** None.

Digital Abstract Session

P144. Therapies For Stroke: Animal Models

Program #/Poster #: P144.04

Topic: C.09.Stroke

Support: The General Research Fund grant from the Research Grant Council of the Hong Kong Special Administrative Region Government (Ref. No.: 11100318).

Title: Low dose ionizing radiation reverses motor deficits and promotes brain rewiring after ischemic stroke in mice

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Abstract: Stroke is the most common form of acquired brain damage caused by blockade of cerebral artery. Patients surviving from stroke often experience life-long motor impairment and disability which require long-term rehabilitation. Motor functional restoration is limited even after intense rehabilitation. Approximately 30% of the stroke survivors cannot walk properly without assistance, and show high dependence in their daily lives. Currently, there is no effective treatment to reverse the motor deficits after ischemic stroke. Growing evidence suggested that low dose of ionizing radiation (LDIR) displayed strong neuroprotective effects on various neurological disorders such as retinitis pigmentosa, spinal cord injury, glaucoma and Parkinson's disease. Our pilot study also demonstrated that LDIR accelerated motor functional recovery after traumatic brain injury in mice via promoting anti-inflammatory M2 polarization in microglia. Here, we extended our study to investigate the therapeutic potential of LDIR on ischemic stroke. We induced a focal ischemic stroke in adult mice by photothrombotic lesion on the right motor cortex using Rose Bengal, and observed an increase in microglial clustering associated with the reduced deposition of chondroitin sulfate proteoglycans at the lesion in irradiated mice shortly after stroke induction. LDIR also markedly reduced the wound area at 7 days post stroke. After confirming the beneficial effects of LDIR on ischemic stroke, we then assessed motor functional recovery by narrow beam walking, pole climbing and grip strength tests over a period of 56 days. While sham-irradiated mice exhibited sustained motor deficit after stroke, the irradiated mice displayed a remarkable restoration in motor function as they returned to their baseline values within 7-21 days in all the behavioral tests. Depletion of the resident microglia in the brain completely abolished the beneficial effects induced by LDIR. Further analysis on the infarct development using TTC staining and magnetic resonance imaging revealed a marked reduction in average infarct volume in the irradiated mice. More importantly, LDIR induced an extensive bi-hemispheric axonal sprouting in the pre-motor cortex area, which led to a marked improvement in average local field potential oscillations in irradiated mice two months after stroke. Taken together, our study demonstrated that promoting microglial M2 polarization using LDIR is a promising therapeutic approach to reverse the motor deficits in a mouse model of ischemic stroke. Further optimization of the dosage of LDIR could pave the path for clinical use in treating patients with ischemic stroke.

Disclosures: G. Kumar: None. **N.P.B. Au:** None. **S.L. Chan:** None. **J.H.C. Lai:** None. **K.W.Y. Chan:** None. **K.N. Yu:** None. **C.H.E. Ma:** None.

Digital Abstract Session

P144. Therapies For Stroke: Animal Models

Program #/Poster #: P144.05

Topic: C.09.Stroke

Title: Translational tools and approaches of cellular therapies in different neurological disorders in drug discovery

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Abstract: Regenerative therapies of central nervous system (CNS) diseases offer innovative strategies to promote tissue and functional repair. As part of cell therapeutic approach, understanding basic biodistribution and ADME profile of studied cell product in early phase is important before proceeding towards more complex and comprehensive study designs. In addition to the ADME profile of the cells, safety and efficacy of cell products are crucial steps in development of novel treatment approaches. Efficacy of cell therapies is highly dependent on route of administration of transplanted cells, biodistribution, graft survival, and integration in the affected brain/spinal cord area. Intra-arterial (i.a.) infusion of cells bypass the filtering organs enabling the cells to transplant the target region more efficiently compared to e.g. intravenous delivery. In addition to used administration route, one approach to affect transplantation efficacy of cell product, can by altered by transducing cells with viral vectors. In this work we studied biodistribution of stem cell with or without viral transduction in rat stroke model. In case of stroke, successful and efficient homing of the cells to traumatic brain area without complications is important for the therapeutic potential of cell therapy. Here we studied the biodistribution of human stem cells after a direct i.a. administration in rat stroke model. Cells were radiolabeled using 111In and biodistribution over time was studied using SPECT/CT imaging. Our results showed that i.a. infusion of cells was a safe and efficient administration route for studied cells resulting in a transient localization of cells in the rat brain. For early distribution studies of cells, radiolabeling and SPECT/CT imaging offers efficient and fully translational tool for early ADME profiling of novel cell products.

Disclosures: K.M. Paldanius: None. **T. Huhtala:** None. **D. Miszczuk:** None.

Digital Abstract Session

P144. Therapies For Stroke: Animal Models

Program #/Poster #: P144.06

Topic: C.09.Stroke

Support: American Heart Association (18AIREA 3399009
Neuroscience program

College of Medicine
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John G. Kulhavi Professorship in Neuroscience at CMU
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Title: Delivery of hSOX2 gene using PAMAM dendrimer nanomolecules to reprogram astrocytes into neurons in ischemic stroke rats

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Abstract: Stroke is the fifth leading cause of death in the US with ischemic stroke accounting for 87% of the cases. A major limitation of currently available treatment strategies is that they are time sensitive and must be administered within 3 hr of the patient's first symptoms to achieve maximum therapeutic efficiency. Alternative treatments are being developed that are not as time sensitive and utilize *in vivo* reprogramming to replenish the lost neurons in sufficient numbers. Our work suggests that the use of dendrimers can facilitate delivery of therapeutic biomolecules and gene vectors. Dendrimers are nanomolecules with a well-established capacity of delivering drugs/ large biomolecules across the BBB, while possessing anti-inflammatory properties themselves. They are comprised of a central core, an interior dendritic structure , and an exterior surface with functional surface groups. The surfaces can be manipulated in order to efficiently enter the cells without damaging the integrity of, thus keeping toxicity to a minimum; they can also be labeled for efficient tracking. The G4 PAMAM dendrimers (4 nm) used in this study have 10% of the surface covered with amine groups and 90% of the surface covered with hydroxyl groups (D-90/10) and labelled with Cy5.5. They have been shown to be less toxic and readily form complexes with plasmids up to 14kb in size while successfully delivering cargo in vitro and in vivo In this study, we tested whether a transcription factor, *SOX2,* when expressed under a glial cell-specific gfa2 promoter, could sufficiently reprogram astrocytes at the site of insult/inflammation to enhance their differentiation into neurons. Four days after inducing strokes via a middle cerebral artery occlusion (MCAo). in Sprague Dawley rats, a novel gfa2-pomoter-containing dendriplex was injected into the corpus callosum. The dendriplex allowed for *hSOX2* gene expression under an astrocyte-specific GFAP promoter which was complexed with a mixed-surface PAMAM G4 90% OH and 10% NH2 dendrimers tagged with Cy5.5 (D-Cy5.5-gfa2-hSOX2-GFP-Luc2 dendriplex). Eight weeks following the injections, the brains were collected and analyzed for the presence of the complex and *hSOX2* gene expression using IHC (Abcam; Anti-SOX2 antibody #ab97959). The stained tissues were mounted on slides and imaged using Zeiss AxioImager M2.0. When injected in the stroked brains complexed with hSOX2, we were able to observe a clear co-localization of the hSOX2 protein, the GFP reporter protein, as well as the PAMAM G4 90/10-Cy5.5 dendrimer. Thus, we conclude that the

dendrimer-plasmid complex was successfully delivered to the cells, giving rise to hSOX2 protein expression at the infarct site.

Disclosures: **A. Kannan-Sundhari:** None. **B. Srinageshwar:** None. **M. Andrews:** None. **J. Stadler:** None. **B. MacDonald:** None. **A. Poudel:** None. **P. Otero:** None. **J.E. Smith:** None. **A. Toth:** None. **N. Singh:** None. **M. Reigler:** None. **R. Schalau:** None. **J. Richmond:** None. **D. Swanson:** None. **A. Sharma:** None. **G. Dunbar:** None. **J. Rossignol:** None.

Digital Abstract Session

P144. Therapies For Stroke: Animal Models

Program #/Poster #: P144.07

Topic: C.09.Stroke

Support: Florida Biomedical Grant, Florida Department of Health.
NIH- Clinical and Translational Science Institute Grant
Anderson Family Gift Fund
Applebaum Foundation
Department of Neurology, University of Miami Miller School of Medicine

Title: Safety and efficacy of intra-arterial mesenchymal stem cell therapy in a canine model of stroke

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Abstract: Introduction: Therapeutic advances for ischemic stroke in the past few decades include intravenous tissue plasminogen activator administered within 4.5 hr and mechanical thrombectomy up to 24 hr after stroke onset. Although this has led to a 30% increase in survival rates after stroke, more than 50% of stroke survivors are chronically disabled. Hence, novel more effective therapies are needed. Research in the last 2.5 decades has shown great promise in the use of cell-based therapies for stroke. Our group and others have shown the safety and efficacy of intra-arterial (IA) stem cell therapy in a rodent model of stroke and safety in a clinical study, RECOVER-Stroke. The IA route of delivery is minimally invasive and allows for targeted delivery of cells. We pursued a pre-clinical project to study the safety, efficacy, and optimum dosage of IA mesenchymal stem cell delivery in a canine model of stroke. This model is well-suited for translation due to its gyrencephalic brain and a white to gray matter ratio that is similar

to humans. The objectives of this study were: 1) Develop a reproducible endovascular model of canine focal cerebral ischemia with reversible middle cerebral artery occlusion (rMCAO). 2) Test the safety, efficacy and optimal dosage of IA MSC therapy in this model. Methods: An endovascular canine rMCAO model using retractable platinum coil for 60-120 min was established. At 48 hr post-rMCAO, canine MSC (10-80 million) were delivered using a microcatheter in the ipsilateral upper cervical internal carotid artery. Serial MRIs and neurological deficit scoring (NDS) were performed over 30 days. Animals were euthanized at 15-30d post-rMCAO and brains were harvested for histology and molecular analyses. Results: Our study yielded a highly reproducible, minimally invasive endovascular large animal model of rMCAO. Significantly higher infarct volume reduction was seen at IA 10 million and 40 million doses in dose dependent manner as compared to the control group. This correlated with improvement in focal neurological deficits with faster and more complete recovery in the treated groups as compared to controls. Delivery of high dose, 80 m cells, led to worsened outcome and new infarct lesions 4 days post-injection with spontaneous resolution at day 15 were observed. Conclusions: In conclusion, our study suggests that IA delivery of MSC at 48 hr post-stroke is safe and effective in a dose-dependent manner up to 40 m MSCs. IA delivery of 80 million MSCs is toxic leading to new severe cerebral ischemia from the cell dose. Collectively, our study has established a successful preclinical canine model of stroke and reported dose-dependent efficacy of IA MSC therapy after stroke.

Disclosures: R.D. Thakkar: A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. **M. Watanabe:** A. Employment/Salary (full or part-time):; Jikei University School of Medicine. **L. Guada:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. **K. Bates:** A. Employment/Salary (full or part-time):; Rapid Medical Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rapid Medical Ltd: Incentive stock options, professional relationship as Clinical Research Manager. **K. Nishimura:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. **V. Saini:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. **P. Bhattacharya:** A. Employment/Salary (full or part-time):; National Institute of Pharmaceutical Education and Research, ahmedabad, India. **C. Sidani:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. **K. Ramdas:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. **E. Howerth:** A. Employment/Salary (full or part-time):; University of Georgia. **A. Khan:** A. Employment/Salary (full or part-time):; Interdisciplinary Stem Cell Institute, University of Miami Miller School of Medicine. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AssureImmune Cord Blood Bank: Equity Aceso Therapeutic: Equity. **J. Hare:** A. Employment/Salary (full or part-time):; Interdisciplinary Stem Cell Institute, University of Miami Miller School of Medicine. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); National Heart, Lung and Blood Institute: Grant Support. Other; a patent for cardiac cell-based therapy, Vestion Inc.: Equity, Longeveron: Equity, co-investor of intellectual property licensed to Longeveron, Heart Genomics: Equity. **D. Yavagal:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine.

Other; Medtronic, Neuralanalytics, Cerenovus, Rapid Medical: Consultant TIGER study, SWIFT Prime, RECOVER-Stroke : Steering Committee Member MR RESCUE: Investigator Steering Committee ESCAPE: DSMB member. Ap. **C. Dong:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine.

Digital Abstract Session

P144. Therapies For Stroke: Animal Models

Program #/Poster #: P144.08

Topic: C.09.Stroke

Support: JES Edwards Foundation
Neurobiology of Aging and Alzheimer's Disease Associate Fellowship (T32 Training Grant)
Sigma Xi Grant-in-Aid of Research

Title: Effect of dietary genistein on functional recovery and chronic poststroke inflammation in ovariectomized middle-aged rats

Authors: *A. OPPONG-GYEBI, D. METZGER, P. VANN, N. SUMIEN, D. SCHREIHOFER; UNT HEALTH SCIENCE CENTER, Fort Worth, TX

Abstract: PURPOSE: Increasing age is associated with an increased risk of stroke in women after the menopausal transition. A drop in circulating estrogens after menopause has been described as a key reason for the age-related risk, considering that estrogen has shown neuroprotection preclinically. However, the use of estrogen therapy for chronic prevention of cardiovascular diseases is limited by inconsistent outcomes including both benefits and detriment. For this, other agents are investigated as possible alternatives to protect women against the stroke-related physiological changes that come with aging and low plasma estrogen concentrations. In the current study, we hypothesized that genistein, a neuroprotective plant-derived estrogen will confer neuroprotection following hypogonadism and experimental stroke. METHOD: We used proven retired breeder Sprague-Dawley rats (aged ~9 months old) that were ovariectomized and categorized into two hypogonadal time points (2weeks= short-term and 12 weeks= long-term) during which they were treated with a soy-free diet. Each time point was followed by treatment with isoflavone-free diet, genistein diet (GEN) or 17-β estradiol (E2) implant. All animals were subjected to intraluminal middle cerebral artery occlusion or sham surgery followed by behavioral tests assessments for motor and cognitive functions over 21-days and brains collected were used for biochemical analysis for chronic poststroke inflammation. RESULTS: Sham-operated animals performed better on the locomotor task with cylinder test compared to stroked animals after both short-term and long-term hypogonadism. Both GEN and E2 improved performance on cylinder test after long-term hypogonadism. GEN but not E2 improved reversal learning on the Morris water maze test after short-term hypogonadism. GEN but not E2 reduced activated calcium-binding adaptor molecule 1 (Iba1) and increased transforming growth factor-β1 (TGF-β1) after short-term hypogonadism. CONCLUSION:

Dietary Genistein may improve locomotor function in the acute phase of stroke following long-term hypogonadism, improve aspects of cognitive function and reduce inflammation after short-term hypogonadism.

Disclosures: **A. Oppong-Gyebi:** None. **N. Sumien:** None. **D. Metzger:** None. **P. Vann:** None. **D. Schreihofer:** None.

Digital Abstract Session

P145. Stroke Recovery: Assessment of Computer Interface and Stimulation

Program #/Poster #: P145.01

Topic: C.09.Stroke

Support: E! 12274 – EUROSTARS
MSCA-RISE grant Pro-Gait (No. 778043)

Title: Brain-computer interface system for lower extremity rehabilitation of chronic stroke patients

Authors: ***C. GUGER**[1,2,3], M. SEBASTIAN-ROMAGOSA[3], R. ORTNER[3], W. CHO[2]; [1]g.tec neurotechnology GmbH, Schiedlberg, Austria; [2]g.tec medical engineering GmbH, Schiedlberg, Austria; [3]g.tec medical engineering Spain S.L., Barcelona, Spain

Abstract: Neurorehabilitation based on Brain-Computer Interfaces (BCIs) show important rehabilitation effects for patients after stroke. Previous studies have shown improvements for patients that are in a chronic stage and/or have severe hemiparesis and are particularly challenging for conventional rehabilitation techniques.
For this publication ten stroke patients in chronic phase with hemiparesis in the lower extremity were recruited. All of them participated in 25 BCI sessions about 3 times a week. The BCI system was based on the Motor Imagery (MI) of the paretic ankle dorsiflexion and healthy wrist dorsiflexion with Functional Electrical Stimulation (FES) and avatar feedback. Assessments were conducted to assess the changes in motor improvement before, after and during the rehabilitation training. Our primary measures used for the assessment were 10-meters walking test (10MWT), Range of Motion (ROM) of the ankle dorsiflexion and Timed Up and Go (TUG). Results show a significant increase in the gait speed in the primary measure 10MWT fast velocity of 0.16 m/s (SD = 0.14). This improvement is above of the minimally clinically important difference (MCID). The speed in the TUG was also significantly increased by 0.06 m/s, P = 0.002. One patient was not able to perform TUG assessment before the rehabilitation training but was able to perform it after the BCI treatment with time 92.2 seconds. The passive ROM assessment increased 8.61° (SD = 6.54), P = 0.002, and active ROM increased 8.50° (SD = 7.23) after rehabilitation training, P =.008.
These outcomes show the feasibility of this BCI approach for chronic stroke patients, and further support the growing consensus that these types of tools might develop into a new paradigm for rehabilitation tool for stroke patients. However, the results are from only ten chronic stroke

patients so the authors believe that this approach should be further validated in broader randomized controlled studies involving more patients.
MI and FES-based non-invasive BCIs are showing improvement for the gait rehabilitation of the patients in the chronic stage after stroke. This could have in impact on the rehabilitation techniques used for these patients, especially when they are severely impaired, and their mobility is limited.

Disclosures: C. Guger: None. **W. Cho:** None. **M. Sebastian-Romagosa:** None. **R. Ortner:** None.

Digital Abstract Session

P145. Stroke Recovery: Assessment of Computer Interface and Stimulation

Program #/Poster #: P145.02

Topic: C.09.Stroke

Support: NIH grant NS115759
Paul Kalmanovitz Central Nervous System Repair Research Program

Title: Improved Functional Outcome after Peripheral Nerve Stimulation of the Impaired Forelimb Post-Stroke

Authors: *S.-Y. TSAI[1], J. A. SCHREIBER[2], N. S. ADAMCZYK[1], J. Y. WU[1], S. T. TON[1], R. C. HOFLER[2], J. S. WALTER[1], T. E. O'BRIEN[3], G. L. KARTJE[1,4], R. P. NOCKELS[2];
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Abstract: Lack of blood flow to the brain, i.e., ischemic stroke, results in loss of nerve cells and therefore loss of function in the effected brain regions. There is no effective treatment to improve lost function except restoring blood flow within the first several hours. Rehabilitation strategies are widely used with limited success. The purpose of this study was to examine the effect of electrical stimulation on the impaired upper extremity to improve functional recovery after stroke. We developed a rodent model using an electrode cuff implant onto a single peripheral nerve (median nerve) of the paretic forelimb and applied daily electrical stimulation. The skilled forelimb reaching test was used to evaluate functional outcome after stroke and electrical stimulation. Anterograde axonal tracing from layer V pyramidal neurons with biotinylated dextran amine was done to evaluate the formation of new neuronal connections from the contralesional cortex to the deafferented spinal cord. Rats receiving electrical stimulation on the median nerve showed significant improvement in the skilled forelimb reaching test in comparison with stroke only and stroke with sham stimulation. Rats that received electrical stimulation also exhibited significant improvement in the latency to initiate adhesive removal from the impaired forelimb, indicating better sensory recovery. Furthermore, axonal tracing analysis showed a significant higher midline fiber crossing index in the cervical spinal cord of

rats receiving electrical stimulation. Our results indicate that direct peripheral nerve stimulation leads to improved sensorimotor recovery in the stroke-impaired forelimb, and may be a useful approach to improve post-stroke deficits in human patients.

Disclosures: S. Tsai: None. **J.A. Schreiber:** None. **N.S. Adamczyk:** None. **J.Y. Wu:** None. **S.T. Ton:** None. **R.C. Hofler:** None. **J.S. Walter:** None. **G.L. Kartje:** None. **R.P. Nockels:** None. **T.E. O'Brien:** None.

Digital Abstract Session

P146. Cellular and Molecular Mechanisms of Brain Injury and Disease

Program #/Poster #: P146.01

Topic: C.10. Brain Injury and Trauma

Support: DoD Grant W81XWH-18-1-0811
Roskamp Institute

Title: Temporal profiles of microglial genes driving neuroinflammation in the acute to chronic aftermath of repetitive mild traumatic brain injury

Authors: *A. J. PEARSON, C. ORTIZ, M. BROWNING, F. CRAWFORD, J. OJO;
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Abstract: Repetitive mild traumatic brain injury (r-mTBI) is a strong risk factor for the development of neurodegenerative diseases such as Alzheimer's disease. One of the hallmark features of r-mTBI is persistent neuroinflammation typified by the activation of microglial cells, which serve as the innate immune cells of the brain. Microglia represent a promising target to beneficially alter the disease progression of r-mTBI, however, attempts to broadly target immune activation have been so far unsuccessful in improving clinical outcomes, due in part due to a lack of understanding of the precise molecular mechanisms driving the inflammatory changes at chronic time points post-injury. To better understand the sequelae of inflammatory changes in r-mTBI, we performed next-generation RNA sequencing of CD11b+ microglia from our mouse model of chronic r-mTBI, and analyzed the temporal course of changes in microglial gene expression at 14, 90, and 180 days post-injury. We exposed animals to their injuries at 3 and 12 months of age, to explore the impact of age on TBI mediated microglial inflammation. We reveal multiple time-dependent and injury responsive changes in microglial gene expression profiles. In the young cohort, at acute stages of injury, we identified an increase in genes associated with chemokine and cytokine signaling, suggesting a role of microglia in the initiation of inflammation and the recruitment of peripheral immune cells, notably, neutrophils into the CNS. At 3 months post-injury we noted that microglia maintained their upregulation in several pro-inflammatory genes notably IFNγ and TNFά. We also noted a distinct change in lipid and lipoprotein metabolism indicative of a disease associated microglia signature. At 6 months post-injury we noted a distinct change in the metabolic profiles of isolated microglia with a downregulation in oxidative phosphorylation accompanied by a shift towards glycolytic

metabolism, indicative of chronically activated microglia. Further analysis of these transcriptomic changes identified PPARγ as a key upstream regulator driving these metabolic changes in microglia at chronic time points after injury, to confirm this we undertook a treatment study utilizing the potent PPARγ agonist Pioglitazone and analyzed its effects on glial pathology and the ability to reduce learning deficits following r-mTBI. Further analyses of the aged cohort are underway. Thus far, these data identify putative microglia specific targets with the potential to beneficially alter r-mTBI mediated neuroinflammation.

Disclosures: A.J. Pearson: None. **C. Ortiz:** None. **J. Ojo:** None. **F. Crawford:** None. **M. Browning:** None.

Digital Abstract Session

P146. Cellular and Molecular Mechanisms of Brain Injury and Disease

Program #/Poster #: P146.02

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant AG052460
NIH Grant NS113879
NIH Grant NS101167

Title: Characterization of neuroinflammatory markers in the fluid percussion injury model of traumatic brain injury and posttraumatic epilepsy

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Abstract: Traumatic brain injury (TBI) is a leading cause of morbidity and mortality, worldwide. Over 1.4 million TBIs result in 50,000 deaths, annually in the USA, and survivors frequently suffer long-term disability due to neuropsychiatric or neurological disorders. Studies in patients indicate that neuroinflammation, broadly defined as induction of inflammatory mediators, glial activation and oxidative stress in the brain following TBI, exacerbate the disease pathology promoting posttraumatic epileptogenesis. However, precise nature and temporal profile of epileptogenic neuroinflammation is not known. In this study, we utilized a well-characterized rostral parasagittal-fluid percussion injury (*rp*-FPI) rat posttraumatic epilepsy model to investigate the expression pattern of inflammatory mediators, oxidative stress, astrogliosis and microglial activation markers from 3h to 24h following FPI injury. Our results show FPI in rats results in increased mRNA expression of proinflammatory cytokines, enzymes, receptors, markers of oxidative stress and gliosis in cortex. However, there are notable differences in expression pattern among these markers. Specifically, the inflammatory mediators COX2, EP2, IL1β, IL6, TNFα, COX1, and mPGES1 were highly induced 3h post FPI, with expression returning to basal levels by 8h post-FPI for all but TNFα, which returned to basal

levels by 16h post FPI. Markers of oxidative stress (NOX2), astrogliosis (GFAP) and microglial activation (CD11b) started to show increased levels beginning 8h post FPI, with expression remaining elevated 24h after injury. Continuing our investigation at protein level, we observed a significant increase in expression of COX2 at 3h post FPI with levels staying significantly elevated until 24h post FPI. Our results indicate that the inflammatory pathways are induced first followed by gliosis and oxidative stress in FPI rats.

Disclosures: V. Rawat: None. **T. Ganesh:** None. **R. Dingledine:** None. **C.L. Eastman:** None. **R. D'Ambrosio:** None.

Digital Abstract Session

P146. Cellular and Molecular Mechanisms of Brain Injury and Disease

Program #/Poster #: P146.03

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS095850
NIH Grant NS105864
NIH Grant NS109585

Title: Sleep fragmentation following traumatic brain injury alters neuroinflammation and hippocampal neuronal activity

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Abstract: Traumatic brain injury (TBI) impairs the body's ability to restore homeostasis in response to stressors, reflecting dysregulation of the hypothalamic-pituitary-adrenal (HPA)-axis. We hypothesize that sleep fragmentation (SF) is a physiologically relevant stressor that engages the HPA-axis after TBI and upon a dysregulated stress response promotes increased neuroinflammation. To test this, male and female mice were given moderate lateral fluid percussion TBI or control sham-surgery and either left undisturbed or exposed to SF for 7 or 30 days. At 7 days post-injury (DPI), TBI increased cortical Iba1 labelling regardless of SF. NanoString nCounter analysis of ipsilateral cortex revealed that pro-inflammatory gene expression was decreased with sham SF but increased with TBI SF, indicating differential effects of SF with injury. Further, interferon-associated genes were uniquely increased in TBI SF mice compared to TBI control. TBI also increased hippocampal Iba1 labelling regardless of SF; however, this was associated with a striking increase in phosphorylated glucocorticoid receptor (GR) and CA1 neuronal activity in TBI SF mice, indicative of GR hypersensitivity in areas important for inhibitory HPA axis regulation. We next determined if these changes in inflammation and neuronal circuitry persist with chronic SF. At 30 DPI, TBI SF exacerbated cortical Iba1 labeling near the site of injury that was associated with increased expression of

genes encoding proteins and transcription factors that, when dysregulated, are associated with an exacerbated pro-inflammatory and neurodegenerative state. Hippocampal Iba1 labelling remained elevated 30 DPI following TBI; however, TBI SF mice displayed a suppression in CA1 neuronal activity compared to sham SF mice. This was associated with decreased neuronal activity in the paraventricular nucleus of the hypothalamus (PVN), indicating persistent HPA axis dysfunction with post-TBI SF. These data indicate that post-TBI SF is a physiologically relevant stressor that engages the dysfunctional HPA axis to differentially influence inflammation and neuronal responses sub-acutely and chronically after injury. Hippocampal circuitry seems to be particularly vulnerable to influences of post-TBI SF as the ipsilateral CA1 is highly activated at 7 DPI but then suppressed at 30 DPI and may influence behavioral recovery after TBI. Further elaboration of how post-TBI stressors, such as SF, influence outcome may be critical in promoting post-injury recovery.

Disclosures: **Z.M. Tapp:** None. **J. Kumar:** None. **S. Cornelius:** None. **A. Oberster:** None. **R. Atluri:** None. **K. Wticher:** None. **J. Velasquez:** None. **C. Bray:** None. **J.F. Sheridan:** None. **J.P. Godbout:** None. **O. Kokiko-Cochran:** None.

Digital Abstract Session

P146. Cellular and Molecular Mechanisms of Brain Injury and Disease

Program #/Poster #: P146.04

Topic: C.10. Brain Injury and Trauma

Support: NIH NS098170, JC and CS
Kentucky Spinal Cord and Head Injury Research Trust 16-3A, JC and CS
NeuroCures Foundation, Inc., JC

Title: Activation of hypoxia-inducible factor-1 alpha signaling and oxidative stress response in the developing mice after rotational acceleration-deceleration traumatic brain Injury

Authors: T. CHU[1], W. ZENG[1], N. MELLEN[2], Y.-P. ZHANG[3], L. B. E. SHIELDS[3], C. B. SHIELDS[3], **J. CAI**[1];
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Abstract: Shaken baby syndrome (SBS) or shaken impact syndrome is a form of abusive head injury (AHT), in which a child is subjected to severe repetitive rotational acceleration-deceleration (RAD) forces without blunt impact to the head. We previously reported that RAD injury (RADi) results in subdural/subarachnoid hemorrhage, brain-blood barrier (BBB) damage, parenchymal edema, global cerebral blood hypoperfusion, inflammatory response, neuronal degeneration, cognitive deficits, and anxiety-like behavior in the developing mouse brain. However, little is known about the underlying mechanisms and preventive/therapeutic interventions are lacking. Here we test the hypothesis that early ischemic hypoxia mediates the secondary RAD induced brain injury using a mouse RADi model. Anesthetized neonatal mice at

12 days after birth were subjected to repetitive head rotations with a +55°/-35° angle and 60 fire-fashion hits in sagittal plane. The cerebral hypoxia-inducible factor-1α (HIF-1α) accumulates in cortex at least during the first 3 days after injury, probably due to the inhibited expression of HIF prolyl hydroxylase domain protein 1 (PHD1). The expressions of HIF-1 target molecules, including glucose transporter 1 (Glut 1), nuclear factor like 2 (Nrf2), and heme oxygenase-1 (HO-1), are greatly changed. Meanwhile, nitrotyrosine (3-NT, marker for indirectly detecting $ONOO^-$) and 4-hydroxynonenal (4-HNE, marker for lipid peroxidation) are significantly elevated in RADi cortex as early as 1hr after injury in the developing brain. At the later stage, HIF-1α is significantly down-regulated with the activation of p38 mitogen-activated protein kinase (MAPK). In addition, strong intracellular calcium influx is detected in layer II/III of cortex and hippocampal area starting at 24 hours after RADi, which declines after 7 days after injury. Furthermore, the calcium network-level recordings on ventral hippocampal slices indicate that Ca^{2+} overload in RADi brain induces the electrophysiological changes, which may lead to neurocognitive impairment. These findings suggest that hypoxia-activated HIF-1α signaling and oxidative stress may play a vital role in SBS-related neurological and/or psychological sequelae.

Disclosures: **T. Chu:** None. **W. Zeng:** None. **N. Mellen:** None. **Y. Zhang:** None. **L.B.E. Shields:** None. **C.B. Shields:** None. **J. Cai:** None.

Digital Abstract Session

P146. Cellular and Molecular Mechanisms of Brain Injury and Disease

Program #/Poster #: P146.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant UG3TR002188
MJFF Grant 16561

Title: Development of a Human Brain-Chip Model to Study Neuroinflammatory Diseases

Authors: *I. PEDIADITAKIS, K. KODELLA, D. MANATAKIS, W. TIEN-STREET, C. HINOJOSA, G. HAMILTON, L. EWART, K. KARALIS;
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Abstract: Species differences in brain function and blood-brain barrier often preclude accurate extrapolation from animal models to human patients. There is an unmet need for human relevant systems that can recreate key aspects of brain physiology and pathophysiology of common diseases. We are developing a human Brain-Chip to model neuroinflammation, a hallmark of many neurodegenerative diseases, to enable studies on mechanistic aspects of neural pathology and disease progression. We provide evidence that this complex human Brain-Chip model can support co-culture and establishment of extensive interconnection between human iPSC-derived neurons and primary glial cells (astrocytes and microglia). Human iPSC-derived brain endothelial cells successfully maintained at the vascular channel of the Brain-Chip in the presence of fluidic shear stress while exhibiting hallmark features of the human blood-brain

barrier, such as the formation of specific tight junctions and minimal barrier permeability. Exposure to inflammatory triggers (e.g., TNF-α) or toxic protein oligomeric species (e.g., alpha-synuclein), resulted in neuronal death, glia activation, increased secretion of the corresponding proinflammatory cytokines, and a compromised barrier function. In summary, our current findings demonstrate that human Brain-Chip could support the development of models for the study of neuroinflammation and blood-brain barrier dysfunction in neurological disorders.

Disclosures: I. Pediaditakis: A. Employment/Salary (full or part-time):; Emulate Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emulate Inc. **K. Kodella:** A. Employment/Salary (full or part-time):; Emulate Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emulate Inc. **D. Manatakis:** A. Employment/Salary (full or part-time):; Emulate Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emulate Inc. **W. Tien-Street:** A. Employment/Salary (full or part-time):; Emulate Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emulate Inc. **C. Hinojosa:** A. Employment/Salary (full or part-time):; Emulate Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emulate Inc. **G. Hamilton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emulate Inc. **L. Ewart:** A. Employment/Salary (full or part-time):; Emulate Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emulate Inc. **K. Karalis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emulate Inc..

Digital Abstract Session

P146. Cellular and Molecular Mechanisms of Brain Injury and Disease

Program #/Poster #: P146.06

Topic: C.10. Brain Injury and Trauma

Support: PTSD Systems Biology Consortium

Title: Overlapping and distinct molecular signatures between recent and chronic combat PTSD: clinical and multi-omics integration

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Abstract: Introduction: Post-Traumatic Stress Disorder (PTSD) is one of the major disorders affecting many veterans and active duty soldiers. However, objective diagnosis and effective

therapies for PTSD are still unmet needs. Identification of altered molecular signatures and signaling pathways could aid in diagnosis, treatment decision-making, and risk evaluation. **Methods:** In the search for molecular basis and markers for PTSD, we carried out blood based proteomic, metabolomic and epigenomic (genome wide methylation and microRNA) assays. Multi-omics assays were carried out on samples collected from well characterized cohorts of 340 veterans with chronic PTSD (40F – 21P-/19P+; 300M – 148P-/152P+) and 180 active-duty service-members with recent PTSD (21F – 13P-/8P+; 159M – 44P-/47P+/68 subthreshold). Vitals and clinical assessments, blood chemistry and endocrine assays were also carried out. Molecular and clinical datasets from each cohort were computationally integrated. **Results:** We identified both inhibited and activated molecular pathways that were persistent and distinct between the two-cohort participants with recent and chronic combat-PTSD. Cellular processes and pathways that are important in wound healing, vasculature development (angiogenesis) and coagulation were persistently inhibited across cohorts. On the other hand, pathways and processes that are important in inflammatory response, age-rage signaling, and oxidative stress were activated more significantly in veterans compared to active duty participants. Molecules and pathways that were consistently altered across cohorts were correlated with severity and chronicity of the different facets of PTSD symptoms. **Discussion:** Significantly altered multimodal molecular signatures convergently indicate impaired wound healing, and speculatively with an implication for stress caused vasculature damage with delayed healing, which in turn acting as the basis for the persistence of the disorder. **Conclusion:** We have identified altered molecular and pathway signatures that can potentially link stress related disorders with that of impaired wound healing and vascular morphogenesis. Our observations support that inflammation and oxidative stress potentially dysregulate multiple biological processes, and hence might be involved in the chronicity of PTSD. Profiled multimodal molecules and pathways were correlated with core symptoms of PTSD and its somatic comorbidities. **Disclaimers:** The views, opinions, and findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision.

Disclosures: S. Muhie: None. **A. Gautam:** None. **M. Jett:** None. **R. Yang:** None. **R. Hammamieh:** None. **P. Consortium:** None.

Digital Abstract Session

P147. Mechanisms of Axonal Degeneration and Regeneration After Injury

Program #/Poster #: P147.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Adelson Medical Research Foundation (LB)
MTEC (LB: J.L. Goldberg)
Viral Vector Core (P30EY012196)
NIH IDDRC imaging core (HD018655)
NINDS P30 Core Center grant #NS072030

Title: Chemokine CCL5 promotes optic nerve regeneration and mediates the major effects of CNTF gene therapy

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Abstract: Ciliary neurotrophic factor (CNTF) is a leading therapeutic candidate for several ocular diseases and induces optic nerve regeneration in animal models. Paradoxically, however, although CNTF gene therapy promotes considerable regeneration, the recombinant CNTF protein (rCNTF) has little effect (1). Because intraocular viral vectors induce inflammation and because CNTF is an immune modulator, we investigated whether CNTF gene therapy acts indirectly through other immune mediators. The beneficial effects of CNTF gene therapy were unaffected by deleting the CNTF receptor CNTFR-alpha (CNTFRα) in retinal ganglion cells (RGCs), the projection neurons of the retina, but were diminished by immune depletion of neutrophils (anti-Ly6G) or by genetically suppressing monocyte infiltration (deletion of CCR2) (2). CNTF gene therapy increased expression of C-C motif chemokine ligand 5 (CCL5) in immune cells and retinal glia, and recombinant CCL5 induced extensive axon regeneration. Conversely, CRISPR-mediated knockdown of the cognate CCL5 receptor (CCR5) in RGCs or treating wild-type mice with a CCR5 antagonist (DAPTA) strongly repressed the effects of CNTF gene therapy. Thus, CCL5 is a previously unrecognized, potent activator of optic nerve regeneration and mediates the major effects of CNTF gene therapy.

Disclosures: **L. Xie:** None. **Y. Yin:** None. **L.I. Benowitz:** None.

Digital Abstract Session

P147. Mechanisms of Axonal Degeneration and Regeneration After Injury

Program #/Poster #: P147.02

Topic: C.10. Brain Injury and Trauma

Support: R01EY027347
R01EY030883
R01EY031779
K99EY031742

Title: Polycomb group protein Ezh2 promotes mammalian axon regeneration in central nervous system

Authors: ***X.-W. WANG**[1], C. ZHANG[1], M.-W. HU[1], S.-G. YANG[1], A. KOSANAM[1], J. QIAN[1], C.-M. LIU[1,2], F.-Q. ZHOU[1];
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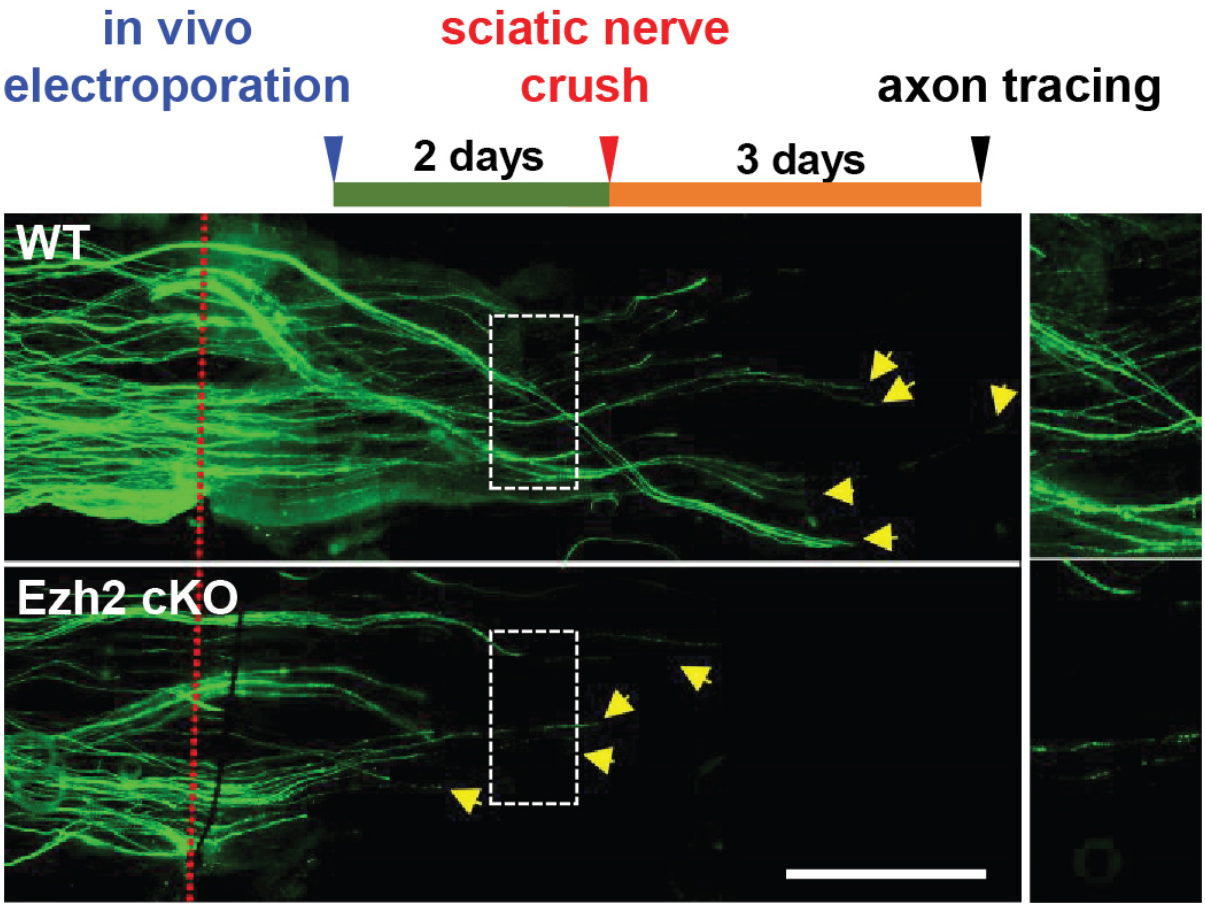
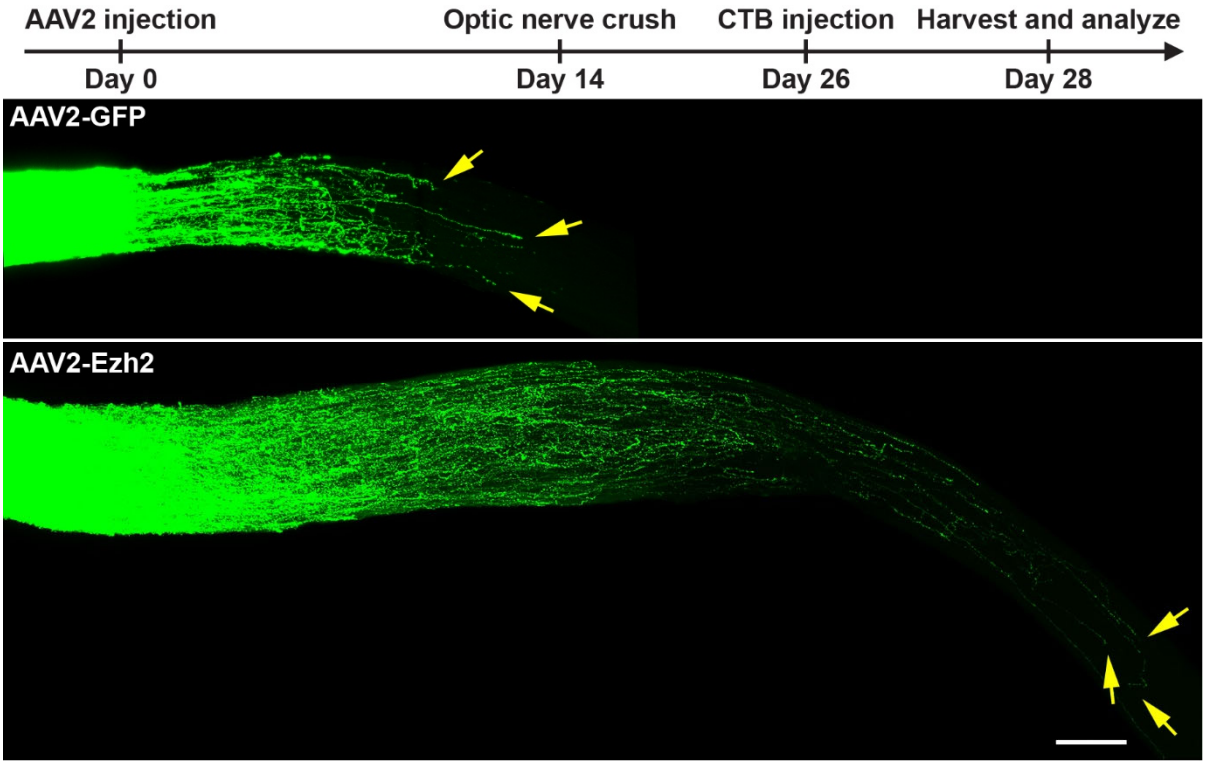
Abstract: In mammals, neurons in the central nervous system (CNS) gradually lose their axon growth capacity as they mature. On the contrary, mature neurons in the peripheral nervous system (PNS) still possess such a capacity and can spontaneously regenerate their axons upon axonal injury by initiating a regenerative response. Emerging evidence suggests that the switch from non-regenerative state to regenerative state of injured PNS neurons largely involves the epigenetic regulation of chromatin accessibility. Here we first investigated the role of Ezh2, a histone methyltransferase that adds methyl groups onto lysine 27 on histone H3 (H3K27) to suppress gene transcription, in PNS axon regeneration, and then tested if its modulation could promote CNS axon regeneration. We found that Ezh2 level was upregulated in mouse lumbar 4 and 5 dorsal root ganglia (L4/5 DRGs) 3 days following sciatic nerve axotomy. Functionally, Ezh2 knockdown/knockout in DRG neurons impaired axon growth speed *in vitro*. Consistently, knockdown/knockout of Ezh2 in L4/5 DRGs or conditional knockout of Ezh2 in sensory neurons impaired sensory axon regeneration *in vivo*. In addition, Ezh2 overexpression in retinal ganglion cells (RGCs) both before and after optic nerve injury significantly promoted axon regeneration. RNA-seq of RGCs enriched by fluorescence-activated cell sorting revealed that Ezh2 might promote axon regeneration by suppressing transcription of various neurotransmitter transporters.





Disclosures: **X. Wang:** None. **C. Zhang:** None. **M. Hu:** None. **S. Yang:** None. **A. Kosanam:** None. **J. Qian:** None. **C. Liu:** None. **F. Zhou:** None.

Digital Abstract Session

P147. Mechanisms of Axonal Degeneration and Regeneration After Injury

Program #/Poster #: P147.03

Topic: C.10. Brain Injury and Trauma

Title: NMN, NAD^+, and SARM1 signaling in Wallerian degeneration of mammalian axons: temporal dynamics and interactions

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Abstract: Wallerian degeneration (WD) is an evolutionary conserved axon self-destruction program that is activated in mechanically and/or metabolically compromised axons and as such contributes to diverse neurological disorders, including traumatic brain injury, peripheral neuropathies and possibly neurodegenerative diseases. WD has several and not fully understood decision points including the degradation of nicotinamide mononucleotide adenylyltransferase (NMNAT2), the built up of its substrate NMN, and the activation of the NAD^+ degrading sterile α and Toll/interleukin-1 receptor (TIR) motif containing 1 (SARM1) protein, all of which are

necessary for the eventual axon fragmentation hours or days after injury. Yet, the time course of these events and critical interactions are still unclear. Here we utilized an *in vitro* mouse dorsal root ganglion axotomy model to interrogate the timing and interplay between two critical molecular pathways/cascades (MAPK stress cascade and SARM1 pathway) by pharmacological means, including inhibition of the NMN-synthesizing enzyme NAMPT and the NMNAT2-degrading MAPK stress pathway. Axons were grown in microfluidic devices or drop cultures, cut with a razor blade and treated with drugs or vehicle at different time points. Images of axons were taken at different intervals and the degeneration index was calculated. Axonal preparations at different time points post-injury were also used for metabolomic and proteomic profiling of injury-related signals. We found that NMN plays a critical role in SARM1-dependent axon fragmentation within a time window corresponding to 2-4 hours post-injury. We also found that the protective effect of lowering NMN with NAMPT inhibition also depends on axonal NAD^+ levels and is enhanced by nicotinic acid riboside supplementation. Moreover, we found a temporal correlation of these events with NMNAT2 degradation by the MAPK pathway. In conclusion, we demonstrated that the decision to commit to WD is temporally separated from the time of injury, evolves within a narrow window determined by the degradation of NMNAT2, and depends on the effects of both NMN and NAD^+ on SARM1 signaling. The existence of a time window of commitment and the availability of NMN-lowering compounds, i.e. NAMPT inhibitors, may have translational significance for the treatment of traumatic and other axonopathies.

Disclosures: **A. Alexandris:** None. **J. Ryu:** None. **Y. Wang:** None. **L. Rajbhandari:** None. **A. Venkatesan:** None. **V. Koliatsos:** None.

Digital Abstract Session

P147. Mechanisms of Axonal Degeneration and Regeneration After Injury

Program #/Poster #: P147.04

Topic: C.10. Brain Injury and Trauma

Support: NIH 1R21NS095158
NJ CBIR16PIL026

Title: Neuronal network changes after in vitro stretch injury

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Abstract: To understand acute changes in neuronal network dynamics after traumatic injury, Ca^{2+} imaging was used to record individual neuron responses during spontaneous and triggered activity in cultures of rat neocortical neurons and glia in the presence of bicuculline. Triggered activity was initiated by single-cell photolysis of caged-glutamate (photostimulation) to reveal

connections, or links, between neurons. We integrated this technique with a stretch injury model that mimics mild traumatic brain injury (mTBI).Parameters were derived to quantify changes in network dynamics post-injury at two different strain and strain rate levels, 45%, 20 s^{-1} (n = 18 cultures) or 70%, 35 s^{-1} (n = 13). Compared to non-injured controls (n = 10), stable links, number of responsive neurons to photostimulation, and participation of neurons in and amplitude of spontaneous, synchronous activity (i.e. bursting activity) was significantly decreased, while lost links were significantly increased after injury at both strain levels. These results (Table 1) showed that network activity was acutely depressed after injury, consistent with prior reports, but more prominent was the appearance of network instability characterized by variability, shown as interquartile range (IQR), in link properties when compared to controls. Additional experiments were undertaken to determine if network instability was due to changes in neuron excitability and/or synaptic transmission properties. Ca^{2+} response amplitude of the photostimulated neuron, a measure of cell excitability, was significantly decreased after injury in a strain-dependent manner. Furthermore, variability of link properties between some neurons was consistent with a depression of synaptic transmission. These results suggest that acute mTBI decreases cell excitability acutely after injury and alters synaptic transmission between neurons. Overall, our novel approach integrating photostimulation, Ca^{2+} imaging and stretch injury enables us to investigate drivers of injury-induced neuronal network changes. In future work, this approach will be used to investigate interventions that can confer neuroprotection to the network after injury.

Table 1. Percent Changes in Network Parameters after Injury

Parameter	**No Injury**	**Injury: 45%, 20 s^{-1}**	**Injury: 70%, 35 s^{-1}**
	Median (%) ± IQR	Median (%) ± IQR	Median (%) ± IQR
Parameters from Spontaneous Activity			
Amplitude	15.2 ± 15.3	-30.7 ± 34.9 **	-61.6 ± 60.4 **
Frequency	-22.5 ± 25.5	-12.1 ± 32.0	-60.7 ± 43.2
Participation Rate	2.6 ± 4.3	-19.4 ± 23.9 **	-29.1 ± 29.8 **
Parameters from Photostimulation			
Total Links	11.3 ± 16.3	11.5 ± 59.2	-29.2 ± 89.4
Responsive Neurons	-0.7 ± 5.7	-8.9 ± 18.5 **	-39.1 ± 43.1 **
***k** (links per responsive neuron)*	1.0 ± 28.9	26.9 ± 55.9	-5.8 ± 59.7
Stable Links	81.3 ± 9.8	64.3 ± 21.4 **	53.6 ± 42.9 **
Lost Links	18.7 ± 9.8	35.7 ± 21.4 **	46.4 ± 42.9 **
New Links	30.7 ± 20.1	47.8 ± 44.5	18.5 ± 37.5
** Significantly different from controls (no injury) , post-hoc $p < 0.0167$			

Disclosures: **C. Rojvirat:** None. **T. Nguyen:** None. **J. Berlin:** None.

Digital Abstract Session

P148. Histology and Cellular Markers of Traumatic Brain Injury

Program #/Poster #: P148.01

Topic: C.10. Brain Injury and Trauma

Support: Chronic Brain Injury (CBI) Pilot Award Program

Title: Effects of reproductive experience on acute neuroimmune outcomes following traumatic brain injury in mice

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Abstract: Intimate partner violence is a leading cause of TBI in women, with pregnant women or women with children in the home at the highest risk for acquiring a TBI (Sharps et al., 2007; Peek-Asa et al., 2017; Valera et al., 2018). Although pregnancy induces profound acute and long-term physiological changes (Duarte-Guterman et al, 2019), the consequences of such changes on brain injury susceptibility and outcomes are unclear. Here, we examined the effects of parity (previous pregnancy and maternal experience) on the neuroinflammatory response to TBI induced by lateral fluid percussion injury (FPI). Multiparous females (2-3 pregnancies and motherhood experiences) and age-matched nulliparous (no previous pregnancy and motherhood experience) females were administered either FPI or sham injury. Brain tissue was collected 3 days post-injury (DPI). Our results show that cortical Iba1 immunolabeling increased following injury independent of parity. The number of deramified Iba1+ cells near the site of injury did not differ between TBI mice. Similarly, TBI increased cortical GFAP immunolabeling 3 DPI. While no differences in cortical CD68 immunolabeling were identified between TBI groups, an interaction between parity and injury was evident in cortical CD45 labeling. Specifically, the number of $CD45^+$ cells near the site of injury was higher in nulliparous compared to multiparous animals. Together, these data confirm that FPI induces a cortical immune response in brain resident microglia and astrocytes and show that this effect is independent of parity. Notably, these are the first data to demonstrate that previous parity may act as a factor in attenuating leukocyte recruitment to the site of injury following TBI. Together these data suggest that previous pregnancy and maternal experience can influence the acute neuroinflammatory response to TBI.

Disclosures: R. Gilfarb: None. **E. Lemanski:** None. **J. Velasquez:** None. **Z. Tapp:** None. **S. Cornelius:** None. **O.N. Kokiko-Cochran:** None. **B. Leuner:** None.

Digital Abstract Session

P148. Histology and Cellular Markers of Traumatic Brain Injury

Program #/Poster #: P148.02

Topic: C.10. Brain Injury and Trauma

Support: Swiss National Science Foundation number P2ZHP3_191255 (NLA)

Title: Traumatic brain injury-like pathology in a muskox brain and implications for the natural occurrence of TBI

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Abstract: Muskoxen (*Ovibos moschatus*) are large tundra-dwelling bovids that can strike heads at speeds around 30 mph (48 Km/h). Their wide horn base armors the skull against such impacts, however, it is unknown if this successfully prevents brain trauma. Our research explores the brain and skull anatomy of combative bovids, focusing on whether these animals sustain traumatic brain injury (TBI) after head-butting, and if not, what features most likely provide protection. Achieving a better understanding of brain injury in animals with extreme adaptations will provide insight to develop strategies for the reduction of traumatic brain injury in humans. In a preliminary study, the brain of an adult male muskox was collected after humane euthanasia and preserved in formalin. It had been observed clashing heads in the wild moments before its death. Brains from two female muskoxen were used as comparisons. The brains were MR scanned and the right prefrontal cortex was examined histologically for evidence of traumatic brain injury using a variety of exploratory histological protocols to investigate abnormalities in neurons, microglia, astrocytes, and blood vessels. Specifically, Tau protein, a biomarker found in the cerebrospinal fluid and in neurodegenerative lesions, was used to detect any cellular consequence of brain trauma related to chronic or acute head clashing. Microglial morphology appeared normal and the MRI scan revealed no abnormal neuropathological changes. However, in the bull's brain, tau antibodies AT8 and CP13 highlighted the presence of neuronal plaques and damaged neurons on the surface of the white matter and in sulcus depths. The presence and distribution of these abnormalities resembles that of early chronic traumatic encephalopathy (CTE) in humans. Although our small sample size does not allow us to make sweeping assumptions, these preliminary findings lead us to believe that muskoxen may suffer from chronic or acute brain trauma after head clashing despite their imposing headgear. If TBI occurs frequently in these animals, it has consequences for sexual selection and evolutionary strategies. Furthermore, if widespread, this type of injury would be an interesting as a model to compare to human TBI and CTE.

Disclosures: N.L. Ackermans: None. **T. Williams:** None. **A. Alipour:** None. **B. Priti:** None. **M. Varghese:** None. **J. Reidenberg:** None. **P.R. Hof:** None.

Digital Abstract Session

P148. Histology and Cellular Markers of Traumatic Brain Injury

Program #/Poster #: P148.03

Topic: C.10. Brain Injury and Trauma

Support: PAPIIT IN228320

Title: The effect of darkness on recovery from traumatic brain injury

Authors: R. MARTÍNEZ-TAPIA[1], *F. E. ROJO[1], I. M. ANCONA-MARTÍNEZ[2], L. NAVARRO[1], C. DANIELA NAYELI[2], S. NIÑO-CARVAJAL[2], D. MONROY-MATAMOROS[2];
[1]Phisiology, Facultad de Medicina UNAM, Mexico City, Mexico; [2]Escuela Nacional Preparatoria 8, UNAM Cd Mx, Mexico

Abstract: The effect of darkness on recovery from traumatic brain injury (TBI).Ancona Martínez Isis Monserratt[1], Córdova Balcázar Dania Nayeli[1], Monroy Matamoros Danna Paola[1], Niño Carbajal Salvador Usiel[1], Navarro Luz[2], Martínez Tapia Ricardo de Jesús[2] and Estrada Rojo Francisco[2]1.- Escuela Nacional Preparatoria, Plantel No.5 “José Vasconcelos” UNAM2.- Lab. de Neuroendocrinología, Departamento de Fisiología, Facultad de Medicina, UNAM Traumatic brain injury (TBI) is a severe health problem that involves people's death and disability, with high costs for society and health systems.When a TBI occurs, brain mechanisms are activated, leading to further damage to the brain and mechanisms that protect it from injury (neuroprotection); the latter should be favored to establish a better and speedy recovery. Several investigations look for therapeutic strategies that increase neuroprotection. Recently in animal models, it has been reported that exposure to darkness for several days allows the recovery of damaged visual pathways, even at the visual cortex level. This work proposes to explore this approach as a therapy for recovery from TBI.Mus musculus, CD1 strain (50 gr) were used, acclimatized first for 8 days to a 12:12 light/dark cycle with lights on at 8 AM, later they were randomly divided into 4 groups: 1, control 12:12 light/dark; 2, control in continuous darkness; 3, TBI 12:12 light/dark; 4, TBI in continuous darkness. These conditions were maintained for 10 days after TBI, during which food intake, weight, and water consumption were measured, and a neurobehavioral scale was applied. The TBI was produced by means of a "weight drop" type model in the motor cortex with lateral coordinates of 2 mm and anteroposterior coordinates of -1.4 mm. Subsequently, the individuals were sacrificed, and their brains were extracted to make histological sections stained with Hematoxylin-Eosin. Cell counting was made in the damaged area. Cell counting, behavior, and metabolic data were statistically analyzed. Our data show that the TBI maintained in continuous darkness had a better behavioral and histological recovery than the 12:12 light/dark cycle TBI group. Project supported by PAPIIT IN228320

Disclosures: R. Martínez-Tapia: None. **F.E. Rojo:** None. **I.M. Ancona-Martínez:** None. **L. Navarro:** None. **C. Daniela Nayeli:** None. **S. Niño-Carvajal:** None. **D. Monroy-Matamoros:** None.

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.01

Topic: C.10. Brain Injury and Trauma

Support: CDMRP GW120037
CDMRP GW180106
CDC-NIOSH Intramural

Title: Projectile Concussive Impact as a Preclinical Model for Traumatic Brain Injury

Authors: *L. T. MICHALOVICZ, K. A. KELLY, J. P. O'CALLAGHAN;
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Abstract: Traumatic brain injury (TBI) is as a major cause of death and disability experienced by nearly 3 million people per year. These injuries can be experienced in any environment and can result from falls, vehicular accidents, or from being struck by or against an object. While TBIs can be severe and/or fatal, the majority of injuries are considered to be mild. However, even mild TBI has the potential to have long-lasting neurological effects including headaches, cognitive/memory impairments, mood dysfunction, and fatigue. By using animal models of TBI, we can investigate the underlying pathophysiology that can lead to neurological dysfunction in the absence of serious trauma (i.e. penetrating injuries). Here, we used a projectile concussive impact (PCI) model of mild TBI developed at Walter Reed Army Institute of Research, where a ball bearing is propelled at the head by air pressure. While several studies using PCI have employed helmets to mitigate direct damage from impact, we needed to utilize a non-helmeted model of PCI-induced TBI to evaluate the protective nature of different helmet materials for the mitigation of work-related TBI. Adult male Sprague-Dawley rats were evaluated for neurobehavioral, neuroinflammatory and neural damage end points up to 72 hours post-TBI using different types of ball bearings. Animals that received TBI using aluminum or steel ball bearings took approximately 1.7 and 7.3 times longer, respectively, to recover from anesthesia compared to sham controls. While rats impacted with aluminum were behaviorally normal even 1 hr following TBI, those impacted with the steel ball bearing had an impaired neurobehavioral score for up to 24 hrs post-TBI which correlated with the presence of subdural hematoma, neurodegeneration, and significant markers of neuroinflammation including increased brain inflammatory cytokine mRNA expression and gliosis. Interestingly, while rats impacted with the aluminum ball bearings did not display markers of neurodegeneration or changes in brain cytokine mRNA expression, they did display minor activation of astrocytes and microglia. These data support the use of this non-helmeted variation of the PCI model for the future evaluation of helmet materials and demonstrates that even minor TBI can produce measurable brain cellular changes.

Disclosures: L.T. Michalovicz: None. **K.A. Kelly:** None. **J.P. O'Callaghan:** None.

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.02

Topic: C.10. Brain Injury and Trauma

Support: VA Grant I01RX002334

Title: Proteomic and Phosphoproteomic Identification of Upregulated Mechanisms in Two Mouse Models of r-mTBI and in Human CTE, and Their Response to Delayed Treatment with the Potential Therapeutic Anatabine

Authors: *A. MORIN[1,2], R. DAVIS[1], T. DARCEY[1], B. MOUZON[1,2], M. BROWNING[1], N. SALTIEL[1], S. FERGUSON[1,2], D. PARIS[1,2], M. MULLAN[1], F. CRAWFORD[1,2];
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Abstract: Heterogeneity of mild traumatic brain injury (mTBI) is recognized as a factor that hinders the development of efficient therapeutic strategies. At the preclinical phase, this heterogeneity is driven by high variability across animal models of mTBI; at the translational stage - by differences between animal and human physiological responses. Our study attempted to bridge these gaps by conducting phospho- and total proteomics of cortical tissue from two well-characterized mouse models of repetitive mTBI (r-mTBI) and identifying the overlapping mechanisms. Mice with 5 (over 9 days) or 24 (over 90 days) repetitive closed-head hits were euthanized at 6 months after the last injury to target chronic aspects of mTBI pathology. Their proteomic profiles were further compared to results from human samples of chronic traumatic encephalopathy (CTE, n=11) (Proteome Central: PXD007694). We identified 5 biological functions that were upregulated in the two preclinical models and all human samples of CTE - *Transport, RNA splicing, Axonogenesis, Glycolytic processes, GTPase signaling*. In the second part of our study, we introduced a delayed treatment with an anti-inflammatory compound anatabine, an agonist of α7 nicotinic acetylcholine receptors, which has previously exhibited therapeutic properties in models of r-mTBI and neurodegeneration. Mice of both r-mTBI models (5 and 24 hits) were treated with anatabine starting at 6 months after the last injury to explore the therapeutic potential of its late administration, to mimic typical treatment interventions in human mTBI. Anatabine treatment was successful at suppressing the 5 biological processes triggered by r-mTBI. The delayed treatment with anatabine also improved cognitive functions, however the relationship of this to proteomic responses requires additional research. Identification of the mechanism of action of anatabine in these models will identify novel therapeutic targets for the chronic consequences of r-mTBI. Overall, our study revealed 5 mechanisms common to two preclinical models of r-mTBI and to human CTE that could be claimed to be validated targets for further investigation.

Disclosures: A. Morin: None. **R. Davis:** None. **T. Darcey:** None. **B. Mouzon:** None. **M. Browning:** None. **N. Saltiel:** None. **S. Ferguson:** None. **D. Paris:** None. **M. Mullan:** None. **F. Crawford:** None.

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.03

Topic: C.10. Brain Injury and Trauma

Support: NINDS R56-NS-090311
NIA R01-AG-051902
NINDS P30-NS-045758
P30-CA-016058
T32-DE-014320

Title: Tbi-induced functional deficits associated with inflammation are augmented by secondary immune challenges and ameliorated by forced turnover of microglia

Authors: *C. E. BRAY[1], K. G. WITCHER[1], D. ADELKUNLE-ADEGBITE[1], E. HANS[1], F. ZHAO[1], T. CHUNCHAI[3], J. E. DZIABIS[4], Z. M. TAPP[1], C. ASKWITH[1], D. S. EIFERMAN[2], J. P. GODBOUT[1], J. P. GODBOUT[1];
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Abstract: Traumatic brain injury (TBI) induces microglia mediated neuroinflammation that leads to acute symptoms and dysfunction. Though acute complications often resolve, cognitive and psychiatric impairments may develop or persist years after injury. Chronic microglial priming/reactivity may be a major issue that sets the stage for exaggerated responses to secondary challenges that augment inflammation and neuropathology. Our goal was to induce forced turnover of microglia after TBI and determine the degree to which reactive microglia profiles were reversed. Here, mice were injured by midline fluid percussion injury and 7 d later microglia were subjected to forced turnover. At 30 dpi, cortical gene expression was determined using NanoString's Neuropathology gene expression assay (760 genes). Many inflammatory genes that persisted at 30 dpi in the cortex (C1qc, Tlr2) were reversed following microglia turnover. Moreover, microglia repopulation attenuated TBI associated astrocyte gene expression (Aqp4, Gfap, S100b). Deficits in neuronal connectivity (N1 and N2 compound action potentials) in the corpus callosum were rescued by microglia forced turnover. Cognitive dysfunction 30 dpi was reversed by microglia turnover. Additionally, repopulation of microglia eliminated chronic TBI-induced depressive-like behavior. Next, we investigated if functional responses to a secondary immune challenge through intraperitoneal injection of LPS were attenuated. As expected, LPS caused exacerbated sickness behavior 30 dpi in mice subjected to TBI. Forced Microglia turnover after TBI alleviated prolonged sickness behaviors associated with microglial priming. Together, these data indicate that post-injury turnover of microglia attenuates persistent inflammatory effects and functional deficits sustained by TBI.

Disclosures: **C.E. Bray:** None. **K.G. Witcher:** None. **D. Adelkunle-Adegbite:** None. **E. Hans:** None. **F. Zhao:** None. **T. Chunchai:** None. **J.E. Dziabis:** None. **Z.M. Tapp:** None. **C. Askwith:** None. **D.S. Eiferman:** None. **J.P. Godbout:** None. **J.P. Godbout:** None.

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.04

Topic: C.10. Brain Injury and Trauma

Support: Phoenix Children's Hospital Mission Support
Barrow Neurological Institute at Phoenix Children's Hospital

Title: Mice born to mothers with gravida traumatic brain injury have distorted brain circuitry and altered immune responses

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Abstract: Intimate partner violence (IPV) is a world-wide epidemic that affects 1 in 4 women in their lifetime. The COVID-19 pandemic has restricted personal movement and confined people to their homes, which elevated IPV incidence and severity. As a result of IPV, upwards of 90% of victims receive assaults directed towards the head, neck, or face that likely induce traumatic brain injury (TBI). And, incidence and prevalence of IPV rises for pregnant women, with unknown consequences on fetal development. Here, we studied the impact of gravida TBI (gTBI) on cortical circuitry, cognition, depressive- and anxiety-like behavior, inflammation, and gut histology on the offspring of pregnant mice who suffered a TBI. We expected that gTBI would lead to altered cognition, increased depressive- and anxiety-like behavior, susceptibility to infection, dysbiosis in gut microbiome, and distorted brain circuitry in offspring. Pregnant dams received either diffuse TBI or sham injury 12 days post-coitum. After giving birth, a subset of mixed-sex pups from TBI and sham control mothers were assessed for cortical circuitry using laser scanning photostimulation (LSPS) at post-natal day (PND) 28. All other pups were assessed for cognitive, anxiety-like, and depressive-like behaviors from PND30-80. At PND80, pups received LPS (1 mg/kg, i.p.) to induce an immune response and blood was drawn 6hrs post-LPS. After 24hrs, pups were euthanized and blood and tissue were harvested. Litter size was similar between gTBI and control offspring. Male gTBI offspring had significantly lower body weights at weaning compared to male control offspring. LSPS functional circuit mapping revealed significantly weaker intralaminar connectivity onto layer 5 pre-frontal pyramidal neurons in male gTBI offspring compared to male control offspring. At PND42 and PND58, circulating neutrophil and monocyte populations were significantly lower in male gTBI offspring than male control offspring. And, after an LPS injection, gTBI offspring had significantly smaller

neutrophil populations. Analysis of gut histology failed to reveal significant differences in mucosal layer thickness. Moreover, there were no overt behavioral differences on depressive-like or cognitive behavior tests. However, during the open field (OF) task to measure anxiety-like behaviors, male gTBI offspring spent more time in the OF center and crossed into the OF center more often than male controls. This study is the first to show that gTBI in mice leads to altered cortical functional connectivity and altered immune response in offspring. The social impact of gTBI necessitates further investigation to aid recovery in mothers and children.

Disclosures: J.B. Ortiz: None. **M. Saber:** None. **L.M. Rojas:** None. **X. Ma:** None. **B.R. Tallent:** None. **P.D. Adelson:** None. **S. Qiu:** None. **R.K. Rowe:** None. **J. Lifshitz:** None.

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.05

Topic: C.10. Brain Injury and Trauma

Support: CIHR grant 1422720
CIHR-GSK grant 387577
Dr. Adil Nazarali Multidisciplinary Scholarship

Title: Characterizations and Targeting of the Endocannabinoid System in Traumatic Brain Injury.

Authors: *T. BLACK;
Univ. of Saskatchewan.

Abstract: Traumatic Brain Injuries (TBI) are considered one of the leading causes of death and disability worldwide. One emerging area of TBI research is the involvement of the endocannabinoid system (ECS) in response to brain injury. The ECS is modulated by exogenous cannabinoids such as Δ^9-tetrahydrocannabinol (THC) found in *Cannabis sativa.* THC is a partial agonist of both cannabinoid receptors CB1R and CB2R. CB1R activation contributes to analgesia and anxiolytic effects, whereas CB2R activation reduces inflammation. In the wake of *Cannabis* legalization in Canada, and as awareness surrounding post-traumatic complications increases, the need for research examining this intricate relationship between exercise, the ECS, and TBI is apparent. To date there has been a lack of research into the effects of TBI and exercise on the ECS, pre-clinical or otherwise. We **hypothesized that treatment of rats subjected to TBI with THC post-injury will restore locomotor function and improve behavioral and molecular profiles of injured rats.** **Methods:** 4-5 Sprague-Dawley rats of both sexes aged 8-12 weeks at the time of TBI were randomly assigned to: 1) No TBI + Vehicle; 2) No TBI + 1 mg/kg THC *i.p.*; 3) 300 g weight drop TBI + Vehicle; or 4) 300 g weight drop TBI + 1 mg/kg THC *i.p*. Rats were trained on the rotarod 4 days pre-injury, and tested for 7 days post-injury, with a break from rotarod at days 5 and 6. Behavioral and physiological changes were assessed using the tetrad battery: catalepsy, body temperature, open-field, and antinociception.

Y-maze was used to assess spatial learning and memory. Inflammatory cytokine profiles and CB1R receptor quantity were performed post-mortem on samples from the cortex. **Results:** 1 mg/kg of THC decreased male, but not female, rotarod performance between TBI treatment groups, and non-TBI controls. However, THC had no significant effect on catalepsy, temperature regulation, or tail-flick across all treatments. There were no significant differences between treatment groups in the open field or Y-maze. According to the post-mortem analysis, there were no significant differences in inflammatory cytokine profiles or CB1R concentrations between groups.
Conclusions: According to the results, 1 mg/kg THC exhibited a significant decrease in locomotor performance in male TBI and non-TBI rats in comparison to controls across the test period. These locomotor differences were not reciprocated in female rats and were not mirrored by any other behavioural or molecular differences. Therefore, 1 mg/kg THC may limit motor coordination in male rats following TBI, but the underlying mechanisms for this deficit remain unclear.

Disclosures:

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.06

Topic: C.10. Brain Injury and Trauma

Support: PAPIIT IN228320

Title: Effect of the estrous cycle on motor recovery from a traumatic head injury (TBI)

Authors: *L. NAVARRO, **F. ESTRADA-ROJO**;
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Abstract: The estrous cycle involves a series of events in various female systems regulated by the hypothalamic-pituitary-gonad axis. The various phases of this cycle involve considerable changes in the release of hormones such as FSH, LH, estradiol, and progesterone. Relatively a few years ago, it was known that women had lower mortality compared to men of the same age in the event of a head injury (TBI) of similar magnitude, which suggested that the presence of estradiol and progesterone provided some neuroprotection before the damage. Additionally, it is known that in animal models, the administration of both hormones after a TBI is associated with a reduction in apoptosis and neuronal death and better behavioral recovery after the damaging event. This work analyzes the motor recovery of rats subjected to TBI at different phases of the estrous cycle. Female Wistar rats of between 250 and 300 grams were used, divided into four groups (n = 6), one group for each phase of the estrous cycle. The estrous cycle was monitored by vaginal smears. Once the phase had been established, a TBI was induced through a weight drop model at the motor cortex level; the site of damage was chosen using a stereotaxic system and coordinates established in the Paxinos atlas (2010). Subsequently, a neurobehavioral test of

21 points was applied during the following eight days. The results were statistically analyzed. Our data show that the group in which TBI was induced during the diestrus, had a better recovery than the control group, followed by the group in the proestrus phase. In both cases, this coincides with peaks of progesterone release.

Disclosures: L. Navarro: None. **F. Estrada-Rojo:** None.

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.07

Topic: C.10. Brain Injury and Trauma

Support: DoD Grant W81XWH-16-1-0724-PRARP-CSRA
Roskamp Institute

Title: Influence of repetitive brain trauma on extracellular tau elimination at the blood brain barrier

Authors: *M. EISENBAUM, J. OJO, F. CRAWFORD, C. BACHMEIER;
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Abstract: Introduction. Exposure to repetitive head injuries sustained in the military or contact sports has been associated with the accumulation of extracellular tau in the brain, which may contribute to the pathogenesis of neurodegenerative tauopathies. Several studies have observed an interaction between extracellular tau and neurovascular damage in the context of repetitive mild traumatic brain injury (r-mTBI). What remains unclear is the effect of neurovascular dysfunction on the elimination of tau from the brain following head trauma. These studies will investigate the role of the cerebrovasculature in the elimination of extracellular tau from the brain, and the influence of r-mTBI on these processes. **Aims.** 1) Evaluate cerebrovascular tau uptake in the context of repetitive mild traumatic brain injury (r-mTBI), 2) examine the influence of r-mTBI on exogenous tau elimination from the brain, and 3) interrogate the role of the blood brain barrier (BBB) in tau elimination. **Methods.** *In vivo,* TBI was administered to wild-type mice 2 times per week for 12 weeks using a of closed head injury model. To determine the interaction between cerebrovascular cells and extracellular tau, tau uptake and caveolin-1 expression was evaluated in isolated r-mTBI cerebrovessels at 24 hours, 3, and 6 months post injury. Tau elimination from the brain was examined following stereotaxic injection of exogenous tau in mice 6 months post last r-mTBI. Tau transcytosis across the blood-brain barrier (BBB) was examined in the presence of a caveolin-1 modulator in an *in vitro,* BBB model. **Results.** Cerebrovascular tau uptake and caveolin-1 expression progressively decreased in r-mTBI cerebrovessels relative to r-sham animals. Exogenous tau levels residing in the brain following intracranial injection were increased in r-mTBI mice compared to r-sham mice. Caveolin-1 inhibition significantly decreased the basolateral-to-apical transit of tau across the BBB model. **Discussion.** Cerebrovascular uptake of extracellular tau is mediated by caveolin-1

and represents a mechanism for tau elimination across the BBB. In the chronic phase following r-mTBI, cerebrovascular caveolin-1 levels progressively decrease, which may contribute to the accumulation of tau in the brain following head trauma.

Disclosures: M. Eisenbaum: None. **J. Ojo:** None. **F. Crawford:** None. **C. Bachmeier:** None.

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.08

Topic: C.10. Brain Injury and Trauma

Support: NIH Training Grant 5T32HL007953-20

Title: Altered Orexin Function Following Mild Traumatic Brain Injury

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[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Anesthesiol. and Critical Care Med., Children's Hosp Philadelphia, UPENN, Bala Cynwyd, PA; [3]Dept. of Neurol., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Every year, approximately 2 million people in the United States alone will experience a Traumatic Brain Injury (TBI). Between 30-70% of patients that have a TBI will also develop a sleep disorder. These can include hypersomnia and narcolepsy, where patients have difficulty staying awake. While prior research on TBI induced sleep disorders have identified potential treatments of the symptoms, the underlying causes of these disorders are not known. The neuropeptide orexin is critical in regulating arousal and is decreased in non-TBI occurrences of hypersomnia and narcolepsy. Studies in humans and rodents have shown that following TBI, there is a decrease in the levels of orexin in the blood and brain. To further study this, our lab employed the lateral fluid percussion injury (LFPI) method to induce a mild injury in mice 6-8 weeks of age. We recorded sleep behavior in mice 1 week after injury and found that the animals had an inability to maintain wakefulness. We used immunohistochemical staining to count the number of orexin neurons after injury to determine if orexin cell death was the cause of excessive sleepiness. We found that there was no meaningful reduction in the number of orexin neurons after a mild TBI. We stained for the immediate early gene cFOS to determine the activity of orexin neurons after injury. We found that there was a smaller percentage of active orexin neurons in the injured animals. To directly assess the activity of orexin neurons after TBI, we used slice electrophysiology to measure their activity. To locate orexin neurons within the lateral hypothalamus, we used a mouse model with an orexin-EGFP reporter and confirmed their identity using immunohistochemical methods. Once identified, we used whole cell patch clamp to record synaptic currents and action potential dynamics in orexin neurons. This research will greatly improve our understanding of the cellular mechanisms of TBI induced sleep disorders.

Disclosures: R.T. Somach: None. **I. Jean:** None. **A.S. Cohen:** None. **M. Lim:** None.

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.09

Topic: C.10. Brain Injury and Trauma

Support: An endowment for the Richard T. Anderson Chair in Neurosciences, University of Oklahoma College of Pharmacy (KMS)

Title: Sex differences in neurobehavioral responses to mild and moderate traumatic brain injury (TBI) in rats

Authors: *O. N. AL YACOUB, M. P. BAIER, K. M. STANDIFER, H. O. AWWAD;
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Abstract: Traumatic Brain Injury (TBI) is a condition of disruption of normal brain function due to an impact, penetration or jolt to the head. Male bias in clinical and preclinical studies is a pressing issue in neuroscience and TBI research. Sex differences in pathophysiology and recovery following TBI of varying severity are poorly understood, which limits delivery of efficient care and development of successful therapeutics for TBI. The goal of this study was to investigate sex differences in response to either mild TBI (mTBI) or moderate TBI (ModTBI) by assessing neurological, behavioral and nociceptive responses. Wistar male (9-16 weeks) and female (12-13 weeks) rats received either a sham craniotomy, mTBI or modTBI controlled cortical impact to the left cerebral hemisphere. Injury severity and recovery were assessed using modified neurological severity scores (mNSS) on days 1 and 8 post-TBI. Vestibulomotor function was determined using the rotarod. Nociceptive sensitivity was assessed by measuring hind paw withdrawal threshold (PWT) from mechanical pressure and tail flick latency (TFL) from radiant heat on days 2, 4, and 7 post TBI. The presence of anxiety-like symptoms was assessed using the elevated plus maze (EPM) on day 7 post-TBI. Two-way ANOVA with or without repeated measures (as appropriate) was performed on data from each sex. Results: Both male and female rats displayed similar neurological deficits per TBI severity; with validated mNSS scores on day 1 post-TBI (mTBI: 1-6 and modTBI: 7-12). Both sexes showed full neurological recovery on day 8 following mTBI (mNSS <1) and partial neurological recovery from ModTBI (mNSS, mean ± SD, M : 4.07 ± 2.2, F: 2 ± 1.3; n=5-7). mTBI induced transient vestibulomotor deficits on days 1 - 4 post-TBI in both sexes and rats recovered by day 8 post-TBI. However, modTBI induced vestibulomotor deficits from day 1 with no recovery by day 8 post-TBI in male rats, and full recovery in females. Female rats showed impaired rotarod performance on days 1-4 after mTBI and 1-7 after ModTBI compared to sham rats. mTBI, modTBI and sham groups were significantly different from each other on all days tested in male rats. Female rats with mTBI and ModTBI were equally impaired, with no statistical difference between them on days 1-8. mTBI and ModTBI induced significant thermal hyperalgesia and mechanical allodynia compared to sham rats in both sexes to a similar extent. No differences in anxiety-like behaviors between sham and TBI groups were detected on day 7 post-TBI in either

sex. In conclusion, mTBI and ModTBI produced similar deficits in neurological functions and pain sensitivity, but different patterns of vestibular deficits in male and female rats.

Disclosures: **O.N. Al Yacoub:** None. **M.P. Baier:** None. **K.M. Standifer:** None. **H.O. Awwad:** None.

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.10

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01 NS095803

Title: Conditional ablation of ApoE in astrocytes reduces hippocampal newborn neuron complexity and impairs spatial learning and memory following traumatic brain injury.

Authors: ***T.-S. YU**, Y. TENSAOUTI, E. STEPHANZ, E. RAFIKIAN, M. YANG, S. KERNIE;
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Abstract: Polymorphisms in the apolipoprotein E (APOE) gene confer a major genetic risk for the development of late-onset Alzheimer disease (AD), with the APOE4 allele providing an increased risk relative to the more common E3 allele. Despite its clinical implications, the function for APOE in the brain remains largely unknown but is believed to be critical for repair and remodeling lipid membranes, organelle biogenesis, and dendritogenesis because of its role in cholesterol and lipid transport and metabolism. We have recently demonstrated that APOE is critical in both dendritogenesis and spine development of adult hippocampal newborn neurons in naïve and injured brains using conventional APOE null mice.
In the adult brain, APOE is synthesized by astrocytes, microglia, and pericytes. Among them, the major source of APOE is the astrocyte. To reveal the specific role of APOE synthesized by astrocytes, we generated astrocyte-specific ablation of ApoE using floxed ApoE mice crossed to Aldh-Cre transgenic mice to allow for inducible ablation of APOE in astrocytes.
Mice received tamoxifen at 3-weeks of age and then underwent controlled cortical impact (CCI) to mimic traumatic brain injury 3 weeks later. At the time of injury, a GFP-expressing retrovirus (MMLv) was injected into the dentate gyrus to allow for visualization of newborn neurons and subsequent analysis of morphology. Four weeks after surgery, an approximately 80% reduction in APOE-expressing cells in the hippocampus was observed in conditional knockouts. Using the Golgi-Cox method, stained cells in the dentate gyrus in the absence of astrocytic APOE had unchanged dendritic structures when compared with controls in both sham-operated and injured brains. In hippocampal newborn neurons, no difference was observed in the sham-operated mice despite the reduction in astrocytic APOE. However, in the injured mice, reduction of astrocytic APOE resulted in less complex newborn neurons revealed by Sholl analysis. The deficits in newborn neurons in the dentate gyrus resulted in impairments in hippocampal-dependent

behavioral tasks. Using a reversal learning version of Morris water maze, the injured conditional knockout mice learned to reach the hidden platform in the learning phase, but failed at retrieving the location of the hidden platform. This demonstrates that astrocytic ApoE is required for functional injury-induced neurogenesis following traumatic brain injury.

Disclosures: T. Yu: None. **S. Kernie:** None. **Y. Tensaouti:** None. **E. Stephanz:** None. **E. Rafikian:** None. **M. Yang:** None.

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.11

Topic: C.10. Brain Injury and Trauma

Support: NS1108098
SAP4100077079
SAP4100079710

Title: The nicotinic acetylcholine receptor modulator AVL-3288 attenuates hippocampal-based cognitive deficits following repeated mild TBI in adolescent rats

Authors: *T. A. MCCORKLE[1], R. RAGHUPATHI[2];
[2]Neurobio. and Anat., [1]Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Sports-related concussions (SRC, a subset of mild Traumatic Brain Injury) in adolescents is a leading cause of long-term cognitive deficits. We have developed an age-appropriate and clinically-relevant model of SRC in adolescent (35-day old) male and female Sprague Dawley rats by subjecting them to 3 separate episodes over 7 days of an impact to the intact skull (2.0mm depth at 5.5m/s velocity). Rats were tested for hippocampal-dependent cognition at 4 weeks post-injury in the Morris Water Maze (MWM) and Novel Object Location (NOL) tasks. Male and female brain-injured animals exhibited significant deficits in locating the hidden platform in the MWM over the 5-day testing period compared to the uninjured (sham) rats. Similarly, both male and female brain-injured rats had significant difficulty recognizing the new location of an object they had been familiarized with in an earlier trial. Based on previous studies, we hypothesized that impaired cholinergic transmission may be a mechanistic basis for these cognitive deficits. Positive allosteric modulation of nicotinic acetylcholine receptors (nAChR) via AVL-3288 was reported to diminish spatial learning deficits in brain-injured adult male rats. Male and female rats underwent injury or sham surgery in adolescence and then were injected with AVL-3288 at 30 minutes prior to testing the animals in either the MWM or NOL tasks at 4 weeks post-injury. Preliminary data indicate that treatment with AVL-3288 attenuated injury-induced cognitive deficits in both tasks suggesting that reduced activity of the nAChR may underlie post-traumatic cognitive deficits.

Disclosures: T.A. McCorkle: None. **R. Raghupathi:** None.

Digital Abstract Session

P149. Traumatic Brain Injury: Animal Models and Molecular Mechanisms

Program #/Poster #: P149.12

Topic: C.10. Brain Injury and Trauma

Support: NS1108098
SAP4100077079
SAP4100079710

Title: The role of hippocampal glucocorticoid receptor dysfunction in long term outcomes of pediatric Traumatic Brain Injury

Authors: *D. LENGEL[1], J. W. HUH[2], R. RAGHUPATHI[3];
[1]Drexel Univ., Philadelphia, PA; [2]Children's Hosp. of Philadelphia, Philadelphia, PA; [3]Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Children that sustain a Traumatic Brain Injury (TBI) during preschool-age (younger than 4 years old) are more likely to suffer from cognitive and psychosocial deficits that can emerge during adolescence/adulthood. We evaluated the effects of pediatric TBI in 11-day-old rats on cognitive and emotional behaviors between 4-6 weeks post-injury (adolescence). Brain-injured animals exhibited more open arm time in the elevated plus maze (EPM) at 4-weeks post-injury. Pediatric TBI also resulted in impairments in spatial learning in the Morris water maze at 6-weeks post-injury along with deficits in long-term potentiation in the hippocampal CA1 field. Prior studies have demonstrated that suppression of glucocorticoid receptors (GR) within the hippocampus impairs spatial learning and hippocampal synaptic plasticity, and results in more active behaviors in the EPM. Thus, we investigated whether pediatric TBI influences the expression and function of hippocampal GRs at 4-weeks post-injury by exposing sham and brain-injured animals to the EPM and measuring corticosterone, corticotropin-releasing hormone (CRH), and the mRNA expression of GRs and GR target gene, serum- and glucocorticoid-inducible kinase 1 (sgk1). Pediatric TBI did not influence corticosterone reactivity to the EPM, but resulted in an impairment in the transcriptional activity of GRs within the dorsal and ventral hippocampus at 4 weeks post-injury, as evidenced by an attenuation of the stress-induced increase in skg1. In order to determine whether impaired function of GRs influences LTP function, we bath-applied corticosterone (100nm) onto hippocampal slices during recording. Corticosterone restored short-term but not long-term potentiation, further pointing to impaired function of GRs. These results demonstrate that pediatric TBI results in long-term deficits in GR function within the hippocampus, which may underlie the cognitive and behavioral deficits observed following injury. Future directions include investigating whether GR overexpression in the hippocampus can reverse these cognitive and behavioral deficits following TBI.

Disclosures: D. Lengel: None. **J.W. Huh:** None. **R. Raghupathi:** None.

Digital Abstract Session

P150. Biomarker and Functional Correlates of Traumatic Brain Injury in Animal Models

Program #/Poster #: P150.01

Topic: C.10. Brain Injury and Trauma

Support: EU FP7/2007-2013 Grant 602102 (EPITARGET)
Academy of Finland Grant 272249
Academy of Finland Grant 273909
Academy of Finland Grant 2285733-9
Sigrid Juselius Foundation

Title: Quantitative PCR validation of plasma microRNAs as potential diagnostic biomarkers of epileptogenesis after traumatic brain injury

Authors: M. HEISKANEN, S. DAS GUPTA, X. EKOLLE NDODE EKANE, N. LAPINLAMPI, N. PUHAKKA, A. PITKÄNEN;
Univ. of Eastern Finland, Kuopio, Finland

Abstract: Over 50 million people suffer traumatic brain injury (TBI) each year, and it is a major cause of death and disability. Patients with TBI often suffer from comorbidities, such as post-traumatic epilepsy (PTE). There is an urgent need for molecular biomarkers to improve prediction of TBI outcome. We studied plasma microRNA (miRNA) expression in an animal model of PTE. We hypothesized that expression levels of specific plasma miRNAs at acute post-TBI time point will predict epileptogenesis. We present our preliminary results of plasma miRNA validation by PCR methods.
RT-qPCR validation: Tail vein plasma was sampled at 48 h after lateral fluid-percussion injury (LFPI)-induced TBI (n=16) or sham operation (n=4). TBI group included 7 rats that during a 6-months follow-up developed epilepsy (TBI+) and 9 that did not (TBI-). Based on DESeq2 analysis (TBI vs. sham) of small-RNA sequencing data from the present and two other cohorts, we selected 5 miRNAs for RT-qPCR validation using miRCURY LNA RT-qPCR assays.
Droplet digital PCR (ddPCR) validation: Four miRNAs validated in qPCR, and 3 miRNAs selected from sequencing data were measured in plasma of 5 TBI (48 h post-injury) and 5 naïve rats by ddPCR using miRCURY LNA miRNA assays with EvaGreen chemistry.
RT-qPCR showed upregulation of miR-9a-3p (FC=9.3, $p<0.001$), miR-434-3p (FC=4.2, $p<0.01$), miR-323-3p (FC=4.6, $p<0.05$), and miR-136-3p (FC=4.4, $p<0.01$) in TBI animals as compared to sham. Difference in miR-129-5p expression did not reach statistical significance (FC=1.9, $p=0.09$). RT-qPCR found no difference in miRNA expression between TBI+ and TBI- rats.
ddPCR showed upregulation of miR-9a-3p (FC=27.7, $p<0.01$), miR-434-3p (FC=14.6, $p<0.01$), miR-323-3p (FC=34.9, $p<0.01$), miR-136-3p (FC=10.6, $p<0.01$), miR-124-3p (FC=5.5, $p<0.05$), miR-212-3p (FC=1.5, $p<0.01$) and miR-132-3p (FC=3.5, $p<0.01$) in TBI animals as compared to naïve.
We validated 7 differentially expressed plasma miRNAs after TBI, indicating them as potential biomarkers for injury effect. RT-qPCR analysis did not find differences in miRNA expression

between TBI+ and TBI- rats, however, miR-124, miR-212, and miR-132 remain to be analyzed. Next, we will measure the 7 potential miRNA biomarker candidates in a larger EPITARGET cohort, including 115 TBI, 23 sham, and 13 naïve rats, using ddPCR. We will also investigate if miRNA expression is linked to epileptogenesis, or histopathological and behavioral outcome measures after TBI.

Disclosures: M. Heiskanen: None. **S. Das Gupta:** None. **X. Ekolle Ndode Ekane:** None. **N. Lapinlampi:** None. **N. Puhakka:** None. **A. Pitkänen:** None.

Digital Abstract Session

P150. Biomarker and Functional Correlates of Traumatic Brain Injury in Animal Models

Program #/Poster #: P150.02

Topic: C.10. Brain Injury and Trauma

Title: Effects of Traumatic Brain Injury on Neural Signatures Mediating Impulse Control in Rats Performing a Probabilistic Reversal Learning Task.

Authors: M. J. FRANCOEUR[1], L. FAKHRAEI[1], T. TANG[2], S. HULYALKAR[2], D. RAMANATHAN[1];
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Abstract: Traumatic brain injury (TBI) has been linked with chronic behavioral impairments seen in disorders such as depression, anxiety and addiction. Poor impulse control is a central feature of many psychiatric disorders and a common symptom post-TBI, yet the manifestation of impulsivity in TBI animals and its underlying neural mechanisms remain unknown. Impulsivity generally describes acting prematurely without consideration of behavioral consequences and can be debilitating to everyday life. Here, we examine chronic behavioral deficits resulting from bilateral frontal controlled cortical impact during a probabilistic reversal learning (PRL) task while simultaneously recording “brain-wide” local field potentials (LFPs). In the PRL task rats choose to respond in one of two noseports: one leads to a high-probability reward (“target“) and the other to a low-probability reward (“non-target”). Rewards are probabilistically delivered at an 80:20 ratio and a reversal of “target” and “non-target” noseports occurs when the rat makes 80% correct responses over a 10 trial moving window. Following recovery from TBI surgery, we implant electrodes across 30 brain regions to record LFPs from sensorimotor, attention, visual, reward, limbic, and cognitive brain regions, allowing for an unbiased sampling across neural networks. Comparing the behavior and physiology of control (N=10) and TBI (N=12) rats, we hypothesize that rats with injury will demonstrate dysregulated impulse control on the PRL task and show neural signatures associated with this impairment. Rats with injury overall perform fewer reversals and take longer to correctly respond to contingency changes. We found that reward-related beta-oscillations in orbitofrontal cortex signal a match in expected and actual reward on the PRL task. Specifically, reward-related beta activity is greater for “targets” compared to “non-targets” in both the initial block and subsequent reversal blocks. Similarly, theta activity in medial prefrontal cortex provides a neural signature for errors (“non-target”) that

switches to the new "non-target" after reversal. This activity likely mediates optimal win-stay behavior for the "target" while allowing animals to ignore misleading feedback from probabilistic reward delivery and may serve as a bio-marker for disrupted impulse control in rats with brain injuries.

Disclosures: M.J. Francoeur: None. **L. Fakhraei:** None. **T. Tang:** None. **D. Ramanathan:** None. **S. Hulyalkar:** None.

Digital Abstract Session

P150. Biomarker and Functional Correlates of Traumatic Brain Injury in Animal Models

Program #/Poster #: P150.03

Topic: C.10. Brain Injury and Trauma

Support: NIH R56NS101909
NIH R01NS101909
NIH T32-GM08433

Title: Linking brain and serum lipidome alterations following mild traumatic brain injury

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Abstract: Traumatic brain injury (TBI) is an extremely heterogeneous disease, which makes clinical diagnosis challenging, especially for mild TBI (mTBI). Current methods to diagnose mTBI rely heavily on self-reported subjective symptoms. Serum-based lipid biomarkers may be a more objective tool to diagnose and discriminate between different injury phenotypes because of the relative ease with which lipids transport across the blood brain barrier into systemic blood flow compared to protein biomarkers. In this study, we aimed to identify a lipid biomarker panel that is both brain-specific and TBI-specific using a clinically relevant closed head injury model. Male (n=14) and female (n=18) rats obtained sham-procedures (n=9), a single impact (n=10; head displacement= 5 mm), or 3 impacts (2 min interval; head displacement= 5 mm, 2 mm, 2mm) (n=11) by a controlled cortical impact device (velocity = 5 m/s). Whole blood was collected at baseline, 30 min, 4 hrs, and 24 hrs post-injury and brains were collected 24 hrs post-injury. Untargeted ultra-performance liquid chromatography (UPLC-MS) in negative and positive modes was used to analyze lipidome changes after injury in serum and brain samples. UPLC-MS of serum in both positive and negative modes yielded 14,119 features. Principal component analysis showed separation between uninjured and injured serums and clustering of pooled quality control samples. Welch's t-tests were conducted and features with q-values below 0.05 and fold change above 1.5 were identified as features of interest for further MS/MS experiments. Twenty-one candidate lipid biomarkers were selected with genetic algorithms for discriminating injured from uninjured samples in female and male sexes independently, including phosphatidylcholine and

sphingomyelin. These features were used in a binary comparison of injured versus uninjured serum and performed with high sensitivity, specificity, and accuracy (94.1%, 97.1, 93.6%) for males and (91.7%, 93.8%, 92.7%) for females, respectively. Orthogonalized partial least squares discriminant analysis with venetian blinds cross validation and modeling of each timepoint as a separate class showed 77% proper class assignment over all four timepoints. Most misclassified samples were collected at baseline and 30 mins post-injury, which may suggest minimal lipidome changes at early time points. These results show the ability of serum lipids to differentiate between TBI and uninjured rodents. Comparison to brain lipids is ongoing. Together, these results will indicate if dysregulated lipids in the serum reflect brain-specific changes following TBI and guide further refinement of a novel biomarker panel.

Disclosures: A. Pulliam: None. **E. Gier:** None. **D. Gaul:** None. **F. Fernandez:** None. **M.C. LaPlaca:** None.

Digital Abstract Session

P150. Biomarker and Functional Correlates of Traumatic Brain Injury in Animal Models

Program #/Poster #: P150.04

Topic: C.10. Brain Injury and Trauma

Support: Wellcome Trust Grant 212430/Z/18/Z

Title: Computational prediction of vascular injury after traumatic brain injury

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Imperial Col. London, London, United Kingdom

Abstract: Introduction: Mechanical forces acting on vessels during traumatic brain injury (TBI) often damage blood-brain barrier (BBB) and cause haemorrhage. A key question is how mechanical forces disrupt the vascular network. We hypothesised that large tensile forces acting on vessels can produce BBB disruption. To test this, we combined finite element (FE) modelling with quantitative histopathology in a controlled cortical impact (CCI) model of TBI in rats. **Methods:** High resolution synchrotron images of a Sprague Dawley (SD) rat brain (Zhang et al., Sci. Rep., 2015) was used to develop a detailed FE model incorporating vascular network (Fig. A-C). The model was used to predict maximal tensile strain in vessels during CCI. CCI was performed on SD male rats (sham n=4, CCI n=8). Three days post-injury, sections from the contusion core were stained for fibrinogen. The staining was quantified in 5 segments in the ipsilateral corpus callosum (CC) and cortex (Fig. F). Linear mixed effects modelling was used to test the relationship between strain in vessels and fibrinogen extravasation. **Results:** The FE model predicted large strains in ipsilateral vessels particularly in the cortex and near the edges of the impactor (Fig. E). The empirical data also showed pronounced vascular damage, as indicated by a strong increase of Fibrinogen cortex and subcortical structures (Fig. F). Quantification of Fibrinogen optical density showed an increase of 150% in the cortex and 90% in CC (Fig. G). A

linear mixed effects model incorporating strain and ROI (CC and cortex) as the fixed effects predicted 30% of the variation in Fibrinogen optical density (Fig. H). Removing strain from the model slightly reduced the prediction to 28%. **Conclusion:** We found a link between mechanical strain in vessels and BBB disruption post-injury. Our results show that fibrinogen extravasation increases by increasing strain. They also show more BBB disruption in the cortex than CC when we control for strain.

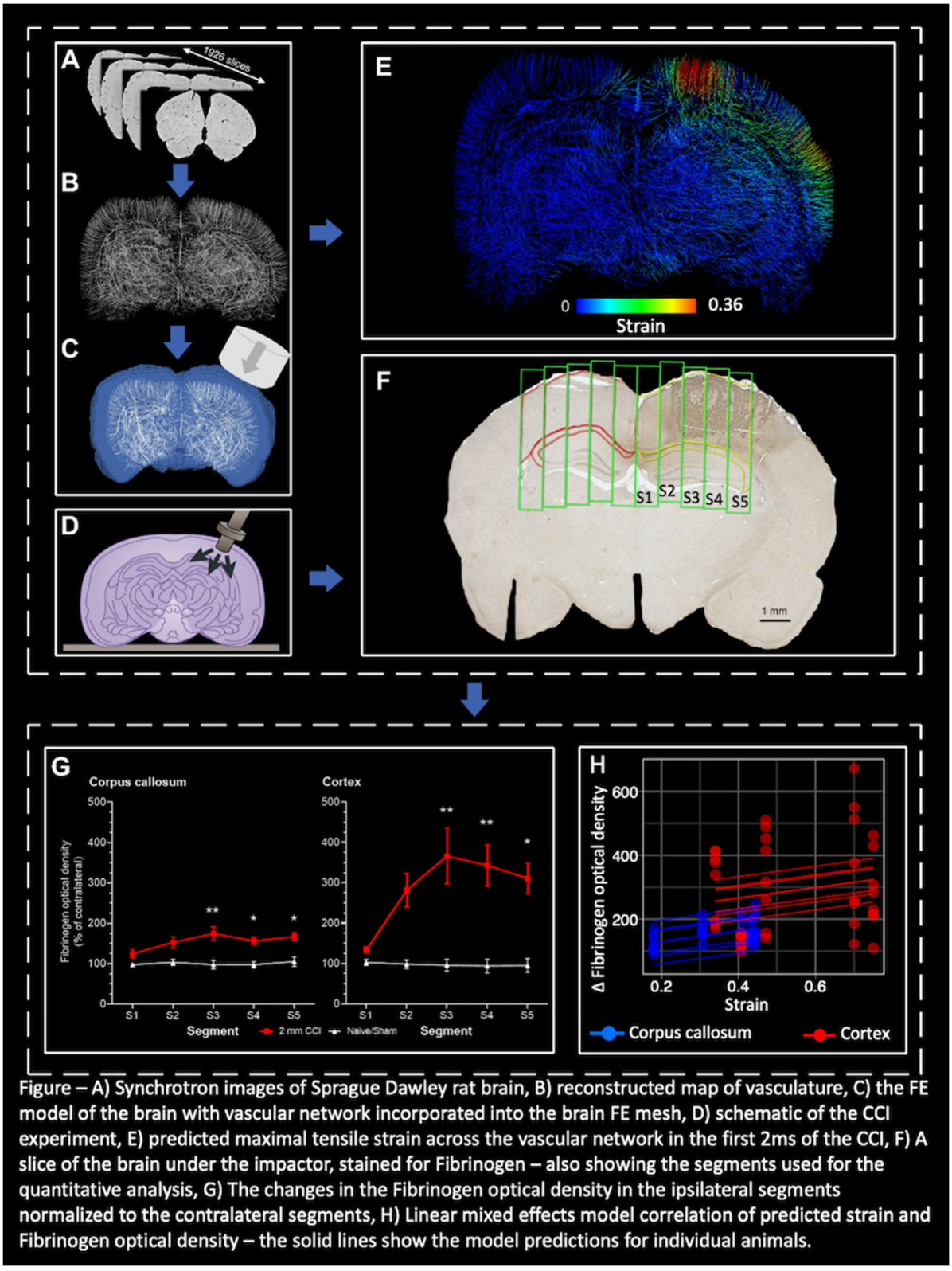

Figure – A) Synchrotron images of Sprague Dawley rat brain, B) reconstructed map of vasculature, C) the FE model of the brain with vascular network incorporated into the brain FE mesh, D) schematic of the CCI experiment, E) predicted maximal tensile strain across the vascular network in the first 2ms of the CCI, F) A slice of the brain under the impactor, stained for Fibrinogen – also showing the segments used for the quantitative analysis, G) The changes in the Fibrinogen optical density in the ipsilateral segments normalized to the contralateral segments, H) Linear mixed effects model correlation of predicted strain and Fibrinogen optical density – the solid lines show the model predictions for individual animals.

Disclosures: **M. Ghajari:** None. **C.K. Donat:** None. **A. McGarry:** None. **O. Porat:** None. **M. Sastre:** None. **D. Sharp:** None. **S.F. Khosroshahi:** None.

Digital Abstract Session

P150. Biomarker and Functional Correlates of Traumatic Brain Injury in Animal Models

Program #/Poster #: P150.05

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R37HD059288
NIH Grant T32HL07713
Pennsylvania Department of Health Grant SAP #4100077078
University of Pennsylvania Center for Undergraduate Research and Fellowships
2020 Grant for Faculty Mentoring Undergraduate Research

Title: Using an Elevated Plus Maze assay to measure anxiety-like behaviors in a mouse model following mild traumatic brain injury

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Abstract: Every year in the United States traumatic brain injury (TBI) results in 2.5 million emergency department visits, hospitalizations, and deaths. Millions more may be affected that do not seek care. Victims of TBI can suffer from a multitude of chronic symptoms including sensory and motor deficits and neuropsychiatric symptoms, such as anxiety. The neurobiology underlying the symptoms of anxiety disorders after TBI remains largely unknown. Since anxiety disorders have such a high prevalence and lead to prominent reductions in productivity and quality of life for TBI sufferers, it is important to investigate their causes and manifestations. Modeling these symptoms in animal models of TBI affords the opportunity to determine mechanisms underlying behavioral pathologies and to test potential therapeutic agents. This study examined the effect of mild TBI on anxiety-like behaviors in a mouse model. Mild lateral fluid percussion injury (LFPI), or sham surgery, was induced in 8-week-old male C57B/L6 mice. An Elevated Plus Maze (EPM) protocol was adapted and performed at 24 hours, 3 days, and 2 weeks after injury in separate cohorts of mice. The EPM is an elevated, 1 meter high platform divided into four equal quadrants arranged in a plus sign. Two opposing quadrants are open while the remaining two closed quadrants are surrounded by dark, opaque walls. As a test of anxiety-like behavior, the EPM relies on a rodent's natural tendency to avoid exposed, bright areas where they may be vulnerable to predators. Each mouse was placed in the center of the EPM apparatus and allowed to freely explore for 5 minutes. ANY-maze software was used for mouse tracking and videos were analyzed for the number of entries and amount of time spent in

each quadrant by each mouse. Our results demonstrated increased anxiety behaviors in the sham (non-injured) compared to the injured animals. However, injury condition was significantly correlated with total distance traveled and average speed, suggesting that motor deficits must be examined as a potential confounding factor. Further research is needed to determine if the observed behavioral alterations have electrophysiological correlates in brain structures mediating anxiety.

Disclosures: **M. Mojena:** None. **K.M. Best:** None. **H. Metheny:** None. **A.S. Cohen:** None.

Digital Abstract Session

P150. Biomarker and Functional Correlates of Traumatic Brain Injury in Animal Models

Program #/Poster #: P150.06

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R37HD059288
Pennsylvania Department of Health Grant SAP#4100077078
NIH Grant T32HL07713

Title: Pain sensitivity and aversion after traumatic brain injury (TBI): A behavioral and histological analysis

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Abstract: Traumatic brain injury (TBI) affects more than 2.5 million people in the United States each year and is associated with multiple long-term sequelae that impact functional ability and quality of life. Chronic pain is a significant morbidity after TBI, with up to 90% of veterans reporting chronic headache and a similar incidence in civilian populations. Pain may occur in other body areas not affected by the primary head trauma, including neck, back and limb pain, and is hypothesized to arise from TBI-induced neuroplasticity and central sensitization. This study examined the effect of mild TBI on pain sensitivity and pain-related aversive behaviors in a mouse model of brain injury, lateral fluid percussion injury (LFPI). Under anesthesia, mild LFPI was induced in 6- to 8-week-old male C57B/L6 mice and behavioral experiments were performed at one or two weeks after injury. A three-day conditioned place aversion (CPA) protocol was adapted from Cunningham et al. (2006), using 1% formalin injection as the aversive stimulus, to allow for simultaneous assessment of evoked pain behaviors and aversion. Following completion of behavioral testing, animals underwent live perfusion with 4% paraformaldehyde (PFA), and fixed brains were sliced into 50 μm thick sections. Immunohistochemical (IHC) staining for cFos was performed to identify brain regions activated

by the CPA protocol. All experiments were carried out under protocols approved by the Institutional Animal Care and Use Committee. Mouse tracking during CPA was performed using ANY-maze software (Wood Dale, IL), and evoked pain behaviors were manually coded in 10 second epochs each minute for 60 minutes after formalin injection. Data were analyzed with two-way repeated measures ANOVA and Sidak's multiple comparison test in order to evaluate for effects by injury condition and test duration. Our results showed increased pain-related aversion at one week following TBI. Further research is needed to determine whether the observed behavioral alterations have electrophysiological correlates in brain structures mediating nociceptive processing and aversion.

Disclosures: K.M. Best: None. **M.M. Mojena:** None. **H. Metheny:** None. **A.S. Cohen:** None.

Digital Abstract Session

P151. Unravelling Mechanisms in Disorders Affecting The Human Brain

Program #/Poster #: P151.01

Topic: C.10. Brain Injury and Trauma

Support: Sunnybrook Foundation

Title: Neurocovid19: impact of the virus on the brain

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Abstract: Background: The symptoms of coronavirus disease 2019 (COVID-19) are highly variable, ranging from minor to potentially life threatening - the latter requiring intensive critical care medical response at prevalence levels that can strain healthcare systems. Significant concern is also emerging over the potential for lingering symptoms in COVID-19 survivors. In particular, numerous cases of hospitalized COVID-19 patients with neurological symptoms are being reported, including deficits in sensation, cognition and consciousness, as well as vascular and inflammatory lesions observed by magnetic resonance imaging (MRI). Very little is known about how these brain effects persist or resolve - and the extent of the effects in the much larger population of COVID-19 survivors, who self-isolated while infectious.
Objectives: To fill this knowledge gap, we have created NEUROCOVID19: a multidisciplinary study involving neuroimaging scientists and academically-oriented clinicians, that aims to: **1.** Test whether COVID-19 survivors have incident brain lesions compared to matched COVID-19 negative controls; **2.** Evaluate neuroanatomy and neurophysiology, clinical, sensory, and

behavioural findings when COVID-19 survivors are no longer infectious (at baseline) and at follow-up; **3.** Test for multivariate relationships between these measurements across COVID-19 survivors, including associations with risk variables such as age and indices of cerebrovascular health. **Methods:** The study involves ongoing recruitment of COVID-19 survivors in two groups: those that were hospitalized, and those that self-isolated; and a control group of participants that tested negative for COVID-19 and self-isolated consequent to cold or flu-like symptoms. NEUROCOVID19 measurements include comprehensive, state-of-the-art brain MRI at 3 Tesla, including acquisitions and associated metrics probing brain anatomy and physiological function, lung MRI, olfactory and behavioural (NIH toolbox) assessments, and electroencephalography. Baseline measurements are undertaken within 3 weeks after hospital discharge or leaving quarantine, with 3-month follow-up. **Significance:** Our initial pilot data (20 cases, 10 controls) strongly support our hypothesis that neuroinvasion occurs and produces brain lesions in some COVID-19 survivors with and without hospitalization. Knowledge translation of our protocol and findings to radiology, neurology and COVID-19 recovery clinics will help to address on-going health needs of COVID-19 survivors, and will help to understand the mechanisms that underpin the heterogeneous brain symptoms.

Disclosures: S.J. Graham: None. **J.J. Chen:** None. **A. Gilboa:** None. **B.J. Macintosh:** None. **A.B. Sekuler:** None. **S.E. Black:** None. **J.A. Chad:** None. **I. Cheng:** None. **R. Fowler:** None. **F. Gao:** None. **M. Goubran:** None. **C. Heyn:** None. **A. Jegatheesan:** None. **X. Ji:** None. **B. Lam:** None. **M. Masellis:** None. **J.S. Rabin:** None.

Digital Abstract Session

P151. Unravelling Mechanisms in Disorders Affecting The Human Brain

Program #/Poster #: P151.02

Topic: C.10. Brain Injury and Trauma

Support: Israel Ministry of Science and Technology, Levi Eshkol Ph.D. fellowship
Teva, The BioInnovation Fellowship & Mentorship
Elton Laboratory
The Lily and Avraham Gildor Chair
ERA-NET neuron, ADNPinMED
AMN Foundation
MAFAT

Title: Cytoskeletal and inflammatory putative gene somatic mutations in the blood of PTSD-symptomatic soldiers

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Abstract: Motivation/problem statement: We have recently discovered autism/intellectual disability somatic mutations in postmortem brains, presenting higher frequency in Alzheimer's disease subjects, compared with the controls. We further discovered high impact cytoskeletal gene mutations, coupled with potential cytoskeleton-targeted repair mechanisms (Ivashko-Pachima, Hadar⋯Gozes, Molecular Psychiatry, 2019 EPUB). One of the identified genes/proteins exhibiting somatic mutations was activity-dependent neuroprotective protein (ADNP), also linked with stress (Sragovich⋯Gozes, 2019, Transl Psychiatry. 9(1):235). We now investigate if somatic mutations in brain diseases are specific to the brain, or phenocopied in the blood in Alzheimer's disease risk populations, such as posttraumatic stress syndrome (PTSD) individuals, toward potential diagnostics and preventative measures. **Methods/ approach:** To identify high and moderate impact somatic potential mutations, we performed variant calling analyses on an RNA-seq database including peripheral blood samples from 85 soldiers (58 controls and 27 with symptoms of PTSD (Boscarino et al., 2019, G3 (Bethesda) 9, 463-471). **Results:** High (e.g. protein truncating) as well as moderate impact (e.g. single amino acid change) putative somatic mutations in thousands of genes as well as germline mutations were identified. Further crossing the mutated genes with databases including genes driving autism, intellectual disability, cytoskeleton, inflammation and DNA repair (Venn diagrams), identified the highest number of cytoskeletal-mutated genes (187 high and 442 moderate impact). Most of the mutated genes were not specific to PTSD. However, the inflammation database exhibited a larger number of putative high impact mutated genes specific to the PTSD-symptom cohorts vs. the controls (14 vs. 13), highlighting tumor necrosis factor specifically in the PTSD-symptom cohort. **Conclusion/implications:** With microtubules and neuro-immune interactions playing essential roles in brain neuroprotection and Alzheimer-related neurodegeneration, these findings contribute to mechanistic understanding of PTSD and brain protection, as well as provide future diagnostics toward personalized military deployment strategies and drug design.

Disclosures: S. Sragovich: None. **M. Gershovis:** None. **J.C.K. Lam:** None. **V.O.K. Li:** None. **I. Gozes:** A. Employment/Salary (full or part-time):; Coronis Neurosciences. F. Consulting Fees (e.g., advisory boards); Osteop.

Digital Abstract Session

P151. Unravelling Mechanisms in Disorders Affecting The Human Brain

Program #/Poster #: P151.03

Topic: C.10. Brain Injury and Trauma

Support: C.G.G. Supported in part by the Valley Baptist Legacy Foundation Institute for Neuroscience Grant
A.O.& M.E.A. Supported by King Abdullah Scholarship Program, Saudi Government, Ministry of Education

Title: Can alternative medical methods evoke neuro-functional somatosensory responses? A case study suggesting functional improvement.

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Abstract: Somatosensory pathways act as the avenue in transferring information concerning the body and its interaction with the external environment to the brain. We aim to demonstrate that through studying somatosensory, motor cortical and subcortical networks, we can explain functional recovery after stimulations applied as an alternative medical treatment. Those stimulations might have evidenced neural pathways and networks important in recovery of function. Materials and methods: The de-identified medical reports of nine patients with initial presentations of cerebral trauma or stroke inducing paralysis were studied.These included the alternative treatments they received and other available materials such as videos and photographs. Patients were either males or females and their ages ranged from 20 to 95-year old. All patients, were first treated through conventional medical interventions, including physical therapy. Patients consulted for alternative medical treatment, one year or more, after the initial diagnosis**.** The alternative medical treatment consisted in multiple points stimulations. Twelve points of stimulation on the skull were used. Additional 4 points of stimulation were located at the right and left calves and at the right and left forearms. All stimulations had nociceptive and proprioceptive components. The stimulations were applied successively one by one (legs, arms, skull). The treatment consisted in a one-time (exceptionally two) session. The duration of each stimulation was about 0.5 s. Results show that in all 9 cases, patients had improvement in their motor skills, including gain of weight support and unassisted small walks, independent and voluntary movements of limbs. Improvement was steady over a period of one to several years. We believe that whether lesions included prefrontal cortical areas, or motor and sensory areas, the alternative treatment triggered existing or new neuronal networks as well as synaptic efficiency or reactivation, through highly increased, sensory nociceptive coupled to proprioceptive, afferences. Those results highlight the need to further investigate neural function of cortical and subcortical areas through non-invasive and indirect pathways stimulations, during a stable chronic phase after a CNS insult.

Disclosures: A. Otaif: None. **M.E. Alshammari:** None. **C.G. Gerin:** None.

Digital Abstract Session

P151. Unravelling Mechanisms in Disorders Affecting The Human Brain

Program #/Poster #: P151.04

Topic: C.10. Brain Injury and Trauma

Title: Loss of consciousness in National Football League players is associated with high strain in the thalamus and brainstem.

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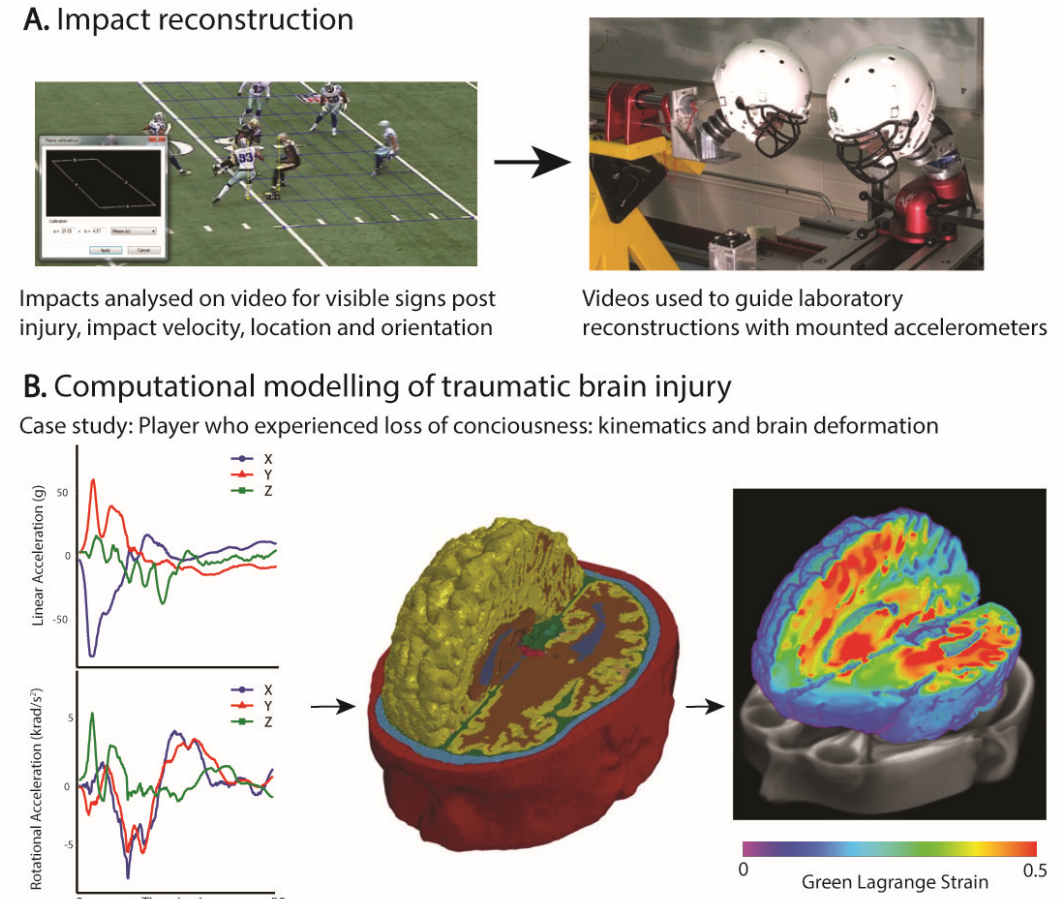
Abstract: Background: Sports traumatic brain injury (TBI) can produce transient neurological signs such as loss of consciousness (LOC) and dystonic posturing. However, it is unknown why impacts produce a range of neurological effects. Video surveillance allows the biomechanics of impacts to be calculated from impact reconstructions. The biomechanics can then be used to estimate the patterns of strain produced in different brain regions. Here we use a 3D model of brain injury biomechanics to investigate the strains produced by National Football League impacts that produce mild TBI leading either to LOC, dystonic posturing, or producing no neurological signs. We test the hypotheses that LOC is associated with high strain within the brainstem and thalamus, whereas dystonic posturing is association with high strain in the motor cortex and/or corticospinal tracts.

Methods: 82 videos of mild TBIs in the NFL were classified as showing either LOC (20), posturing (21) or no neurological signs (No Signs - 41). Methods are highlighted in Fig A & B. The estimated magnitude and location of mechanical strain in specific brain regions were then statistically compared across the groups. Results: Impacts leading to LOC had significantly higher impact kinematics and whole brain measures of brain deformation compared to No Signs impacts. A voxelwise analysis of brain deformation corrected for the magnitude of strain showed regions of disproportionately higher strain in the thalamus, brain stem and cerebellum in impacts leading to LOC compared to No Signs (Fig C). Impacts leading to posturing also showed regions of higher strain, however these areas were constrained to the cortical regions including the motor cortex.

Conclusions: We show that strains are particularly high in the thalamus and brain stem in those players who lose consciousness. In contrast, impacts leading to posturing appear to disproportionately affect cortical regions, including the motor cortex. These results provide evidence that loss of consciousness is produced by head impacts that produce high strain in the brainstem and thalamus.



Disclosures: K.A. Zimmerman: None. **D. Sharp:** F. Consulting Fees (e.g., advisory boards); Received an honorarium from the Rugby Football Union for participation in an expert

concussion panel, which was used to support his research.. **M. Ghajari:** None. **J. Cournoyer:** None. **C. Karton:** None. **T.B. Hoshizaki:** None.

Digital Abstract Session

P152. Biological and Clinical Diagnostic Biomarkers of Human Traumatic Brain Injury

Program #/Poster #: P152.01

Topic: C.10. Brain Injury and Trauma

Support: ERANET Neuron MR/R004528/1
NIHR-RP-011-048
ARUK-CRF2017A-1

Title: Advanced blood and neuroimaging biomarkers of axonal injury after traumatic brain injury in the prospective multi-centre BIO-AX-TBI study

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Abstract: Background: Outcomes after traumatic brain injury (TBI) are highly variable, with many survivors affected by persistent disability, including cognitive problems. Axonal injury is thought be a significant determinant of outcome, which can be assessed using novel techniques such as fluid biomarkers and advanced MRI assessment. Poor long-term outcomes post-TBI may also relate to progressive neurodegeneration, which can be assessed using serial volumetric MRI to quantify brain atrophy longitudinally.
Methods: BIO-AX-TBI (Developing and Validating Blood and Imaging BIOmarkers of AXonal Injury Following Traumatic Brain Injury, NCT03534154) is a large multi-centre cohort study of patients after acute moderate-severe TBI. Patients admitted to major trauma units underwent baseline clinical followed by serial blood biomarker and MRI assessments over the course of a year, with detailed functional outcome ascertainment. Single molecule array assays quantified blood concentrations of neurofilament light (NFL), S100-B, ubiquitin carboxy-terminal hydrolase L1 (UCHL1), glial fibrillary acidic protein (GFAP) and tau.
Results: 197 patients after TBI underwent acute blood biomarker assessment. Clinical outcomes were ascertained at 6 and 12 months. Several control groups were assessed: 95 healthy participants with imaging and blood biomarker assessments, 28 healthy controls with longitudinal neuroimaging and 24 trauma patients with extracranial injuries (non-TBI trauma, 'NTT', London). TBI patients had significant elevations of blood biomarkers compared with

controls and NTTs. GFAP, S100B, Tau and UCH-L1 were elevated rapidly post injury. NFL peaked more gradually and remained chronically elevated. Diffusion tensor imaging (DTI) assessment showed reduced fractional anisotropy in TBI patients, which remained abnormal into the chronic phase. Early cortical atrophy was predicted by acute plasma tau concentration and chronic white matter atrophy was predicted by NFL levels. Cognitive function at 12 months, and functional outcomes at 6 and 12 months were predicted by the acute blood biomarker levels and DTI MRI measures.
Conclusions: In this multi-centre longitudinal of acute moderate-severe TBI, blood assessment of neuronal and glial breakdown products provided a reliable readout of injury severity, predicting functional outcomes at 6 and 12 months. Blood NFL and DTI MRI were particularly sensitive to traumatic white matter injury and predicted accelerated white matter atrophy in the chronic phase.

Disclosures: **N.S.N. Graham:** None. **K.A. Zimmerman:** None. **F. Moro:** A. Employment/Salary (full or part-time):; Mario Negri Institute for Pharmacological Research. **H. Zetterberg:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ERC, Swedish Research Council. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Fujirebio, Alzecure, Biogen. F. Consulting Fees (e.g., advisory boards); Biogen, CogRx, Denali, Roche Diagnostics, Siemens Healthineers, Pinteon, Wave, Samumed. **A. Heslegrave:** A. Employment/Salary (full or part-time):; UCL Dementia Research Institute. **S. Magnoni:** None. **G. Bertolini:** None. **E. Garbero:** None. **G. Nattino:** None. **M. Oddo:** None. **S. Abed-Maillard:** None. **J. Miroz:** None. **P. Gradisek:** A. Employment/Salary (full or part-time):; University Medical Center Ljubljana, Zaloska 7, Sl-1000 Ljubliana, Slovenia. **A. Bernini:** None. **A. Chieregato:** A. Employment/Salary (full or part-time):; Neurorianimazione, ASST Grande Ospedale Metropolitano Niguarda. **E. Fainardi:** None. **C. Baciu:** A. Employment/Salary (full or part-time):; Azienda Socio-Sanitaria Grande Ospedale Metropolitano Niguarda. **D.J. Sharp:** Other; Expert member of UK RFU Concussion Panel. **N. Bourke:** A. Employment/Salary (full or part-time):; Imperial College London.

Digital Abstract Session

P152. Biological and Clinical Diagnostic Biomarkers of Human Traumatic Brain Injury

Program #/Poster #: P152.02

Topic: C.10. Brain Injury and Trauma

Support: DVBIC; Contract HT0014-19-C-0004
HJF/USU Award number: 308811-8.01-60855, CNRM-75-9241
NIH, National Institute of Nursing Research Intramural Research Program

Title: Neurofilament light chain and glial fibrillary acidic protein remain elevated in the chronic phase of recovery following mild, moderate and severe traumatic brain injury in military service members and veterans

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Abstract: Background: Over the last decade, almost 250,000 US service members and veterans (SMVs) have sustained a traumatic brain injury (TBI). Biomarkers that can diagnose TBI and discriminate injury severity, and are associated with long-term neurobehavioral symptom reporting, may shed light on underlying pathological mechanisms and improve clinical care. This study examines the diagnostic utility of blood levels of protein biomarkers linked to brain injury in SMVs with a history of traumatic brain injury (TBI), and examines their relationship with neurobehavioral symptom reporting following TBI. **Methods:** Participants (n=481) were enrolled in the 15-Year Longitudinal TBI Study (Defense and Veterans Brain Injury Center [DVBIC]) from 2011 to 2019, following an uncomplicated mild TBI (n=215), complicated mild TBI (n=49), moderate or severe TBI (Moderate/Severe TBI group, n=69), or orthopedic or soft-tissue injury without TBI (Injured Control group, n=148). Concentrations of neurofilament light chain (NfL), tau and glial fibrillary acidic protein (GFAP) in serum samples were measured using an ultrasensitive assay (SIMOA™). **Results:** Participants were mostly male (93%) and white (73%) with a median age of 36 (IQR=29-44). Within one year after injury, we observed higher levels of NfL in the Moderate/Severe TBI group when compared to the Injured Control ($p < 0.0001$, $d = 1.1$), Uncomplicated mTBI ($p < 0.0001$, $d = 1.02$) and Complicated mTBI ($p = 0.0333$, $d = 0.75$) groups, which was confirmed in logistic regression analysis controlling for demographics, number of TBIs and time since injury. NfL also discriminated the Moderate/Severe TBI group from Injured Control (AUC = 89.8% [95% CI: 81.2-97.9%, $p < 0.0001$), and Uncomplicated mTBI (AUC = 86.6% [95% CI: 77.4-0.94%], $p < 0.0001$) groups with high accuracy. In addition, logistic regression analysis also showed higher levels of GFAP in the Moderate/Severe TBI group when compared to Injured Control and Uncomplicated mTBI groups. One or more years post-injury, GFAP levels were higher in the Moderate/Severe TBI group in comparison to the Injured Control ($p = 0.0093$, $d = 0.66$) and Uncomplicated mTBI groups ($p = 0.0002$, $d = 0.91$). We found no significant group differences for tau at either timepoint. **Conclusion:** Blood levels of NfL, a neurofilament protein predominantly in axons, are elevated in moderate and severe TBIs in the first year after injury, but not one or more years post-injury. GFAP, an astrocytic protein, is elevated within the first-year post-injury, as well as one or more years post-injury. Our results suggest the potential of NfL and GFAP as diagnostic biomarkers in TBI.

Disclosures: V.A. Guedes: None. **R.T. Lange:** None. **E.G. Smith:** None. **R. Vorn:** None. **C. Lai:** None. **J. Leete:** None. **M.E. Glazer:** None. **M.N. Higgins:** None. **C.E. Tippett:** None. **A.M. Byrd:** None. **T.A. Brickell:** None. **S.M. Lippa:** None. **L.M. French:** None. **J.M. Gill:** None.

Digital Abstract Session

P152. Biological and Clinical Diagnostic Biomarkers of Human Traumatic Brain Injury

Program #/Poster #: P152.03

Topic: C.10. Brain Injury and Trauma

Support: Contract W91YTZ-13-C-0015/ HT0014-19-C-0004 with VHA Central Office VA TBI Model Systems Program of Research/DHA Contracting Office (CO-NCR) HT0014
United States Army Medical Research and Material Command and from the United States Department of Veterans Affairs Chronic Effects of Neurotrauma Consortium under Award No. W81XWH-13-2-0095

Title: Exosomal microRNA is associated with sleep quality in military personnel with traumatic brain injury

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Abstract: Traumatic brain injury (TBI) is highly prevalent among military personnel and commonly co-occurs with disturbed sleep and leads to adverse health outcomes. The potential of using exosomal microRNA (exomiRs) as a biomarker is of particular interest in the context of TBI, as it may aid in diagnostics, prediction of patient outcomes, patient stratification for clinical trials, and further understanding of underlying pathophysiology. We used a convenience sample from the baseline measurements of 130 post-9/11 era military personnel and veterans from the Chronic Effects of Neurotrauma Consortium Multicenter Prospective Longitudinal Study (control; *n*=22; TBI poor sleep, *n*=58; TBI good sleep, *n*=50). Participants included in this cross-sectional analysis had clinical sleep data and plasma for exomiR analysis. Sleep quality was assessed using the global score on the Pittsburgh Sleep Quality Index (PSQI) with a cut-off score of >10 to distinguish poor sleepers from good sleepers. ExomiR profiling analysis was performed using nCounter Human v3 miRNA Expression Panels with 798 miRNA probes. Kruskal-Wallis tests followed by the Dunn test and the Benjamini-Hochberg procedure for false discovery rate to correct for multiple comparisons was performed to compare exomiR between groups. Additionally, Spearman's correlations were used to determine the relationship between exomiR and behavioral/psychological scores. After normalization, exomiR expression profiled revealed a total of 8 differentially expressed exomiR among the three groups and the PSQI global score positively. Within the TBI group, the expression levels of 4 exomiRs were significant: hsa-miR-1250-5p (r=0.229, p=0.017); hsa-miR-139-5p (r=0.236, p=0.014); hsa-miR-211-3p (r=0.206, p=0.033); and hsa-miR-4516 (r=0.212, p=0.028). Overall, differentially expressed exomiRs have been reported to play a role in psychiatric disorders, progressive neurodegeneration, and vascular physiology, all of which have been associated with the remote TBI.

Disclosures: **S. Mithani:** None. **J. Leete:** None. **J. Pucci:** None. **V. Guedes:** None. **K. Kenney:** None. **J. Werner:** None. **J. Gill:** None.

Digital Abstract Session

P152. Biological and Clinical Diagnostic Biomarkers of Human Traumatic Brain Injury

Program #/Poster #: P152.04

Topic: C.10. Brain Injury and Trauma

Support: Department of Defense, Chronic Effects of Neurotrauma Consortium (CENC) Award W81XWH-13-2-0095
Department of Veterans Affairs CENC Award I01 CX001135
NIH, National Institute of Nursing Research Intramural Research Program

Title: Exosomal microRNAs and proteins as biomarkers of posttraumatic stress disorder symptoms in veterans with history of mild traumatic brain injury

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Abstract: Symptoms of post-traumatic stress disorder (PTSD) are frequently associated with history of combat-related mild traumatic brain injury (mTBI) in military populations. Finding biomarkers that relate to PTSD symptoms may aid in the identification of therapy targets for those most at risk for poor outcomes following a mTBI. This study examined relationships between levels of exosomal proteins and miRNAs in the peripheral blood and severity of PTSD symptoms in a cohort of combat-exposed service members and Veterans (SMs/Vs) with and without chronic mTBI(s). Participants (n = 141) were all enrolled in the Chronic Effects of Neurotrauma Consortium (CENC) Multicenter Prospective Longitudinal Study, now housed in the Long-Term Impact of Military Brain Injury Consortium (LIMBIC). Participants were divided into four groups based on TBI history and severity of PTSD symptoms as measured by the PTSD Checklist for DSM-5 (PCL-5). We isolated peripherally circulating exosomes and analyzed exosomal levels of 798 miRNAs (miRNAs) with nCounter® Analysis Technology, as well as exosomal levels of neurofilament light chain (NfL), tau, phosphorylated tau (p-tau), Amyloid beta (Aβ) 42, Aβ40, interleukin (IL)-10, IL-6, tumor necrosis factor-alpha (TNFα), and vascular endothelial growth factor (VEGF) by using a Simoa HD-1 analyzer. We observed group differences for exosomal NfL, a protein marker of axonal injury and degeneration ($p = 0.0082$). Pairwise comparison showed higher levels of exosomal NfL in participants with both mTBI

history and more severe PTSD symptoms (PCL-5 scores higher or equal to 50) when compared to those with mTBI and lower PCL-5 scores (p= 0.0110), as well as the control group (no mTBI and no PTSD, p = 0.0221). NfL was also positively correlated with PCL-5 scores (p = 0.0040, r = 0.407573). Pairwise comparisons also revealed 14 differentially expressed miRNAs. Specific miRNAs linked to severity of PTSD symptoms included hsa-miR-139-5p, hsa-miR-103a-3p, hsa-miR-33a-5p, hsa-miR-520f-3p, which have been associated with neurodegenerative diseases. We observed that levels of hsa-miR-139-5p (p = 0.005817, r = 0.254591), hsa-miR-2278 (p = 0.01543, r = 0.2244367), hsa-miR-1827 (p =0.01219, r = 0.2320489) and hsa-miR-11851-3p (p = 0.01014, r = 0.2378501) correlated with PCL-5 scores. Our findings suggest the potential of exosomal NfL and specific miRNAs as prognostic biomarkers in TBI, while also shedding light on possible mechanisms underlying the development of persistent PTSD symptoms in military populations.

Disclosures: V.A. Guedes: None. **C. Lai:** None. **C. Devoto:** None. **K. Edwards:** None. **B. Qu:** None. **S. Mithani:** None. **E.A. Wilde:** None. **W.C. Walker:** None. **R. Diaz-Arrastia:** None. **J.M. Gill:** None. **K. Kenney:** None.

Digital Abstract Session

P152. Biological and Clinical Diagnostic Biomarkers of Human Traumatic Brain Injury

Program #/Poster #: P152.05

Topic: C.10. Brain Injury and Trauma

Title: Validation of a clinical guideline to identify traumatic brain injury patients at risk for in-hospital mortality: Utility of the guideline for prediction of patient disposition.

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Abstract: Over 2.5 million traumatic brain injury (TBI) patients are treated in the United States each year. Rapid diagnosis of TBI and subsequent application of definitive care lead to better outcomes. We previously developed a clinical guideline using patient age, referral status, initial values of systolic blood pressure, heart rate, Glasgow Coma Scale (GCS), and injury severity score (ISS) and CT evidence of contusion and brain edema to calculate in-hospital mortality risk with a logistic regression model (J Emerg Med, 53:18-59, 2017). We now validate this guideline using patient data from Ohio, Maine, and the National Trauma Data Bank (NTDB) and demonstrate the model's utility to predict discharge disposition to home, rehabilitation, nursing home, hospice, or morgue.
TBI patient data for 2017-2019 were obtained from trauma registries at Miami Valley Hospital (Dayton, Ohio, N=2,008), Maine Medical Center (Portland, Maine, N=1,043) and the 2015 NTDB (N=189,907) under IRB-approved protocols. Statistical comparisons were made with SigmaPlot 13 using Chi Square, ANOVA, and t-test. Receiver Operating Curve (ROC) and Hosmer-Lemeshow analyses were performed using R. Significance was indicated for $p<0.05$.

Comparisons of patient data used in the clinical guideline model revealed differences between Ohio and Maine TBI populations. The Maine population was older on average than that of Ohio. The average systolic blood pressure for Maine patients was higher, but the average heart rate was equivalent to that of Ohio patients. Maine patients were more likely to arrive directly from the accident scene. Ohio and Maine populations had different distributions of GCS, ISS, and contusion while the fraction of patients with brain edema was similar. Nevertheless, the mortality rate was equivalent for Ohio, Maine, and NTDB patients (2.8%, 4.2%, 3.4%, respectively).
The guideline predicted in-hospital death with equivalent areas under the ROC (95% CI) of 0.88 (0.84-0.93), 0.91 (0.88-0.96), and 0.91 (0.91-0.92) for Ohio, Maine, and NTDB, respectively. Within ventiles of calculated risk, the model predicted observed rates of mortality. Ohio, Maine, and NTDB populations had different distributions of discharge disposition. The calculated mortality risk was different for each discharge disposition but for each disposition, the risks calculated from Ohio, Maine, and NTDB populations were similar.
We conclude our clinical guideline for calculating in-hospital mortality risk of TBI patients is valid and generalizable across a spectrum of patient populations. The guideline provides early indication of patients at risk for death and can be a tool for predicting patient disposition.

Disclosures: **J.E. Olson:** None. **M. McCarthy:** None. **J. Rappold:** None. **C. Falank:** None. **D. Seder:** None.

Digital Abstract Session

P152. Biological and Clinical Diagnostic Biomarkers of Human Traumatic Brain Injury

Program #/Poster #: P152.06

Topic: C.10. Brain Injury and Trauma

Title: Investigating the Effects of Sport-Related Concussion on Structural Brain Connectivity: Evidence for Altered Local and Global Network Efficiency During Acute Symptom Management

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Abstract: Accumulating evidence indicates that mild traumatic brain injury (mTBI) alters the structural brain connectome by reducing network efficiency at a local and global scale. However, conflicting reports exist as to the time course of these connectivity changes throughout symptom management and initial recovery. Here, we report longitudinal structural connectivity data acquired in 30 student athletes who experienced a concussion while playing NCAA Division I football. Diffusion tensor imaging (DTI) was acquired prior to the athletic season, immediately following exposure to mTBI, and after symptomatic recovery (immediately before return to play). Performing DTI tractography, we identified reliable changes in structural connectivity

between these three timepoints. We find that acute mTBI exposure is initially associated with decreased clustering and local brain network efficiency, followed by a return to baseline after injury management and clearance for return to play ($p < 0.01$; FDR-corrected). However, measures of global network efficiency do not exhibit this pattern of recovery to baseline, instead demonstrating persistent decreases even after injury management and return to play. Our results support a novel dissociation between local and global network efficiency measures of mTBI, suggesting that acute symptomatic recovery is associated with return to baseline in measures of local network efficiency, while alterations to global network efficiency may persist following injury assessment, symptom management, and return to play.

Disclosures: E.D. Anderson: None. **D. Schultz:** None. **Y. Wang:** None. **E. Carlson:** None. **L. Albers:** None. **J. Tuttle:** None. **M. Meyer:** None. **M. Neta:** None. **C.R. Savage:** None. **A.K. Barbey:** None.

Digital Abstract Session

P153. Traumatic Brain Injury: Pre-Clinical Therapeutic Strategies

Program #/Poster #: P153.01

Topic: C.10. Brain Injury and Trauma

Support: Medical Research Council, London, United Kingdom (MR/N027736/1)
Association of Paediatric Anaesthetists of Great Britain & Ireland.
Royal Centre for Defence Medicine, Birmingham, United Kingdom.
Royal British Legion Centre for Blast Injury Studies, Imperial College London, United Kingdom.
The Gas Safety Trust, London United Kingdom

Title: Xenon is neuroprotective and promotes beneficial early neuroinflammation in a rat model of severe traumatic brain injury

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Abstract: Background: Traumatic brain injury (TBI) affects both young and elderly populations and results in a significant global healthcare burden. TBI patients often suffer from costly long-term neurological and cognitive problems that reduce their quality of life and ability to work, including motor deficits such as gait abnormalities, memory impairments and anxiety. Current clinical practice for TBI patients is largely supportive, centred on non-specific endpoints such as management of tissue oxygenation, cerebral perfusion pressure and intracranial pressure[1]. At present there are no clinically validated drug treatments aimed specifically at preventing neuronal loss following TBI[2]. Xenon is a noble gas used medically as a general

anesthetic and in MRI imaging[3]. We have previously shown that xenon-treatment improves short and long-term outcomes, prevents late-onset cognitive impairments and improves survival after moderate TBI in mice[4, 5]. The aim of this study is to evaluate the efficacy of xenon in a second species, rats, and in a severe injury model.
Methods: Adult male Sprague Dawley rats (N=22) underwent controlled cortical impact (CCI) brain trauma or sham surgery. Animals were randomised to receive either 50%Xe:25%O_2:25%N_2 or 75%N_2:25%O_2. Locomotor function (CatwalkXT) and histological outcomes [lesion volume, neuronal cell count (NeuN), microglia (Iba1) and astrocytes (GFAP)] were assessed at 24 h by blinded observers.
Results: The xenon-treated group exhibited a reduction in locomotor deficits. Lesion volume was reduced in the xenon-treated group. Xenon-treatment resulted in preservation of neurons in cortical and subcortical regions that was associated with increases in numbers of resting microglia and reactive astrocytes.
Conclusions: We show for the first time that xenon is neuroprotective after severe TBI in rats. Functional improvement and neuronal preservation was associated with a xenon-induced enhancement of resting microglial cell numbers and astrocyte activation. These findings are consistent with a role for early beneficial neuroinflammation in xenon's neuroprotective effect. Xenon may be of benefit in the treatment of clinical brain trauma.
[1] Hutchinson, P.J., et al. BMJ, 2013. **346**: p f1000. [2] Warner, D.S. et al l, Anesth Analg, 2004. **99**(4): p1208-10. [3] Dickinson, R. et al Crit Care, 2010. **14**(4): p229. [4] Campos-Pires, R., et al., Crit Care Med, 2015. **43**(1): p149-158. [5] Campos-Pires, R., et al., Br J Anaesth, 2019. **123**(1): p60-73.

Disclosures: R. Campos-Pires: None. **H. Onggradito:** None. **E. Ujvari:** None. **S. Karimi:** None. **F. Valeo:** None. **J. Aldhoun:** None. **C. Edge:** None. **N. Franks:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nicholas Franks has disclosed being a named inventor on a number of patents relating to the use of xenon as a neuroprotectant. **R. Dickinson:** None.

Digital Abstract Session

P153. Traumatic Brain Injury: Pre-Clinical Therapeutic Strategies

Program #/Poster #: P153.02

Topic: C.10. Brain Injury and Trauma

Support: National Institute of Neurological Disorders and Stroke of the National Institutes of Health (R01NS109488)
Nebraska Research Initiative fund

Title: Core-cross-linked nanoparticles reduce oxidative stress and cell death following traumatic brain injury in mice

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Abstract: Following a traumatic brain injury (TBI), cell death from the initial injury releases excess reactive oxygen species (ROS) and lipid peroxidation products (LPoX). ROS and LPoX induce mitochondrial and cell membrane damage in surrounding cells causing secondary cell death and release of ROS and LPoX. There is currently no clinically available treatment shown to reduce secondary injury, which might be due to poor retention of small molecule drugs in TBI. However, the blood-brain barrier permeability following TBI opens the opportunity for improved retention of nanoparticles (NPs). Here, we used a controlled cortical impact (CCI) mouse model of TBI (4 m/s impact velocity, 2.5 mm impact depth) on 6-wk old male C57BL/6J mice; animal procedures were approved by IACUC. We immediately administered core-cross-linked NPs (ANPs, 0.2 mg IV) with similar properties to our previously reported NPs (24 nm) synthesized through thiol-ene and thiol-Michael reactions and dialysis-purification that rely on their thioether bond to scavenge ROS (9.93 μmol/mg NPs). MnTMPyP (0.5 mg IP), an antioxidant small molecule drug and no additional treatment were used for comparisons. To compare ROS levels following CCI, we administered dihydroethidium (DHE, 625 μg IV) at 3 h post-CCI and harvested the brain at 4 h post-CCI for confocal microscopy. The DHE fluorescence mean intensity at the perilesional was normalized to the contralateral hemisphere. We found a significant increase in ROS in the CCI mice (mean ± SEM: 1.44 ± 0.06) compared to the CCI+MnTMPyP mice (1.19 ± 0.05, $p<0.05$) and the CCI+ANP mice (1.03 ± 0.07, $p<0.01$), as determined by one-way ANOVA and Tukey's post hoc test; n = 3 for each treatment group. To compare cell death following CCI, we performed Western blot of α-II-spectrin breakdown products (SBDPs) that correlate to total cell death (150 kDa), necrosis (145 kDa), and apoptosis (120 kDa) from perilesional brain lysate at 3 d post-CCI; SBDPs were normalized to β-actin and SBDPs in the control mice. We found a significant increase in 150 and 145 kDa SBDPs of the CCI mice (2.31 and 5.11-fold increase) compared to the CCI+ANP mice (1.42 ($p<0.001$) and 3.76-fold increase ($p<0.05$)), while there was no significant difference of 120 kDa SBDP across the treatment groups, as determined by two-way ANOVA and Tukey's post hoc test; n = 3 for each treatment group. Our results suggest that ANP treatment reduces ROS and necrosis in the CCI mouse model of TBI likely because of better ANPs retention in TBI. ANPs are a promising candidate to reduce ROS, LPoX, and secondary injury in TBI.

Disclosures: A.W. Tarudji: None. **A.M. Priester:** None. **A.J. Convertine:** None. **F.M. Kievit:** None.

Digital Abstract Session

P153. Traumatic Brain Injury: Pre-Clinical Therapeutic Strategies

Program #/Poster #: P153.03

Topic: C.10. Brain Injury and Trauma

Support: NS094950

NS099683
NS110609

Title: Positive allosteric modulation of alpha7 nicotinic acetylcholine receptors enhances sustained attention and decreases anxiety-like behavior after experimental traumatic brain injury

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Abstract: Introduction: Traumatic brain injury (TBI) is a leading cause of cognitive disability. Although long-term impairments in higher-order executive functioning such as sustained attention and goal-directed behavior are common clinically, they are not well established in preclinical models of TBI. Additionally, TBI-associated psychopathologies like anxiety and depression can further exacerbate cognition. Moreover, decreased acetylcholine (ACh) after TBI is known to cause cognitive impairment and thus pharmacological strategies that enhance ACh may ameliorate cognitive disruptions. Hypothesis: NS-1738, a novel positive allosteric modulator at the α7 nicotinic ACh receptors will restore sustained attention and reduce affect after TBI. Methods: Anesthetized adult male rats received either a controlled cortical impact of moderate severity (2.8 mm cortical deformation at 4m/s) or sham injury and then were randomly assigned to NS-1738 (3 mg/kg; i.p.) or vehicle (1.0 mL/kg; i.p.). Treatments began 24 h post-surgery and were given once daily for 7 days. Behavioral assessments were evaluated utilizing a complex and novel battery of paradigms. Specifically, assessment of sustained attention and impulsivity was carried out on post-operative days 14-24 using the 3-choice serial reaction time task (3-CSRT), which is analogous to the Continuous Performance Test often used in the clinical setting. Anxiety-like responses (open field test; OFT) and passive/active avoidance (shock probe defensive burying test; SPDB) were conducted on post-operative days 28 and 29, respectively. Results: The preliminary data suggest that NS-1738 ameliorates TBI-induced deficits in the 3-CSRT and the OFT, while also enhancing measures of active coping in the SPDB, which supports the hypothesis and promotes a novel pharmacotherapy for attenuating cognitive deficits and affect disorder for TBI patients. Statistical analyses comprise of repeated measures ANOVAs followed by Newman-Keuls post hoc tests, where appropriate. Conclusion: Subacute augmentation of cholinergic function after TBI may be efficacious at restoring neurobehavioral performance. Significance: Integrating animal models of higher-order attention, motivation, and anxiety in the battery of laboratory tests after TBI, as well assessing cholinergic regulation in cortical regions mediating these outcomes is critical for developing novel treatment approaches that can be successfully translated to the clinic.

Disclosures: E.H. Moschonas: None. **R. Reddy:** None. **C. Sunleaf:** None. **T. Ranellone:** None. **A. Benbourenane:** None. **A. Patel:** None. **S. Balaji:** None. **T. Magdelinic:** None. **S. Sha:** None. **J. Cheng:** None. **C.O. Bondi:** None.

Digital Abstract Session

P153. Traumatic Brain Injury: Pre-Clinical Therapeutic Strategies

Program #/Poster #: P153.04

Topic: C.10. Brain Injury and Trauma

Support: This project is supported by the Academy of Finland and co-funded by Horizon 2020 Framework Programme of the European Union (Marie Sklodowska Curie grant agreement No 740264)

Title: Systems biology-based drug repurposing to improve recovery from traumatic brain injury: in vitro and in vivo validation

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Abstract: Background: Almost 50 million people suffer from traumatic brain injury (TBI) each year and over 40% TBI survivors develop long-term post-TBI complications such as epileptogenesis. To date, no treatment can alleviate progression of TBI sequalae, and identification of novel treatments to improve post-TBI outcome is a major unmet medical need. Hypothesis: Systems biology approach will offer an unbiased approach to identify and select drugs for repurposing to enhance recovery from TBI by promoting neuroprotection and alleviating inflammation. Objective: This study aims at mitigating the severity of pathological and functional post─TBI outcomes by using systems biology -identified drugs. Methods: Selected drugs were tested in cortical neuron-BV2 microglia co-cultures to assess in vitro efficacy of the drugs on: (a) Neuronal survival using microtubule associated protein 2—based neuronal survival assay (b) Neuroprotective potential from nitric oxide mediated neurotoxicity using Griess reagent-nitrite assay (c) Anti-inflammatory effect using tumour necrosis factor alpha ELISA. Preliminary results: Our in vitro results revealed that 50 nM of trichostatin and 100 µM of FBA improved neuronal survival by 72.9% (p=0.000) and 50.6% (p=0.0007) respectively. At 50 nM trichostatin reduced nitrite levels to 27.6% (p=0.000) and 100 µM FBA reduced nitrite levels to 47% (p=0.000). Neuroinflammation was reduced to -139% (p=0.000) by 50 nM trichostatin and to -23.2% (p=0.0084) by 100 µM FBA. Conclusion and prospects: Our in vitro findings suggest that systems biology approach helps identification of drugs that have a potential to treat post-TBI complications. In vivo experiments in a clinically relevant rat model of TBI are currently ongoing to assess the therapeutic effect of drugs that showed the best efficacy in in vitro testing. If effective, these drugs could promote the recovery of TBI patients and prevent post-traumatic epileptogenesis.

Disclosures: **N.P.T. Kajevu:** None. **A. Lipponen:** None. **N. Puhakka:** None. **T. Natunen:** None. **M. Hiltunen:** None. **A. Pitkänen:** None.

Digital Abstract Session

P153. Traumatic Brain Injury: Pre-Clinical Therapeutic Strategies

Program #/Poster #: P153.05

Topic: C.10. Brain Injury and Trauma

Support: NIH/NIA 1R01AG049722

Title: A Rodent Model of Traumatic Brain Injury Impairs Object-Place Paired-Associates Learning

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Abstract: Approximately 2.8 million Americans suffer from a traumatic brain injury (TBI) each year. As a result, TBI disables six times more people annually than breast cancer, multiple sclerosis, spinal cord injuries, and HIV/AIDS combined (Colantonio et al., 2009). Animal models continue to be of paramount importance in elucidating the mechanisms of dysfunction following TBI. Here, we used a rodent model of closed cortical impact (CCI) to assess cognitive dysfunction following either frontal (n=6) or parietal (n=6) cortical injury, and frontal (n=2) or parietal (n=2) sham. Importantly, our assessment of cognitive dysfunction is through a rodent adaptation of a clinically relevant task: object-location paired-associates learning (PAL) (Bussey et al., 2008). PAL is a part of the Cambridge Neuropsychological Test Automated Battery (CANTAB), which uses a variety of assessments to examine cognitive dysfunction in disease states (Robbins & Sahakian, 1994). In humans, the CANTAB PAL task is used to assess cognitive deficits in object and spatial recognition in diseases such as schizophrenia, Alzheimer's, and TBI. To date, our rodent version of PAL has not yet been employed in a rodent model of TBI, and its usage will be important for understanding clinical consequences of cognitive impairment post-injury and for testing the efficacy of potential treatments and rehabilitation strategies. We found that parietal injury impaired performance on PAL compared to sham controls, but frontal injury did not. This was indicated by a greater number of days to criterion (t_6= 3.56; p = 0.01) as well as a larger number of errors post-surgery on the PAL task (t_6 =3.61; p = 0.01). An in-depth trial-by-trial analysis of bias (tendency to stick with an object/place regardless of correctness) and strategy usage (tendency to utilize heuristics such as staying with an object that was correct in a previous trial even though it is no longer correct) was also performed. There was no significant difference in place or object bias between injury groups (frontal vs. parietal, injury vs. sham control), indicating that performance decreases in the parietal injury group were not due to differences in bias. The current data show the utility of PAL for quantifying TBI-related cognitive dysfunction and future studies will test intervention strategies for mitigating TBI-induced cognitive decline.

Disclosures: **S.M. Smith:** None. **N. Ferekides:** None. **A. Hernandez:** None. **S. Lovett:** None. **J.F. Abisambra:** None. **S.N. Burke:** None.

Digital Abstract Session

P154. Spinal Cord Injury: Cellular and Molecular Mechanisms

Program #/Poster #: P154.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant NS10052
NIH Grant NS104177
NIH Grant NS08220
NIH Grant NS108521
NIH Grant NS115523

Title: Loss of mammalian target of rapamycin in NG2 cells reduces acute white matter sparing and locomotor recovery after spinal cord injury

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Abstract: Chronic demyelination slows conduction velocity and impairs recovery after spinal cord injury (SCI), and is partially due to the loss of oligodendrocytes (OLs). Surviving OLs are post-mitotic and thought to not remyelinate spared axons; thus, the robust endogenous repair that occurs after SCI is mainly from NG2+ glial cells that proliferate then differentiate into mature OLs. Since NG2 cells are the primary source of new myelin, it is critical to understand the mechanisms that regulate NG2 cell function post-injury. Previous work from developmental and chemical demyelination studies show that the mammalian target of rapamycin (mTOR) is necessary for OL differentiation and myelination. However, whether mTOR impacts NG2 cell function and recovery after SCI is unknown. Here, we tested the hypothesis that **mTOR activation in NG2 cells promotes OL differentiation and myelination after SCI.** In rat and mouse SCI models, we show mTOR activation in NG2 cells rises for 7-14 days post-injury (dpi) and declines to baseline thereafter. Using conditional transgenic mice, mTOR deletion in NG2 cells resulted in fewer mature OLs, decreased white matter sparing, and worse locomotor recovery at 14 dpi. However, if mice were allowed to survive to 35 d or 84 dpi, chronic OL genesis equalized the ultimate number of OLs, extent of myelination, and locomotor recovery compared to controls. Overall this work suggests that mTOR signaling is important in the acute SCI setting for maintaining OLs and myelin, but it is dispensable for long-term OL genesis and remyelination. These data highlight that cellular mechanisms driving cell repair after SCI fluctuate over time and suggest mTOR-directed therapies should be cognizant of possible effects on myelination in the acute SCI setting.

Disclosures: **N. Pukos:** None. **M.A. Jeffries:** None. **T.L. Wood:** None. **D.M. McTigue:** None.

Digital Abstract Session

P154. Spinal Cord Injury: Cellular and Molecular Mechanisms

Program #/Poster #: P154.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: ERA-Net NEURON
FRQS

Title: Tlr4 signaling in spinal cord injury contributes to neuroinflammation, alterations in extracellular matrix, and reduced locomotor recovery.

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Abstract: Tissue damage after spinal cord injury (SCI) triggers inflammation and alteration in extracellular matrix (ECM). Toll-like receptors (TLRs) are key mediators of innate immunity. They respond to molecules released by damaged tissue, which trigger and subsequently amplify inflammation that may cause further damage to cells and ECM. We studied the role of TLR4 on inflammation and ECM composition after SCI by comparing wildtype (WT) and TLR4 knockout (KO) mice. We also aimed to find whether TLR4 has effects on neuronal survival, myelination and locomotor recovery. To do this, spinal cord contusion injuries were done in female TLR4 KO and WT mice using the Infinite Horizon impactor. mRNA levels of proinflammatory chemokines and cytokines showed no differences between genotypes at 7 d post injury (PI) but they were reduced in KO mice at 8 weeks PI. Immunofluorescence staining of chondroitin sulfate proteoglycans (CSPG), a key component of the ECM, showed a small but significant reduction in TLR4 KO mice. In addition, CSPG deposition is located in closer proximity to macrophages than to microglia. We also observed significant increase in labeling with Wisteria floribunda agglutinin (WFA), a marker of perineuronal nets (PNN), and Aggrecan, a major constituent of PNNs, in TLR4 KO mice at 8 weeks after SCI. These changes were accompanied with significant decrease in mRNA and protein expression of matrix metallopeptidase 9 (MMP9). In addition, there was a significant reduction of neuronal and myelin loss in TLR4 KO mice at 8 weeks PI. mRNA and protein expression of key necroptotic markers were notably reduced in KO mice at this timepoint. There was a significant improvement of locomotor recovery in TLR4 KO mice at 8 weeks. Taken together, our results highlight that TLR4 signaling is detrimental to recovery at later time points after SCI.

Disclosures: F. Ryan: None. **E.J. Bradbury:** None. **S. David:** None.

Digital Abstract Session

P154. Spinal Cord Injury: Cellular and Molecular Mechanisms

Program #/Poster #: P154.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS 2R01NS079702-06
NINDS R01NS110385
NINDS R56NS096028
Paralyzed Veterans of America Research Foundation grant #3054

Title: Modulating the EphB2-NMDA receptor interaction in the superficial dorsal horn attenuates neuropathic pain following cervical spinal cord injury

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Abstract: A large percentage of spinal cord injury (SCI) patients suffer from chronic neuropathic pain (NP). Importantly, pharmacological therapies for NP often are ineffective or have severe adverse effects, highlighting the critical need to identify novel, effective treatments. Central sensitization, or the hyperexcitability of dorsal horn circuitry, is a major substrate for SCI-induced NP. Studies have shown that NP is linked to EphB/ephrinB signaling through potentiation of NMDAR function, suggesting that the EphB-NMDAR interaction may be an important therapeutic target for NP. We previously reported that EphB2 activation stimulates a direct interaction between EphB2 and the NMDAR via the extracellular Y504 residue of EphB2, thus promoting NMDAR synaptic localization and excitatory synapse function. We hypothesized that modulating the EphB2-NMDAR interaction via knockdown of EphB2 would attenuate NP by preventing EphB2-NMDAR localization at excitatory synapses. Our rodent model of unilateral cervical contusion shows a persistent NP phenotype in the form of forepaw thermal hyperalgesia and mechanical allodynia, as well as spontaneous, supraspinal aspects of pain as assessed by the grimace test. Using this model, we find increased EphB2 expression in the dorsal horn two weeks post-injury, as well as increased colocalization of EphB2 and GluN1 (NMDAR subunit) at vGlut2-positive synapses in dorsal horn neurons after cervical SCI, indicating an enhanced interaction between EphB2 and NMDARs at putative excitatory glutamatergic synapses. Furthermore, RNAscope *in situ* hybridization analysis revealed upregulated EphB2 mRNA expression in neurons 2 weeks post-SCI, and specifically upregulated EphB2 mRNA in *tacr*1+ neurons in the superficial dorsal horn. When we knockdown expression of EphB2 7 days post-SCI using an shRNA-EphB2 lentivirus delivered via intraspinal DH injections, we are able to reverse the already-established NP-like phenotype of thermal hyperalgesia. Collectively, these findings suggest that enhanced EphB2-NMDAR interaction underlies alterations in excitatory synaptic transmission in the dorsal horn and consequently persistent NP following SCI.

Disclosures: **N.M. Heinsinger:** None. **W. Zhou:** None. **J.L. Watson:** None. **A. Falnikar:** None. **R. Cain:** None. **G. Spagnuolo:** None. **R.V. Allahyari:** None. **D. Jaffe:** None. **T. Fox:** None. **E.V. Brown:** None. **B.A. Charsar:** None. **M.B. Dalva:** None. **A.C. Lepore:** None.

Digital Abstract Session

P154. Spinal Cord Injury: Cellular and Molecular Mechanisms

Program #/Poster #: P154.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Deutsche Forschungsgemeinschaft

Title: Transneuronal delivery of hyper-interleukin-6 enables functional recovery after severe spinal cord injury in mice

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Abstract: Spinal cord injury (SCI) often causes severe and permanent disabilities due to the regenerative failure of severed axons. Here we report significant locomotor recovery of both hindlimbs after a complete spinal cord crush. This was achieved by the unilateral transduction of cortical motoneurons with an AAV expressing hyper-IL-6 (hIL-6), a potent designer cytokine stimulating JAK/STAT3 signaling and axon regeneration. We find collaterals of these AAV-transduced motoneurons projecting to serotonergic neurons in both sides of the raphe nuclei. Hence, the transduction of cortical neurons facilitates the axonal transport and release of hIL-6 at innervated neurons in the brain stem. Therefore, this transneuronal delivery of hIL-6 promotes the regeneration of corticospinal and raphespinal fibers after injury, with the latter being essential for hIL-induced functional recovery. Thus, transneuronal delivery enables regenerative stimulation of neurons in the deep brain stem that are otherwise challenging to access yet highly relevant for functional recovery after SCI.

Disclosures: M. Leibinger: None. **C. Zeitler:** None. **P. Gobrecht:** None. **A. Andreadaki:** None. **G. Gisselmann:** None. **D. Fischer:** None.

Digital Abstract Session

P154. Spinal Cord Injury: Cellular and Molecular Mechanisms

Program #/Poster #: P154.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation (# 546401)

Title: Expression of gastrin releasing peptide receptors in the spinal ejaculation generator and associated lumbosacral autonomic and motor nuclei after chronic spinal cord injury in male rats.

Authors: W. A. NOFTZ, S. GAIKWAD, T. ETTEY, E. MAUPIN, L. M. COOLEN;
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Abstract: Spinal cord injury (SCI) in men is commonly associated with sexual dysfunction, including anejaculation, and chronic mid-thoracic contusion injury in male rats also impairs ejaculatory reflexes. Ejaculation is controlled by a spinal ejaculation generator (SEG) consisting of a population of lumbar spinothalamic (LSt) neurons, which control ejaculation through release of neuropeptides including galanin and gastrin releasing peptide (GRP) and axonal connections with lumbar and sacral autonomic neurons in sympathetic and parasympathetic nuclei and motor neurons within the sacral nucleus of the bulbocavernosus. In addition, LSt cells are interconnected, while the functional significance of these interconnections is unknown. It was recently demonstrated that mid-thoracic spinal contusion injury in male rats caused reduction of GRP-immunoreactivity and mRNA in LSt cells, while GRP-containing axon projections were unaffected. Moreover, intrathecal infusions of GRP can facilitate ejaculation in SCI males with similar or even greater effects in SCI males compared to sham controls. The current study examined the expression of GRP receptors (GRPR) within LSt cells and autonomic and motor targets and tested if SCI affects GRPR expression. Male rats received mid-thoracic contusion or sham injury and spinal cords were collected and sectioned 4 weeks later. *Galanin, ChAT* and *GRPR* mRNA were visualized in the lumbar spinal cord using fluorescent in situ hybridization using RNAscope. Preliminary analysis showed higher expression of *GRPR* in LSt neurons compared to autonomic and motor targets. Expression of *GRPR* was observed in 25-56% of *ChAT* cells in autonomic and motor targets, where SCI had no effect on *GRPR* or *ChAT* mRNA, except transcript levels in the central autonomic nucleus. *GRPR* was expressed in 90-100% of LSt neurons, where SCI reduced *galanin* but not *GRPR* transcript levels. These results suggest that GRP released from LSt cells can influence ejaculation via actions on subpopulations of lumbosacral autonomic and motor nuclei, and on the LSt cell population. In addition, lack of changes of GRPR receptors after SCI further support development of GRP as a potential novel treatment for sexual dysfunction after SCI.

Disclosures: W.A. Noftz: None. **S. Gaikwad:** None. **T. Ettey:** None. **E. Maupin:** None. **L.M. Coolen:** None.

Digital Abstract Session

P154. Spinal Cord Injury: Cellular and Molecular Mechanisms

Program #/Poster #: P154.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: RTI2018-095872-B-C21/ERDF
Fundació Marató TV3 2017/refs.20172230
Fundació Marató TV3 2017/refs.20172231 and 20172110
INNVAL10/19/047

Title: Transcriptional study of the camp signaling after spinal cord injury (sci). intervention of neural progenitor cell therapy in the restauration of the sci-induced pathway alterations

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Abstract: Spinal cord injury (SCI) is a devastating condition that leads to the loss of locomotor function. Currently, there are no effective treatment for SCI and the subsequent paralysis [1]. The search of an efficient treatment is a challenging task due to the intricated nature of the pathological processes derived from SCI. In order to understand the time-depending transcriptional profile changes after SCI, we performed an analysis of the transcriptome of the spinal cord tissue of rats subjected to severe traumatic SCI. The animals were sacrifice at 4 different time points: 1 week (considered as acute stage), 2 weeks (sub-acute stage) and at 4 and 8 weeks (chronic stages). Moreover, we explored the beneficial molecular mechanism driven by neural progenitor cells (NPCs) transplantation through the analysis of the transcriptome of transplanted animals. We found that several genes belonging to the cAMP signaling pathway (such as EPAC2, BDNF or CAMKK2) are severely downregulated along the different stages after SCI. Interestingly, the NPCs transplantation is able to achieve an increase in mRNA levels of EPAC2, in the area immediately rostral to the injury, and BDNF and CAMKK2 at the injury site. These transcriptional changes enlightened by the microarray analysis were further validated by RT-qPCR. In order to explore the functional relevance of EPAC2 functions over the therapeutical effect of the NPCs therapy we performed an in vivo experiment combining NPCs transplantation with intrathecal administration of EPAC2 agonist/antagonist (S-220/ESI-05). Animals that received NPCs+DMSO, NPCs+S-220 or NPCs+ESI-05 had significantly increased levels of cAMP rostral to the lesion ($p<0.05$) compared to untreated or S-220 treated animals, what suggests that cAMP augmentation is caused solely by NPCs therapy. Additionally the histological quantification of ED-1 showed that NPCs+S-220 treated animals had a reduced ED1+ area what suggest that the NPCs transplantation combined with the activation of EPAC2 lead to a reduced microglia and macrophage infiltration/activation in the injured spinal cord.

Disclosures: V. Moreno: None. **B. Martinez-rojas:** None. **E. Giraldo:** None. **R. Grillo:** None. **F. Garcia:** None. **M. Hidalgo:** None.

Digital Abstract Session

P154. Spinal Cord Injury: Cellular and Molecular Mechanisms

Program #/Poster #: P154.07

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DOD SCI 150225 to LMC

Title: Chronic contusion injury decreases glutamatergic axonal inputs to spinal ejaculation generator in rats: Time course

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Abstract: Chronic spinal cord injury (SCI) results in sexual dysfunction in men and rats, including anejaculation. Ejaculation is controlled by a spinal reflex generator in the lumbosacral spinal cord. This generator consists of a population of lumbar spinothalamic (LSt) cells, located in spinal levels L3-4 and which convert the sensory inputs during sexual activity into a coordinated autonomic and motor output required for ejaculation. Sensory inputs require glutamate-induced activation of NMDA receptors in LSt cells. We previously demonstrated that contusion spinal injury at T5-6 severely disrupted ejaculatory reflexes triggered by stimulation of the dorsal penile nerve in male rats. Therefore, we hypothesize that SCI impaired relay and processing of sensory signals required to trigger ejaculation. Indeed, we showed that contusion injury significantly reduced glutamatergic inputs to LSt cells, evidenced by a significant reduction of axon terminals expressing vesicular glutamate transporter 1 or 2 (VGlut1 or VGlut2). In the current study, we tested the time course by which SCI caused reduction in glutamatergic inputs to LSt cells. Male Sprague Dawley rats received a contusion injury at spinal levels T6 and were perfused 1 day, 1, 2, or 4 weeks later. Controls consisted of animals with sham surgery or no surgery and were perfused at the same time intervals. Spinal cords were immuno-stained for galanin (LSt cell marker) and VGlut1 or VGlut2. Quantitative confocal analysis of putative synaptic inputs to LSt cells showed that SCI significantly reduced VGlut1- and VGlut2-inputs to LSt cells at all time points with increasing further loss of VGlut2-inputs with each time point, while loss of VGlut1 reached statistical significance only at the later time points. In addition, it was determined that loss of glutamatergic inputs wasn't specific to LSt cells, and reductions of VGlut1- and VGlut2-axons were noted throughout the medial laminae at lumbar 3-4 spinal levels. These reductions were again noted at all time points for VGlut2-axons, while reaching statistical significance only at the later time points for VGlut1-axons. Together, these data demonstrate an immediate and long-lasting impact of SCI on glutamatergic inputs to LSt cells and neighboring cells, which may in turn contribute to sexual dysfunction following SCI.

Disclosures: N.P. Ritchey: None. **N.J. Mudrak:** None. **L.M. Coolen:** None.

Digital Abstract Session

P155. Spinal Cord Injury, Repair and Recovery

Program #/Poster #: P155.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH/NINDS NS069551 (Thompson)
NYS SCIRB C029131 (Thompson)
NIH/NICHD P2C HD086844 (Kautz)
NIH/NIBIB P41 EB018783 (Wolpaw)
NYS SCIRB C33279GG (Wolpaw)

Title: The Evoked-Potential Operant Conditioning System (EPOCS)

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Abstract: The Evoked Potential Operant Conditioning System (EPOCS) is an experimental system for improving muscle control in people who have neuromuscular disorders—for example, to improve gait in people who have difficulty walking following an incomplete spinal-cord injury. It works by monitoring the state of specific target muscles (via EMG signals if standing, or by gait cycle measurements if walking on a treadmill), and automatically triggering electrical or mechanical stimulation when the conditions are conducive. It provides two forms of feedback that a researcher or therapist can use to train a patient to increase or decrease the size of the resulting evoked response (for example, the soleus H-reflex). The first feedback channel continuously monitors the amplitude of the ongoing EMG in the target muscle, guiding the patient into a consistent state that is conducive to stimulation; the second channel provides immediate trial-by-trial feedback of the reflex size, relative to the distribution of previous reflexes and the session target. The approach has been shown to be able to improve walking in people with spastic gait due to incomplete spinal-cord injury (Thompson et al., J. Neurosci. 2013).

EPOCS consists of custom-written software paired with carefully chosen and configured hardware. The software is based on BCI2000 (Schalk et al., IEEE Trans. Biomed. Eng. 2004), a widely-used open-source software platform that allows biological signals to be measured and processed in real time for applications in research and in translational clinical neurotechnology. EPOCS replaces BCI2000's usual graphical user interface with a "skin" more suitable for a clinical research workflow. The hardware consists of a biopotential amplifier and data acquisition board capable of delivering surface-electromyography signals together with an auxiliary input, and a constant-current electrical stimulator that can deliver a brief pulse transcutaneously to a peripheral nerve to elicit a reflex. The system can also be configured to trigger a mechanical stimulator (for conditioning natural stretch reflexes) or a transcranial magnetic stimulator (for investigating the role of responses generated by the brain rather than reflexes from the spinal cord).

The software is offered by the National Center for Adaptive Neurotechnologies as part of our NIH-funded core mission to disseminate neurotechnology. It is available without charge to clinical researchers who possess compatible equipment, in conjunction with training. Further information is available at https://neurotechcenter.org/epocs .

Disclosures: **N. Hill:** None. **A. Eftekhar:** None. **K. Luu:** None. **J. Norton:** None. **S. Devetzoglou-Toliou:** None. **J. Carp:** None. **T. Vaughan:** None. **J.R. Wolpaw:** None. **A.K. Thompson:** None.

Digital Abstract Session

P155. Spinal Cord Injury, Repair and Recovery

Program #/Poster #: P155.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: New York State Department of Health Spinal Cord Injury Research Program C32095GG
New York State Department of Health Spinal Cord Injury Research Program C32248GG
NIH/NICHD R01HD100544

Title: Alterations in motor output of multisegmental spinal responses in individuals with SCI after transspinal-transcortical paired associative stimulation combined with locomotor training

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Abstract: Activity-based rehabilitation combined with other therapeutic strategies can promote more robust recovery of sensorimotor function after spinal cord injury (SCI). Transspinal-transcortical paired associative stimulation is a promising neuromodulation strategy that can alter both spinal and corticospinal excitability. In this study, we investigated the changes in the phase-dependent modulation of transspinal stimulation-evoked potentials (TEPs) over multiple spinal segments following transspinal conditioning stimulation, as well as the phase-dependent modulation pattern of the transspinal evoked potentials (TEPs) during robot-assisted stepping in individuals with SCI. In this study, we delivered paired transspinal and transcortical stimuli at an interstimulus interval in which TMS was delivered after transspinal stimulation in seven individuals with chronic motor incomplete and complete SCI (n=1 AIS A, n=1 AIS B, n=2 AIS C, n=2 AIS D) during Lokomat robotic gait training. Paired stimuli were delivered during the stance phase of the step cycle, triggered based on foot switches. Participants received at least 20 sessions (1h/day, 5 days/week) of locomotor training (avg.=25.6). For transspinal stimulation, the cathode electrode was placed over T10-L1, and two anode electrodes were placed bilaterally on the abdominal muscles, while for transcortical stimulation a double cone coil was placed over M1. Both stimuli were delivered at threshold intensities that evoked cortical or spinal motor responses in the soleus muscle. TEPs were recorded bilaterally from locomotor muscles during Lokomat stepping before and after the intervention. TEPs were recorded randomly across the step cycle, which was divided equally into 16 bins with bin 1 corresponding to heel contact, and bins 9 and 16 corresponding approximately to stance-to-swing and swing-to-stance transition, respectively. TEPs during walking were normalized to the homonymous maximal TEP recorded during stepping. The TEPs of the right medial gastrocnemius and medial hamstrings were depressed in the swing phase and the right soleus was depressed during heel contact. In addition,

TEPs from the left soleus, peroneus longus, and vastus lateralis were facilitated in the swing phase while the vastus lateralis was facilitate during the stance phase. We conclude that transspinal-transcortical paired associative stimulation combined with locomotor training may be utilized as a means to alter spinal motor output in people with SCI.

Disclosures: T.S. Pulverenti: None. **M. Knikou:** None. **M. Zaaya:** None. **E. Grabowski:** None. **M. Grabowski:** None. **M.A. Islam:** None.

Digital Abstract Session

P155. Spinal Cord Injury, Repair and Recovery

Program #/Poster #: P155.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Tim Reynolds Foundation

Title: Isolating stimulation artifact to identify motor response during walking

Authors: *K. MOMENI[1], R. PILKAR[2], M. RAVI[1], F. ZHANG[1], G. F. FORREST[1];
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Abstract: Non-invasive spinal cord targeted transcutaneous spinal stimulation (scTS) is applied to one or multiple spinal segments for neuromodulation targeting spinal circuitry. Surface electromyography (sEMG) provides a sensitive technique for analyzing motor activity during scTS. However, the overpowering presence of artifact in the electromyography signal due to scTS limits the ability to measure motor response during different neuromodulation techniques. Recent advances in biomedical signal processing have yielded specific detailed algorithms to successfully remove electrical stimulation artifacts from EMG signals during stimulation. We have previously shown the effectiveness of a custom-developed computational algorithm, utilizing empirical mode decomposition (EMD) and notch filtering, to remove the neuromuscular electrical stimulation artifact from sEMG recordings. In this investigation, we extend our analysis to demonstrate the effectiveness of our custom-developed computational algorithm to isolate the scTS artifact during walking overground in an individual with incomplete spinal cord injury. Our group has been piloting the intervention of scTS with overground and/or exoskeleton training and showing that modulation of spinal networks leads to increased inter-limb reciprocal lower-limb muscle activation. This investigation will help with the modification of individualized scTS parameters to achieve task-specific neuromodulatory effects.

Disclosures: K. Momeni: None. **R. Pilkar:** None. **M. Ravi:** None. **F. Zhang:** None. **G.F. Forrest:** None.

Digital Abstract Session

P155. Spinal Cord Injury, Repair and Recovery

Program #/Poster #: P155.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Tim Reynolds Foundation

Title: Analysis of Transcutaneous Spinal Stimulation induced EMG responses during spinal mapping after SCI.

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Abstract: Activity based training therapies have shown recovery for individuals with SCI. However, more recently, researchers have shown that combining electrical stimulation with the training therapies can lead to significant functional recovery for tasks such as standing and walking. More studies have shown recently that even after an SCI, the spinal sensorimotor networks can be activated when electrical stimulation is applied on the corresponding spinal cord segments. One challenge in using non-invasive spinal stimulation techniques is finding the optimal parameters of stimulation - specifically, frequency and amplitude. We systematically applied non-invasive spinal cord Transcutaneous Spinal Stimulation (scTS) at various locations between C4/5 and Coccyx with anodes placed on the anterior iliac crests and EMG was recorded from lower limb and abdominal muscles. Stimulation waveforms were either monophasic, biphasic or rectified. Carrier-wave frequency was set at 5 kHz for monophasic and biphasic modes of stimulation whereas there was no carrier frequency in the rectified mode. Systematic modulations of amplitude and frequency induced specific EMG responses for different spinal roots. The responses were analyzed by latency - Early Responses (<5ms after stimulation pulse), Middle Responses (5-15ms) and Late Responses (>15ms). Maximum Peak to Peak amplitude (averaged across a time period of at least 3 seconds) was used to compare different muscles and roots. Data was analyzed both normalized and non-normalized. Results show that analyzing the stimulation induced EMG responses during a spinal mapping session, especially the late responses, could be beneficial in finding optimal scTS parameters of stimulation to target specific muscles through existing sensorimotor networks after SCI.

Disclosures: M. Ravi: None. **K. Momeni:** None. **R. Pilkar:** None. **G.F. Forrest:** None. **F. Zhang:** None.

Digital Abstract Session

P155. Spinal Cord Injury, Repair and Recovery

Program #/Poster #: P155.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS096971

Title: Trunk M1 plasticity differences between rehabilitation assisted recovery and spontaneous recovery after SCI

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Abstract: Trunk M1 plays a critical role in the recovery of weight supported stepping after complete mid-thoracic spinal cord injury. After partial SCI, there is a spontaneous recovery of weight supported stepping that can be further enhanced by physical rehabilitation. The difference in cortical plasticity associated with rehabilitation and spontaneous recovery of function is unknown. Hence, we examined trunk M1 reorganization in a clinically relevant injury model where there is scope for substantial spontaneous recovery of function. In this study, the role of physical rehabilitation on locomotor recovery and cortical reorganization was assessed in female Sprague Dawley rats (n=31) with a T10 mid-thoracic contusion (150 Kilodynes). In a subset of animals (SCI-EX, n=15), quadrupedal treadmill exercise therapy was initiated 1-week post-injury for 30 min, five days/week for five weeks post SCI. Locomotor recovery (BBB) was assessed once a week for five weeks post-injury. At week 6, all animals underwent motor cortex mapping (ICMS). The likelihood to activate trunk musculature was evaluated through visual observation and EMG recordings within the trunk and hindlimb M1, at the threshold and suprathreshold currents (100μA). Exercise therapy significantly improved locomotor recovery such that the injured animals could take weight supported steps with consistent co-ordination. SCI shifted trunk M1 Caudal and lateral towards HLM1 with a higher likelihood to activate trunk musculature in HL M1, regardless of therapy. After SCI, there was a loss of cortical control of trunk muscles in putative lower thoracic trunk M1 (LTM1). This loss was rescued by exercise therapy. In LTM1, the likelihood to recruit lower thoracic trunk muscles with ICMS was similar regardless of therapy. However, the recruitment strategy differed. In SCI-EX animals, ICMS exclusively activated lower thoracic trunk musculature below the lesion. In contrast, animals that did not receive therapy (SCI-NX) had a higher likelihood to co-activate with mid-thoracic musculature. Within LTM1, SCI-NX animals also had a higher probability of activating mid-thoracic trunk musculature with ICMS, more than both SCI-EX and Naïve animals. EMG amplitude in the lower thoracic ventral trunk muscles was higher in SCI-EX animals. In contrast, EMG in dorsal mid-thoracic, ventral upper thoracic trunk muscles was higher in SCI-NX animals. These results suggest that cortical plasticity associated with physical rehabilitation is not a mere enhancement of plasticity associated with spontaneous recovery but is relatively novel and relying on establishing cortical control of muscles below lesion rather than muscles above.

Disclosures: B. Nandakumar: None. **G.H. Blumenthal:** None. **K.A. Moxon:** None.

Digital Abstract Session

P155. Spinal Cord Injury, Repair and Recovery

Program #/Poster #: P155.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CH Neilsen Foundation
Duke KURe NIH K12

Title: Effects of epidural kilohertz frequency spinal cord stimulation on lower urinary tract function

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Abstract: Spinal cord injury (SCI) leads to bladder dysfunction, including neurogenic detrusor overactivity (NDO), detrusor-sphincter dyssynergia (DSD), and impaired bladder emptying. Novel approaches are necessary to promote continence and efficient bladder emptying after SCI. Epidural kilohertz frequency spinal cord stimulation (KHF SCS) successfully treats chronic pain, and considering the parallels between the pathophysiology of chronic pain and post-SCI bladder dysfunction, we hypothesized that KHF SCS would treat bladder dysfunction after SCI. The purpose of this study was to determine the parameters of KHF SCS that suppress both NDO and DSD by measuring changes in bladder capacity (BC), voiding efficiency (VE), non-voiding contractions (NVC), and external urethral spincter (EUS) electromyography (EMG) during KHF SCS in anesthetized rats with and without acetic acid cystometry. In the first cohort of healthy male (n=7) and female (n=7) rats, we performed continuous-fill saline cystometry while measuring bladder pressure, EUS EMG, and voided and residual volumes. A two-contact epidural paddle electrode was placed over the S1 cord segment. The effects of KHF SCS were measured using three frequencies {1 kHz, 5 kHz, 10 kHz} and three amplitudes {20, 40, 80% of motor threshold (MT)}, as well as trials with no stimulation (intra-block control) and with 50 Hz SCS at 80% of MT. In the second cohort of healthy male (n=7) and female (n=7) rats, cystometry was performed using 0.1% acetic acid to induce bladder hypersensitivity. BC during saline cystometry was increased with SCS at 1 kHz 80% MT, 5 kHz 20% MT, 10 kHz 80% MT, and 50 Hz 80% MT compared to control. VE was increased with SCS at 5 kHz 20% MT and 5 kHz 80% MT compared to control. Average EUS EMG activity during voiding was significantly increased with SCS at 5 kHz and 10 kHz 20%, 40%, and 80% MT, and there were no changes in EUS burst duration. BC decreased with acetic acid, and the number of NVC increased, but there were no differences in VE, average EUS EMG activity, or burst duration. SCS did not alter BC, and VE was increased with 1 kHz 80% MT SCS. The number of NVC was decreased by SCS at 1 kHz 20% MT, 1 kHz 40% MT, 5 kHz 20% MT, 5 kHz 80% MT, 10 kHz 40% MT, and 10 kHz 80% MT compared to acetic acid control, and the NVC area under the curve was increased with SCS at 5 kHz 20% and 80% MT and 10 kHz 80% MT. Average EUS EMG activity was increased with SCS at 10 kHz 20% and 40% MT. The results suggest that epidural KHF SCS may be a viable approach to modulate lower urinary tract function. SCS at 5 kHz 20% MT, 5

kHz 80% MT, and 10 kHz 80% MT altered bladder function in both healthy and hypersensitive animals, with SCS at 5 kHz 20% MT being the most effective stimulation parameters.

Disclosures: C.J. Steadman: None. **C.L. Langdale:** None. **W.M. Grill:** None.

Digital Abstract Session

P156. Spinal Cord Injury: Animal Models and Human Studies

Program #/Poster #: P156.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: The Miami Project to Cure Paralysis

Title: Comparison of Self-Reported Neurological Status to Evaluated International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) Outcomes in People with Chronic Spinal Cord Injury.

Authors: *N. DATTA, D. C. CILIEN, K. L. GANT;
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Abstract: International Standard for Neurological Classification of Spinal Cord Injury (ISNCSCI) has long been the most reliable screening tool for classification of spinal cord injuries (SCI). The ability to predict ISNCSCI status without performing an in-person ASIA (American Spinal Injury Association) exam can be useful to researchers. This study aims to develop a numerical framework to reasonably predict Neurological status with only information from self-reported SCI levels, including Motor, Sensory, Neurological levels, and ASIA Impairment Scale (AIS) Grade. To this end, a comparison between self-reported levels and those obtained by ASIA certified evaluators was made with data collected over 9 years (2012 -20) from 581 consenting participants. After exclusions (n=567), median distribution and Kruskal-Wallis Tests were studied. We found that (1)94.4% of the study population had an idea about the neurological level of injury component of an ASIA Exam, however only 32.3% had any idea about AIS grade. Further, only 18.9% had any idea about Motor Level, and only 15.6% about sensory level. (2) Overall, participants reported their neurological level of injury as being 1 level below the evaluator rated level. Majority of those with cervical injury (C group) reported their Level accurately, however, majority of those with thoracic injury (T group) reported their Level as being 1 level below the evaluator rated level, and those with lumbar injury (L group) reported their level as being 3 levels below the evaluator rated level. (3) 75^{th} percentile of participants who self-reported their motor level, did so accurately. Among them, C group reported their motor level accurately. T group reported their motor Level as being 1 below the evaluator rated level. None in the L group reported their motor level. (4) 50^{th} percentile of participants who self-reported their Sensory level, did so 1 Level below the evaluator rated sensory level. Among them, C group reported their sensory level accurately, T group reported their Sensory Level as being 1 level below the evaluator rated level. None in the L group reported their sensory level. (5) Self-reported AIS grade can be used to predict complete and incomplete injuries (Sensitivity=

92.15% Specificity= 75.95%) but not sensory incomplete or motor incomplete injuries. The study of the data leads us to surmise that it is possible for an investigator to make a reasonable assumption about the ISCNSCI status of a person with SCI from self-reported data alone. This can significantly impact prescreening protocols, by reducing the need for preliminary in-person visits for ASIA exam, which would benefit both the investigator and the SCI community at large.

Disclosures: N. Datta: None. **D.C. Cilien:** None. **K.L. Gant:** None.

Digital Abstract Session

P156. Spinal Cord Injury: Animal Models and Human Studies

Program #/Poster #: P156.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: R01NS079702
R01NS110385

Title: Differential Activation of Dorsal Horn Neuron Subtypes in a Mouse Model of Spinal Cord Injury Induced Neuropathic Pain

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Abstract: Neuropathic pain is the most common co-morbidity of spinal cord injury (SCI); it is highly debilitating and due to an incomplete understanding of its underlying mechanisms is often refractory to current treatments. SCI induces cellular changes in the CNSthat alters the excitability dorsal horn involved in pain transmission. Primary nociceptors synapse into a complex network of dorsal horn inhibitory and excitatory interneurons, the output of which regulates signaling to supraspinal pain processing areas. The dorsal horn is heterogeneous, with unique microcircuits regulating specific modalities of nociception. To investigate activity changes in the neuron populations that comprise these microcircuits, we are using a clinically relevant C5 hemi-contusion mouse model of SCI. This model induces persistent neuropathic pain-like behavior including mechanical allodynia, and thermal hyperalgesia. Using the immediate early gene c-FOS as a marker, we have spatiotemporally characterized neuronal activity in several general or modality-specific neuronal subtypes of the dorsal horn, including $NK1R^+$ projection neurons, $Pax2^+$ inhibitory interneurons, and $Calretinin^+$ and $PKC\gamma^+$ excitatory interneurons. In addition to immunohistological assessment, we used a c-fos driven transgenic mouse line, to induce fluorescent reporter expression in active populations of neurons. We have initially found that $PKC\gamma^+$ excitatory interneurons become hyperactive following SCI, even without peripheral sensory stimulation. As these neurons are not typically involved in pain circuitry, we hypothesize that this activation may be contributing to the development of neuropathic pain-like behaviors in our SCI model. Further, we are exploring how reduced activation of dorsal horn inhibitory interneurons may contribute to SCI induced mechanical

allodynia. We are continuing to identify activity changes in additional dorsal horn neuron populations following SCI.

Disclosures: E.V. Brown: None. **M.A. DeMarco:** None. **A. Malik:** None. **N.M. Heinsinger:** None. **L. Cheng:** None. **A.C. Lepore:** None.

Digital Abstract Session

P157. Spinal Cord Injury: Therapeutic Strategies – In vivo – Pharmacological

Program #/Poster #: P157.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Ontario Graduate Scholarship
Baxter BioScience Grant
Paralyzed Veterans of America Research Foundation Grant # 3167

Title: Drug Repurposing: Delayed Administration of High Dose Human Immunoglobulin G for Treatment of Traumatic Cervical Spinal Cord Injury

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Abstract: Purpose: Neuroinflammation exacerbates damage caused by initial trauma from spinal cord injury (SCI). Severity of neuroinflammation depends on integrity of the blood-spinal cord-barrier (BSCB), as a compromised BSCB enhances neuroinflammation by facilitating immune cell infiltration. By targeting neuroinflammation, immunosuppressants are used to treat SCI patients. However, as patients experience immune suppression, immunomodulation is more effective than immunosuppression. Human Immunoglobulin G (hIgG) is used in clinic as an immunomodulatory treatment for inflammation. Although we have shown that administration of hIgG (2g/kg) is beneficial after SCI, the optimal time window of administration and mechanism of hIgG are unknown. We hypothesize that hIgG is beneficial when administered at extended time points post-SCI by stabilizing the BSCB. **Methods**: With a clinically-relevant rat model of clip compression-contusion SCI at cervical level 7/thoracic level 1, a single bolus of hIgG (2g/kg) or control buffer was administered intravenously at 15 minutes, 1 hour or 4 hours post-SCI. Spinal cord, serum and spleens were collected to evaluate hIgG's effects. **Results:** Demonstrated by immunohistochemistry, hIgG co-localized with BSCB astrocytes, pericytes and endothelial cells. At 24 hours post-SCI, relative to control buffer, hIgG (2g/kg) significantly enhanced BSCB integrity when administered at delayed time points. This is indicated by Western blot, with greater protein expression of tight junction proteins (ZO-1, occludin) and reduced presence of albumin. In addition, there is reduced extravasation of Evan's Blue. A stronger BSCB was associated with reduced spinal cord neuroinflammation as assessed by cytokine assay. Despite reducing spinal cord inflammation, hIgG (2g/kg) increased levels of

inflammatory cytokines in serum and spleen. With flow cytometry, it was demonstrated that hIgG (2g/kg) reduced neutrophil counts in blood, but increased splenic neutrophil population. Short-term benefits of delayed hIgG (2g/kg) administration correlate with enhanced white and gray matter preservation, higher counts of spinal cord motor neurons and improved sensory and motor function at eight weeks post-injury. **Conclusions:** As a clinically-relevant immunomodulatory treatment, hIgG (2g/kg) shows promise as a therapeutic approach for SCI. The anti-inflammatory effects mediated by hIgG (2g/kg) might be two-fold; enhancing BSCB integrity and trafficking neutrophils to the spleen. To promote clinical translation, future steps include determining which fragment of hIgG mediates the aforementioned immunomodulatory effects.

Disclosures: J. Chio: None. **J. Wang:** None. **V. Surendran:** None. **L. Li:** None. **M. Zavvarian:** None. **K. Pieczonka:** None. **M. Fehlings:** None.

Digital Abstract Session

P157. Spinal Cord Injury: Therapeutic Strategies – In vivo – Pharmacological

Program #/Poster #: P157.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: SC180122
BX005218
S10 OD011981

Title: Time-to-treatment window and cross-sex potential ofβ_2-adrenergic receptor-induced mitochondrial biogenesis-mediated recovery after spinal cord injury

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Abstract: Mitochondrial dysfunction is a well-characterized consequence of spinal cord injury (SCI). We previously reported that treatment with the FDA-approved β_2-adrenergic receptor agonist formoterol beginning 8h post-SCI induces mitochondrial biogenesis (MB) and improves body composition and locomotor recovery in female mice. To determine the time-to-treatment window of formoterol, female mice were subjected to 80 kDyn contusion SCI and daily administration of vehicle or formoterol (0.3 mg/kg) beginning 24h after injury. This delayed treatment paradigm improved body composition in female mice by 21 DPI, returning body weight to pre-surgery weight and restoring gastrocnemius mass to sham levels; however, there was no effect on locomotor recovery, as measured by the Basso-Mouse Scale (BMS), or lesion volume. To assess the cross-sex potential of formoterol, injured male mice were treated with vehicle or formoterol (0.3 or 1.0 mg/kg) beginning 8h after SCI. Formoterol also improved body composition post-SCI in male mice, restoring body weight and muscle mass regardless of dose. Interestingly, however, improved BMS scores and decreased lesion volume was observed only in

male mice treated with 0.3 mg/kg. Additionally, 0.3 mg/kg formoterol induced MB in the gastrocnemius and injured spinal cord, as evidenced by increased MB protein expression and mitochondrial number. These data indicate that formoterol treatment improves recovery post-SCI in both male and female mice in a dose- and initiation time-dependent manner. Furthermore, formoterol-induced functional recovery post-SCI is not directly associated with peripheral effects, such as muscle mass and body weight.

Disclosures: N.E. Scholpa: None. **E.C. Simmons:** None. **J.D. Crossman:** None. **R.G. Schnellmann:** None.

Digital Abstract Session

P157. Spinal Cord Injury: Therapeutic Strategies – In vivo – Pharmacological

Program #/Poster #: P157.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Nielsen

Title: Mu Opioid Receptor Activation Restores Sexual Dysfunction After Chronic Spinal Cord Injury in Male Rats

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Abstract: Spinal cord injury (SCI) significantly affects urogenital functions, including severe deficits in ejaculation in men and male rats, but development of treatment options is limited by a lack of knowledge of effects of SCI on the spinal control of sexual reflexes. The spinal ejaculation generator (SEG) consists of a population of lumbar spinothalamic cells (LSt) that control ejaculation via axonal projections to autonomic and motor centers in the lumbosacral spinal cord upon stimulation of sensory inputs via the dorsal penile nerve (DPN). LSt cells control ejaculation via release of neuropeptides, including enkephalin acting via mu and delta opioid receptors. It was previously shown that in control male rats, intrathecal infusions of mu opioid receptor agonist DAMGO strongly facilitated ejaculatory reflexes, while antagonists prevent ejaculation triggered by stimulation of the DPN. Here, we test the hypothesis that DAMGO infusions may restore ejaculatory reflexes in SCI males. In a first study, male Sprague Dawley rats received either mid-thoracic spinal contusion or sham treatment (n=11 each). Four weeks later, animals received an acute spinal transection to remove remaining supraspinal influence on the SEG and subsequent intrathecal infusions (10 µl) of saline and DAMGO (0.1 nmol) while parameters of ejaculatory reflexes were recorded. In addition, DPN was stimulated at frequencies that normally trigger ejaculation in control males and reflexes were recorded. Findings indicated that DAMGO, but not saline triggered ejaculatory reflexes and facilitated DPN-stimulated reflexes equally in sham and SCI groups. In a second study, the procedures were repeated, but without the acute spinal transection prior to infusions. In this study, DAMGO

triggered and facilitated ejaculatory reflexes in SCI groups, but not in sham males. These results indicate that removal of supraspinal inhibitory inputs is required for facilitatory effects of enkephalin within the SEG and that contusion SCI may at least partially remove such inputs. Together the findings support the hypothesis that Mu opioid receptor agonists may be a target for development of treatment options for sexual dysfunction following SCI.

Disclosures: T. Ettey: None. **S. Gaikwad:** None. **L. Coolen:** None.

Digital Abstract Session

P157. Spinal Cord Injury: Therapeutic Strategies – In vivo – Pharmacological

Program #/Poster #: P157.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life
AbbVie

Title: Blocking inhibitory RGMa promotes tissue preservation and axonal plasticity after cervical spinal cord injury

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Abstract: After traumatic spinal cord injury (SCI) degenerative events including cell death, axonal damage, and the upregulation of inhibitory molecules limit regeneration and recovery. Repulsive Guidance Molecule A (RGMa) is a potent inhibitor of axonal growth and is rapidly upregulated after SCI in both rodents and in humans. Recently, we showed that delayed administration of human monoclonal antibodies that block inhibitory RGMa signaling promote neuroprotective and regenerative effects in a clinically relevant rat model of thoracic SCI. However, it is unknown whether RGMa blocking antibodies are effective for cervical SCI. Also, as the majority of SCI cases are cervical, and spinal cord level-specific heterogeneity in response to surgical and pharmacological interventions have been observed clinically, this warrants investigation. In the present study, we also examined whether systemic administration of RGMa antibodies could be delayed to 3h, a therapeutically relevant time window after cervical SCI. Using a clinically relevant rat model of cervical (C6/7) impact-compression SCI, human RGMa monoclonal antibodies were intravenously injected either immediately following SCI (acute), or 3h, or 24h post-injury, and then weekly for 9 weeks. Control rats received human IgG (hIgG) isotype antibodies recombinantly produced from CHO cells. Plasma levels of antibody were assessed longitudinally, and recovery was examined with weekly behavioral tests and histological analyses at 12 weeks post-SCI. Pharamacokinetic analysis showed human antibodies in rat plasma and in spinal cord injured tissue immunostained with hIgG at all time intervals after administration. Rats with cervical SCI showed improvement in motor function and gait parameters when treated acutely and at 3h post-injury with anti-RGMa antibodies. Lesion analysis showed that inhibition of RGMa promoted tissue preservation, and a 2-fold sparing of

perilesional motoneurons at 12 weeks after cervical SCI in all treated groups. Prior to endpoint, BDA tracer was injected into the sensorimotor cortex for anterograde labelling of motor axons. Rats treated with RGMa antibodies showed a significantly greater density of BDA labeled fibers in the gray matter adjacent to the dorsal corticospinal tract in segments rostral to the cervical lesion. RGMa inhibition also increased serotonergic innervation in the ventral horns caudal to the cervical lesion. These results show that administration of anti-RGMa monoclonal antibodies may be delayed to a therapeutically relevant time window following cervical SCI to promote neuronal and tissue sparing and axonal plasticity to facilitate repair.

Disclosures: A.J. Mothe: None. **P.B. Jacobson:** A. Employment/Salary (full or part-time):; AbbVie. **C.H. Tator:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie.

Digital Abstract Session

P157. Spinal Cord Injury: Therapeutic Strategies – In vivo – Pharmacological

Program #/Poster #: P157.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: RESOLVE (NORTE-01-0246-FEDER-000018)
Morton Cure Paralyis Fund-MCPF
Bluepharma-University of Coimbra Innovation Award
La Caixa Impulse Validate Program (CI20-00266)

Title: Aav-mediated delivery of constitutively active pfn1 induces axon regeneration and functional recovery after spinal cord injury

Authors: *D. MACHADO[1], R. PINTO-COSTA[1], J. NOGUEIRA-RODRIGUES[1], T. SILVA[2], P. BRITES[2], M. SENA-ESTEVES[3], M. M SOUSA[1];
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Abstract: In mammals, axon growth capacity declines after synapse formation, and the CNS fails to regenerate after trauma, causing unrecoverable neurologic deficits. Despite progress in rehabilitative care, no efficient treatment has yet been developed for spinal cord injury (SCI) patients. Using models with different regenerative capacities, we identified profilin1 (PFN1) as a key player in axon elongation and regeneration. *In vitro* results demonstrated that increased expression of constitutively active human PFN1 (S138AhPFN1) tunes the growth cone cytoskeleton towards axon regeneration. Here we present *in vivo* evidence that using AAV.PHP.eB-mediated delivery of S138AhPFN1 to rodents, prior to lesion, promotes axon regeneration, maturation of neuromuscular junctions and functional recovery of injured sciatic nerves and increases the ability of regenerating CNS axons to penetrate the inhibitory spinal cord fibrotic/glial scar. Therapeutic potential of S138AhPFN1 delivery was then assessed at an acute timepoint after injury (2-3 hours) using AAV9-mediated delivery. *In vivo* intravenous delivery of

AAV9.S138AhPFN1 successfully targeted the spinal cord. Spinal cord injured mice injected with AAV9.S138AhPFN1 presented total recovery of mechanical nociception at 16 weeks post-injury. Furthermore, some animals of the treated group were able of gain total weigh support and plantar stepping at the end of the recovery period, in contrast to control animals which were not able to support their own weight during the entire recovery period. At the histological level, sensory axons of the dorsal column tract, traced using cholera toxin, regenerated for longer distances. In summary, our results show that AAV9.S138AhPFN1 intravenous administration presents a very good therapeutic potential for SCI, leading to increased axon regeneration and gain of function.

Disclosures: D. Machado: None. **R. Pinto-Costa:** None. **J. Nogueira-Rodrigues:** None. **T. Silva:** None. **P. Brites:** None. **M. Sena-Esteves:** None. **M. M Sousa:** None.

Digital Abstract Session

P157. Spinal Cord Injury: Therapeutic Strategies – In vivo – Pharmacological

Program #/Poster #: P157.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig Neilsen Foundation

Title: Gastrin releasing peptide alleviates sexual dysfunction after chronic spinal cord injury in male rats.

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Abstract: Spinal cord injury (SCI) has devastating effects on urogenital functions, including severe deficits in ejaculatory function in men and male rats. Surveys among SCI men place recovery of sexual function as high priority, but development of treatment options is limited by a lack of understanding of effects of SCI on the spinal ejaculation generator (SEG). The SEG comprises of a population of lumbar spinothalamic cells (LSt) that regulate ejaculation via release of neuropeptides from axonal projections to autonomic and motor nuclei in the lumbosacral spinal cord upon sensory stimulation of the dorsal penile nerve (DPN). A critical LSt peptide for the control of ejaculation reflex is gastrin releasing peptide (GRP), as intrathecal infusions of GRP facilitate ejaculatory reflexes, while antagonists prevent ejaculation triggered by stimulation of the DPN in control male rats. SCI causes reduction in GRP mRNA and peptide levels in LSt, leading to the hypothesis that this lack of GRP contributes to ejaculatory dysfunction following SCI. Here, we test this hypothesis by studying the effects of GRP infusions on restoration of ejaculatory reflexes in SCI male rats. In a first study, male Sprague Dawley rats received either mid-thoracic spinal contusion injury (n=11) or sham surgery (n=8). Four weeks later, animals received an acute spinal transection to remove remaining supraspinal influences on the SEG and subsequent intrathecal infusions (10 µl) of saline and GRP (0.2 nmol) while parameters of ejaculatory reflexes were recorded. Subsequently, DPN was stimulated and

reflexes were recorded. Findings indicated that GRP, but not saline triggered ejaculatory reflexes and facilitated DPN-stimulated reflexes equally in sham and SCI groups. In a second study, the procedures were repeated, but without acute spinal transection prior to infusions. GRP again triggered and facilitated ejaculatory reflexes but with greater effects in SCI compared to sham groups. In a third study, lower dosages of GRP (0.08 or 0.03 nmol) were shown to have less effect on ejaculation. Together these results support the hypothesis that impairment of ejaculation may be partially due to disruption of GRP synthesis in LSt cells and suggest that GRP may be a potential therapeutic target for sexual dysfunction following SCI.

Disclosures: S.S. Gaikwad: None. **L.M. Coolen:** None. **T. Ettey:** None.

Digital Abstract Session

P158. Autism: Physiology and Systems

Program #/Poster #: P158.01

Topic: A.07. Developmental Disorders

Title: Maturation of the amygdala subnuclei in children and adolescents with Autism Spectrum Disorder and the association with social and repetitive behaviors

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Abstract: The amygdala plays a key role in regulating emotional processes, including emotional recognition and some aspects of fear and anxiety responses. Dysregulated amygdala development has been reported in young children with ASD, while less is known about amygdala maturation in later childhood and adolescence, a sensitive window for social skill development. In this longitudinal magnetic resonance imaging (MRI) study, we assessed macrostructural development of the amygdala subnuclei in older children and adolescents with (n=23) and without ASD (n=15) at 2 timepoints 3 years apart. At the first session, participants were scanned with MRI at a median age of 12 years, with a second scan at a median age of 15 years. The volumes of seven subnuclei (basolateral, central, medial, cortical, paralaminar, corticoamygdaloid area and anterior amygdaloid area)were extracted using an automatic segmentation algorithm for the two timepoints, to examine growth. In adolescents with ASD only, amygdala subnuclear volumetric changes were assessed in relation to ASD symptomology. Participants with ASD were diagnosed using the Autism Diagnostic Observation Schedule, General (ADOS-G) and the Autism Diagnostic Interview, revised (ADI-R). Amygdala subnuclei growth was compared between the participant groups and revealed larger BLA nuclei volumes in adolescents with ASD compared to neurotypical participants (B=46.8, p=0.04). When examining ASD symptomatology in relation to the growth of the amygdala subnuclei, reciprocal social interaction scores on the ADI-R were positively associated with increased growth of the BLA nuclei (B=8.3, p=0.001). Growth in the medial nucleus predicted the communication (B=-46.9,

p=0.02) and social (B=-47.7, p<0.001) domains on the ADOS-G. Growth in the right cortical nucleus (B=26.14, p=0.02) predicted ADOS-G social scores. Central nucleus maturation (B=29.9, p=0.02) was associated with the repetitive behaviors domain on the ADOS-G. Larger BLA volumes may be due to increased neuronal density, consistent with postmortem studies. These findings reveal an association between amygdala subnuclei volumes and ASD symptoms, which may reflect activity-dependent plasticity. Improved understanding of amygdala subnuclei growth trajectories in youth may aid in identifying key windows for targeted interventions, particularly for social communication in adolescents with ASD who display social impairments.

Disclosures: D. Seguin: None. **S. Pac:** None. **J. Chen:** None. **R. Nicolson:** None. **J. Martinez-Trujillo:** None. **E.G. Duerden:** None.

Digital Abstract Session

P158. Autism: Physiology and Systems

Program #/Poster #: P158.02

Topic: A.07. Developmental Disorders

Support: GW Sigelman Undergraduate Research Enhancement (SURE) Award
NIA R01 AG031581
NIA P30 AG019610

Title: Autistic Traits and the Aging Brain

Authors: *A. JOB SAID[1], B. BLAIR BRADEN[2], B. K. WOODRUFF[3], R. J. CASELLI[3], G. L. WALLACE[1];
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Abstract: Introduction: Knowledge of middle and older adulthood in autism spectrum disorder (ASD) is nearly non-existent. The few studies of aging in ASD that have been completed to date are plagued by cohort effects, presenting confounds to understanding contributions of autistic behavior to brain-based outcomes in later adulthood. In three recent studies we have shown that the presence of the Broader Autism Phenotype (BAP), elevated subclinical autistic traits occurring in approximately 5-10% of the population, is associated with increased executive function problems and social cognitive difficulties compared to those without the BAP during older adulthood (60-91 years). The current study, for the first time, examines brain-based associations with the BAP during middle and older adulthood.
Method: 166 community dwelling adults (76% female) ranging in age from 49-81 (M=64) years completed a neuropsychological battery and provided a Magnetization Prepared-RApid Gradient Echo (MP-RAGE) magnetic resonance imaging (MRI) scan. The BAP was assessed using self-ratings from the Autism Quotient (AQ). Brain volumes were quantified using FreeSurfer and compared between the highest quartile of AQ scorers (BAP; n=47) and the other 72% of the sample (non-BAP; n=119) using multivariate analysis of covariance (MANCOVA) accounting

for the effects of age, gender, and total intracranial volume (ICV). In complementary analyses, multiple linear regressions examined links between continuous AQ ratings and brain volumes using the same covariates.
Results: BAP adults had decreased brain volume compared to the non-BAP group in the bilateral hippocampus, right amygdala, left thalamus, and left cerebellar cortex (Fs>5.32, ps<.03), after co-varying the influences of age, gender, and ICV. Similarly, linear regression analyses revealed that increasing continuous autistic traits were predictive of decreased volumes in bilateral hippocampus, left thalamus, and left cerebellar cortex (ts>2.24, ps<.04, $\blacktriangle R^2$s$\geq$.02) after accounting for covariates.
Discussion: This study provides the first evidence that the BAP conveys potential risk to brain health through decreased subcortical (i.e., hippocampal, thalamic, and cerebellar) brain volumes during a period of development (middle and older adulthood) when tissue loss is already occurring. Similar findings of brain volumetric reductions have been shown in studies of children and young adults and middle age adults with ASD. This convergence across subclinical (BAP) and clinical (ASD) samples lends validity to the investigation of the BAP in adult development to inform neurobiological effects of aging in ASD.

Disclosures: A. Job Said: None. **B. Blair Braden:** None. **B.K. Woodruff:** None. **R.J. Caselli:** None. **G.L. Wallace:** None.

Digital Abstract Session

P158. Autism: Physiology and Systems

Program #/Poster #: P158.03

Topic: A.07. Developmental Disorders

Support: DoD AR140105
NIMH 1K01MH116098
NIMH 1F31MH122107

Title: Longitudinal Functional Connectivity of Cognitive Brain Networks in Older Men with Autism

Authors: *M. WALSH[1], B. PAGNI[2], N. GUERITHAULT[1], A. NESPODZANY[3], L. BAXTER[4], C. SMITH[5], B. BRADEN[1];
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Abstract: ***Background***: Emerging cross-sectional research on cognitive aging in ASD shows some evidence of poorer cognitive control in older adults with ASD. Brain functional connectivity may reveal early biomarkers of cognitive aging. In our prior cross-sectional work, we showed age-group differences suggesting exacerbated reductions in functional connectivity affecting the fronto-parietal network (FPN) in adults with ASD (Walsh et al., 2019). This

current, preliminary study extends these findings by longitudinally examining changes in functional connectivity of canonical cognitive brain networks over a two-year follow-up period in a sample of mid to older adults with and without ASD. ***Methods***: Participants included men ages 40-64 years at the start of the study (mean: 50.7[7.1]) of average intellectual ability (IQ>80) with and without ASD (ASD: *n*=13; NT: *n*=18). Follow-up intervals were two years on average. Using a six-minute, eyes closed resting-state fMRI scan, group-ICA was used to identify the FPN, dorsal attention (DAN), default mode (DMN), and salience networks (SN) and main effects of time and group by time interactions were modeled at the whole-brain level with FDR-correction ($p<.05$, cluster-level) with age as a covariate in SPM12. Baseline and follow-up performance on the Wisconsin Card Sorting Task (WCST; Total Errors) was used to examine associations with cognitive control for participants with ASD. ***Results****:* For the right lateralized FPN, a significant time by diagnosis interaction was observed, such that adults with ASD showed declines in connectivity between the left inferior frontal gyrus (IFG; pars triangularis portion) and the rest of the FPN that were not observed in NT participants ($p=.018$, FDR-corrected, cluster level). No other main effects of time or group by time interactions were observed for the right lateralized FPN or other networks investigated (left FPN, DMN, DAN, or SN). No significant effects of time or group by time interactions were found for the Wisconsin Card Sorting Task. However, poorer baseline ($r_9=0.579$, $p=0.062$) and follow-up ($r_9=0.621$, $p=0.042$) WCST performance was marginally and significantly associated, respectively, with larger decreases in FPN-IFG connectivity over time for participants with ASD. ***Conclusions****:* FPN-prefrontal connectivity decreases with age in mid to older adults with ASD, but not NT adults, and this decreasing connectivity predicts poorer follow-up performance on a cognitive control task. Despite the small sample included in this preliminary analysis, these findings suggest a trend toward worse brain aging in ASD, which may predict poorer cognitive outcomes.

Disclosures: **N. Guerithault:** None. **B. Pagni:** None. **M. Walsh:** None. **A. Nespodzany:** None. **L. Baxter:** None. **C. Smith:** None. **B. Braden:** None.

Digital Abstract Session

P158. Autism: Physiology and Systems

Program #/Poster #: P158.04

Topic: A.07. Developmental Disorders

Title: Eeg complexity measures of cognitive states show heterogeneous asd risk with sequence clustering.

Authors: *S. S. WOLFSON, I. KIRK, K. WALDIE, C. KING;
The Univ. of Auckland, Auckland, New Zealand

Abstract: EEG complexity measures of cognitive states distribute heterogeneously to ASD risk measures with some hierarchical sequence clustering.AuthorsS. S. Wolfson, I. Kirk, K. Waldie, S. King; The University of Auckland, Auckland NZDisclosuresS. S. Wolfson: None, I. Kirk: None, K. Waldie: None, S. King: NoneAbstractAutism spectrum disorder is an increasingly

prevalent and debilitating neurodevelopmental condition and an EEG diagnostic challenge. Despite large amounts of electrophysiological research over many decades, an electroencephalogram biomarker for ASD has not been found. We hypothesized that reductions in complex dynamical system behavior in the human central nervous system as part of the macroscale neuronal function during cognitive processes might be detectable in whole EEG for higher risk ASD adults. We compared the medians of correlation dimension, largest Lyapunov exponent, Higuchi's fractal dimension, multiscale entropy, multifractal detrended fluctuation analysis and Kolmogorov complexity during resting, cognitive and social skill tasks in 20 EEG channels of 39 adults over a range of ASD risk. We found heterogeneous complexity distribution with clusters of hierarchical sequences pointing to potential cognitive processing differences but no distinction based on ASD risk.

Disclosures: S.S. Wolfson: None. **I. Kirk:** None. **K. Waldie:** None. **C. King:** None.

Digital Abstract Session

P158. Autism: Physiology and Systems

Program #/Poster #: P158.05

Topic: A.07. Developmental Disorders

Support: Overcome Syngap
CIHR

Title: Sensory brain responses alterations as translational biomarkers for SYNGAP1 haploinsufficiency

Authors: *M. CARRENO-MUNOZ[1,2], B. CHATTOPADHYAYA[1], K. AGBOGBA[1], V. COTE[1,3], S. WANG[4], M. LEVESQUE[4], M. AVOLI[5], J. L. MICHAUD[1], S. LIPPE[1,3], G. DI CRISTO[1,2];
[1]CHU Sainte-Justine Res. Ctr., Montreal, QC, Canada; [2]Neurosciences, [3]Psychology, Univ. of Montreal, Montreal, QC, Canada; [4]Neurol. and Neurosurg., [5]Neurol. and Neurosurgery, and Physiol., Montreal Neurolog. Institute-Hospital, Montreal, QC, Canada

Abstract: Autism-spectrum disorder (ASD) is a range of neurodevelopmental disorders characterized by impaired social interaction, stereotyped behaviours, cognitive impairments and sensory deficits. While there are hundreds of genes involved in ASD, SYNGAP1 gene is particularly gaining attention because of the frequency and penetrance of loss-of-function variants found in patients as well as the wide range of co-morbid brain disorders associated with SYNGAP1 pathogenicity. In both human patients and animal models, SYNGAP1 haploinsufficiency leads to cognitive and social problems, altered neural circuit excitability, spontaneous seizure and unadaptative behaviours. Until now, most of the research on the deficits caused by SYNGAP1 haploinsufficiency has focused on cognitive problems but recent works point to the possibility that altered sensory processing may strongly contribute to cognitive problems and other altered behaviours associated with ASD. Indeed, sensory symptoms are

becoming an important early biomarker, since they are often documented as early as 6 months of age in infants later diagnosed with ASD (considerably earlier than development of other higher cognitive functions as attention). Although sensory processing and sensitivity problems have been largely reported in ASD, whether auditory and visual perception and processing are altered in SYNGAP1 haploinsufficiency is not known. Here we study different aspects of auditory and visual perception in patients and a mouse model of SYNGAP1 haploinsufficiency. Our preliminary results reveal altered electrophysiological activity underlying both auditory and visual perception in both patients and mice, and suggest these could be used as strong and translational biomarkers for SYNGAP1 haploinsufficiency.

Disclosures: **M. Carreno-Munoz:** None. **B. Chattopadhyaya:** None. **K. Agbogba:** None. **V. Cote:** None. **S. Wang:** None. **M. Levesque:** None. **M. Avoli:** None. **J.L. Michaud:** None. **S. Lippe:** None. **G. Di Cristo:** None.

Digital Abstract Session

P158. Autism: Physiology and Systems

Program #/Poster #: P158.06

Topic: A.07. Developmental Disorders

Title: Assessing Glutamate Concentration as a predictor for social behavioral changes in ASD due to Memantine

Authors: *C. RIECKEN[1], D. Q. BEVERSDORF[2], B. FERGUSON[1], N. NAIR[1];
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Abstract: Normal 0 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin:0in; mso-pagination:widow-orphan; font-size:12.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi;} **Background:** Autism spectrum disorder (ASD) is characterized by repetitive behaviors and impaired social communication. Differences in neural excitability/inhibitory ratios have been previously reported in ASD. Magnetic Resonance Spectroscopy (MRS) has been shown as an effective technique to measure concentrations of the neurotransmitter glutamate, which general causes increases in neural excitability. It is therefore possible that this may be used as a predictor for the efficacy of pharmacological interventions in ASD. **Method:** MRS data from adult participants with ASD was processed to determine differences in glutamate concentration in the cerebellum and dorso-lateral prefrontral cortex (DLPFC). Social behavior was assessed before and after a 12 week trial of Memantine using the Clincal Global Impression (CGI) scale. Changes in social behavior were compared to concentrations of glutamate in both ROIs to determine if glutamate concentration

may serve as a predictor of changes in social behavior due to Memantine. **Results:** A positive correlation was discovered between glutamate concentration in left DLPFC and changes in CGI scores related to social interaction. There was no relationship between glutamate concentration in either cerebellum or DLPFC with regard to aberrant or abnormal behavior, repetitive behavior, verbal or nonverbal communication, hyperactivity, anxiety, hypo or hyper sensitivity, or restricted interest. **Conclusions:** More work is needed to further assess the efficacy of biomarkers acquired using MRS in predicting treatment response to Memantine, but the current results indicate that it may be possible that these biomarkers can predict changes in social interaction. <!--EndFragment-->

Disclosures: C. Riecken: None. **D.Q. Beversdorf:** None. **B. Ferguson:** None. **N. Nair:** None.

Digital Abstract Session

P158. Autism: Physiology and Systems

Program #/Poster #: P158.07

Topic: D.01. Sensory Disorders

Support: NIH Grant U54NS092090

Title: Phenotypic variation in neural sensory processing by deletion-size, gender, and age in Phelan McDermid Syndrome

Authors: M. A. REESE[1], E. BERRY-KRAVIS[3], A. THALIATH[4], E. ISENSTEIN[5], A. DURKIN[6], J. FOSS-FEIG[7], P. SIPER[7], C. NELSON[8], L. BACZEWSKI[10], A. LEVIN[9], C. M. POWELL[11], S. WHITE[12], M. MOSCONI[13], A. KOLEVZON[7], ***L. ETHRIDGE**[14,2];
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Abstract: Phelan-McDermid Syndrome (PMS) is a rare genetic condition characterized by deletion or mutation of region 22q13.3, which includes the *SHANK3* gene. Clinical descriptions of this population include mildly dysmorphic features, epilepsy, neonatal hypotonia, severely impaired or absent expressive language, developmental delays, and intellectual impairments. Among these characteristics, individuals with PMS may have autistic-like traits, which include abnormal reactivity to sensory stimuli. However, the neural underpinnings of these sensory processing abnormalities are unknown. This study focuses on comparisons of event-related potential (ERP), event-related spectral perturbation (ERSP), and inter-trial coherence (ITC) between PMS and typically developing (TD) individuals in a standard auditory gating task

measuring attenuation of neural activity to repetitive auditory stimuli. A total of 38 participants, 23 PMS (14 females, age range 96-216 months) and 15 TD (7 females, age range 98-178 months) were included in data analysis. Analysis included a series of general linear models using a region-specific and global (whole head) approach to characterize neural activity between PMS and TD by age, gender, and group. The most notable findings between PMS and TD were in regional analyses, where PMS showed weaker gating than TD for P50 and N1 amplitudes, N1 latency, lower delta ERSP, and lower theta to beta ITC. Within PMS, larger deletion sizes were associated with increased auditory processing abnormalities for P50 and N1 amplitudes and beta, gamma frequencies. Specifically, larger deletion sizes showed greater gating impairment for the P50 in younger individuals and for the N1 in older individuals, suggesting the possibility for developmentally regulated involvement of additional genes in this region. Larger deletion sizes were additionally associated with higher beta and gamma ERSP, in which larger deletions tended toward higher beta for males and toward higher gamma for females, suggesting potential differential effects of deletion size by gender. Results suggest that individuals with PMS exhibit auditory processing abnormalities with complex variation by deletion-size, gender, and age with congruency to impaired early recognition and redundancy attenuation (P50), feature processing (N1), theta to beta synchronization (ITC), information integration (delta), and inhibitory modulation of repeated auditory stimuli (beta, gamma). Findings may provide valuable insight into clinical characterization of sensory and speech behaviors in future studies.

Disclosures: L. Ethridge: None. **M.A. Reese:** None. **E. Berry-Kravis:** None. **A. Thaliath:** None. **E. Isenstein:** None. **A. Durkin:** None. **J. Foss-Feig:** None. **P. Siper:** None. **C. Nelson:** None. **L. Baczewski:** None. **A. Levin:** None. **C.M. Powell:** None. **S. White:** None. **M. Mosconi:** None. **A. Kolevzon:** None.

Digital Abstract Session

P159. Hearing Loss and Cognitive Decline

Program #/Poster #: P159.01

Topic: D.01. Sensory Disorders

Support: Action On Hearing Loss; International Project Grant
Jonathan and Joshua Memorial Graduate Scholarship

Title: Investigating the role of the striatum in the link between noise-induced hearing loss and age-related cognitive decline

Authors: *S. V. PATEL, A. L. SCHORMANS, C. M. DECARLO, S. H. HAYES, S. N. WHITEHEAD, B. L. ALLMAN;
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Abstract: Hearing loss is a chronic and prevalent health condition, affecting ~466 million people worldwide. In addition to age-related hearing impairment, excessive exposure to loud noise is a leading cause of hearing loss. Beyond the devastating effects of hearing impairment itself,

epidemiological studies have identified hearing loss as a major risk factor for age-related cognitive decline, including dementia. At present, the brain regions mediating the link between hearing loss and cognitive decline remain elusive, and it is unclear which brain regions beyond the auditory pathway are particularly susceptible to noise exposure. Although previous studies on rodents have shown that noise exposure alters neurotransmitter systems within the striatum—a brain region involved in stimulus-response habit-learning—it is not known whether such changes contribute to cognitive decline following noise-induced hearing loss. In order to investigate this possibility, we developed a rodent model of noise-induced hearing loss to reliably track cognitive function across aging. To that end, we exposed 6-month old male Fischer 344 rats to broadband noise (100 dB SPL, 4 hours/day x 30 days) or silence. Hearing sensitivity was assessed using the auditory brainstem response; an electrophysiologically-measured evoked potential from the brain in response to sound. Separate cohorts of rats then underwent cognitive-behavioral testing at 7, 10, and 13 months of age on an operant-conditioning based visuomotor associative learning task that relies on normal function of the dorsal striatum. Over 18 consecutive days, rats performed ~150 daily trials of the task in which they received a food pellet upon correctly choosing the left feeder trough when the cue light was steady, and the right feeder trough when the cue light was flashing. Consistent with our effort to model hearing loss often experienced by humans who are exposed to occupational noise, our protocol resulted in a mild, high-frequency hearing loss in the rats. Overall, in contrast to our prediction, noise exposure did not cause deficits in stimulus-response habit-learning at any age. In fact, compared to age-matched shams, the noise-exposed rats performed more accurately on the visuomotor associative learning task at 10 months old, requiring fewer correction trials than the sham-exposed group, and at 13 months old, the noise-exposed rats had faster reaction times for correct responses. Ultimately, the absence of significant noise-induced cognitive deficits on this visuomotor associative learning task suggests a role for brain regions other than the striatum in mediating the link between hearing loss and cognitive decline.

Disclosures: **S.V. Patel:** None. **A.L. Schormans:** None. **C.M. DeCarlo:** None. **S.H. Hayes:** None. **S.N. Whitehead:** None. **B.L. Allman:** None.

Digital Abstract Session

P160. Brain Circuits

Program #/Poster #: P160.01

Topic: D.02. Somatosensation

Title: The functional organization of somatosensory and cortical pathways in the dorsal column nuclei

Authors: *A. UPADHYAY[1], F. IMAI[2], A. YONK[1], D. J. MARGOLIS[1], S. J. BENSMAIA[3], Y. YOSHIDA[2], V. E. ABRAIRA[1];
[1]Cell Biol. and Neurosci., Rutgers Univ., Piscataway, NJ; [2]Burke Neurolog. Inst., White Plains, NY; [3]Dept. of Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: The brainstem dorsal column nuclei (DCN) are the first supraspinal regions for the integration of ascending tactile and proprioceptive information, making them an enticing target to understand precortical sensory processing as well as novel neural prostheses to restore sensory function following injury. However, the underlying cellular and synaptic architecture of the DCN and how they facilitate somatosensory function remains underexplored. Here, we describe the ascending and descending somatosensory pathways of the DCN. We utilized mouse genetic and viral tracing strategies to reveal distinct sensory and cortical projections within the DCN, and used c-fos to investigate the functional role of these nuclei. We show that sensory and cortical pathways primarily converge in the gracile (Gr) and cuneate (Cu) nuclei, which receive primary afferent input from the lower and upper body, respectively. Of the VGlut1+ afferents that define this region, we find that primary afferents (71% of inputs in Gr, 81% of inputs in Cu) make up the majority of inputs to the DCN, with the remainder coming from cortical structures. Given the significance of presynaptic inhibition within the spinal cord, we explored whether sensory and cortical pathways in the DCN also receive axoaxonic contacts. We show that primary afferent terminals more frequently receive axoaxonic contacts than cortical terminals, and have more axoaxonic contacts per terminal. Furthermore, we show that cortical projections to the DCN mainly originate in the primary somatosensory cortex (S1) in a somatotopic manner. Preliminary evidence suggests these cortical projections target both excitatory and inhibitory neurons in the DCN. We used c-fos to investigate the functional role of these nuclei during predominantly sensory or motor behaviors. We found that both punctate stimulation of the hindpaw (44% increase in Gr) and treadmill walking (50% increase in Gr) evoked an increase in c-fos labeling, suggesting these nuclei are recruited during both tactile sensation and locomotion. Finally, we show the DCN project reciprocally to the spinal cord dorsal horn, suggesting a cortico-DCN-spinal pathway for modulating tactile and proprioceptive information. Collectively, these results identify the DCN as a nexus for the processing and distribution of somatosensory information and offers an alternative site for therapeutic intervention to restore sensorimotor function.

Disclosures: **A. Upadhyay:** None. **F. Imai:** None. **A. Yonk:** None. **D.J. Margolis:** None. **S.J. Bensmaia:** None. **Y. Yoshida:** None. **V.E. Abraira:** None.

Digital Abstract Session

P160. Brain Circuits

Program #/Poster #: P160.02

Topic: D.03. Somatosensation – Pain

Support: Dirección de Investigación, Universidad de Monterrey Grant No. UIN20537
Dirección de Investigación, Universidad de Monterrey Grant No. UIN20512
Consejo Nacional de Ciencia y Tecnología, México Ciencia Básica Grant No. A1-S-16282

Title: The transcription factor Ebf2 is a marker for somatosensory, motivation and reward systems of the mouse brain

Authors: *M. B. D. CEPEDA-VARELA[1], L. M. CABRERA-ALVARADO[1], A. C. BARBOZA-CHÁVEZ[1], D. HERNÁNDEZ-MORALES[1], D. A. VILLANUEVA-CUEVAS[1], H. A. GONZÁLEZ-MIRANDA[1], A. G. LOZANO-ULLOA[1], L. O. GUTIÉRREZ-SÁNCHEZ[1], M. M. CISNEROS-CASAS[1], D. S. MORALES-HENRÍQUEZ[1], D. SALAS-LÓPEZ[1], V. ZOMOSA-SIGNORET[2], I. MEESTER[1], R. VIDALTAMAYO[1];
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Abstract: The functions of the brain arise from the connections between neurons and the formation of neural circuits. Pain and its processing, proprioception, and motivated behaviors are some examples of the result of the activity of these circuits. It is relevant to understand the molecular mechanisms leading to the formation of neural circuits; to identify potential modulators that serve as a foundation for therapeutic approaches for pain management and motivated behavior disorders such as addiction, obesity, and eating disorders. Therefore, it is fundamental to find markers that can track specific neural circuits. In this work, we show that the transcription factor Early B-Cell Factor 2 (Ebf2) is a marker of circuits responsible for somatosensory processing, and motivation and reward systems of the brain. By using genetically engineered 129Sv$^{Ebf2/Tau-GFP}$ mice, which express the reporter gene green fluorescent protein fused to Tau protein (TGFP) under the control of the *Ebf2* gene promoter, we detect the expression of Ebf2 in the mouse brain at different stages of development, from late embryonic stage (E14.5) to adult mice (P56) (n=25 individual brains). At all these stages, we observe expression of Ebf2-TGFP in various components of the somatosensory system including, the substantia gelatinosa (SG) of the spinal cord and the central nervous system: the ventral posteromedial nucleus of the thalamus (VPM), the principal trigeminal (PrV) nucleus and the trigeminal lemniscus, and the parabrachial nucleus (PBN) of the pons. The latter has a role in pain and somatosensory processing but, additionally, it participates in the regulation of motivated behaviors. Related to this motivation system of the brain, we also observe Ebf2-TGFP signals in components of the Dorsal Diencephalic Conduction System; such as the bed nucleus of the stria terminalis (BNST), the stria medullaris (sm), the dorsomedial habenula (dMHb), and the interpeduncular nucleus (IPN) of the midbrain. Ebf2-TGFP axons also connect the IPN to the dorsal tegmental nucleus (DTg). Moreover, we can detect Ebf2-TGFP axons arising from the periaqueductal gray matter (PAG) and innervating the ventral forebrain. Another set of Ebf2 axons originate from the lateral hypothalamic area (LHA); and connects it to the amygdala, which suggests these populations have a role in regulating motivation and reward.

Disclosures: M.B.D. Cepeda-Varela: None. **L.M. Cabrera-Alvarado:** None. **A.C. Barboza-Chávez:** None. **D. Hernández-Morales:** None. **D.A. Villanueva-Cuevas:** None. **H.A. González-Miranda:** None. **A.G. Lozano-Ulloa:** None. **L.O. Gutiérrez-Sánchez:** None. **M.M. Cisneros-Casas:** None. **D.S. Morales-Henríquez:** None. **D. Salas-López:** None. **V. Zomosa-Signoret:** None. **I. Meester:** None. **R. Vidaltamayo:** None.

Digital Abstract Session

P160. Brain Circuits

Program #/Poster #: P160.03

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01NS099245
NIH Grant R01NS069568

Title: The parabrachial complex processes dura inputs through a direct trigeminal ganglion to parabrachial connection

Authors: *O. UDDIN[1], M. ANDERSON[1], R. MASRI[2], J. SMITH[1], A. KELLER[3];
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Abstract: Migraines cause significant disability and contribute heavily to healthcare costs. Irritation of the meninges' outermost layer (the dura mater) and trigeminal ganglion activation contribute to migraine initiation. Maladaptive changes in central pain-processing regions are also important in maintaining pain. The parabrachial complex (PB) is a central region that mediates chronic pain. Specifically, amplified PB activity is causally related to increased pain (PMID 32217613, PMID 29862375). PB receives diverse sensory information, including a direct input from the trigeminal ganglion (PMID 29184209). We hypothesized that PB processes inputs from the dura. Using *in vivo* electrophysiology recordings from single units in 22 anesthetized male and female adult rats, we identified 58 neurons in lateral PB that respond reliably and with short latency to electrical dura stimulation. After injecting tracer into PB, anatomical examination reveals retrogradely labeled cell bodies in the trigeminal ganglion. Neuroanatomical tract-tracing revealed a population of neurons in the trigeminal ganglion that innervate the dura and project directly to PB. Based upon these findings, we hypothesize that amplified activity of dura-responsive PB neurons underlies migraine-associated pain. To model the trigeminal sensitization associated with migraine, we applied low pH (4.8) solution to the dura, recording spontaneous and pinch-evoked activity of dura-responsive PB neurons. In preliminary experiments conducted several hours after applying low pH to the dura, we have identified dura-stimulus-responsive PB neurons in which either receptive field or neural activity is increased compared to baseline. These data indicate that PB is strategically placed to process dura inputs and suggest that it is directly involved in the pathogenesis of migraine headaches.

Disclosures: O. Uddin: None. **M. Anderson:** None. **R. Masri:** None. **J. Smith:** None. **A. Keller:** None.

Digital Abstract Session

P161. Itch

Program #/Poster #: P161.01

Topic: D.02. Somatosensation

Support: T32NS086749
F32NS110155
T32NS073548
R01NS096705

Title: The neurokinin-1 receptor is expressed with gastrin-releasing peptide receptor in spinal interneurons and modulates itch

Authors: *T. SHEAHAN[1], C. WARWICK[1], L. FANIEN[1], S. ROSS[2];
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Abstract: The neurokinin-1 receptor (NK1R, encoded by *Tacr1*) is expressed in spinal dorsal horn neurons and has been suggested to mediate itch. However, previous studies relied heavily on neurotoxic ablation of NK1R spinal neurons, which limited further dissection of their function in spinal itch circuitry. Thus, we leveraged a newly developed $Tacr1^{CreER}$ mouse line to characterize the role of NK1R spinal neurons in itch. We show that pharmacological activation of spinal NK1R and chemogenetic activation of $Tacr1^{CreER}$ spinal neurons increases itch behavior, whereas pharmacological inhibition of spinal NK1R suppresses itch behavior. We use fluorescence *in situ* hybridization to characterize the endogenous expression of *Tacr1* throughout the superficial and deeper mouse dorsal horn, as well as the lateral spinal nucleus of mouse. Notably, *TACR1* expression was evolutionarily conserved in the human spinal cord dorsal. Retrograde labeling studies from the parabrachial nucleus show that less than 20% of superficial $Tacr1^{CreER}$ dorsal horn neurons are spinal projection neurons, and thus the majority of $Tacr1^{CreER}$ are local interneurons. We then use a combination of *in situ* hybridization and *ex vivo* two-photon Ca^{2+} imaging of the spinal cord to establish that NK1R and the gastrin-releasing peptide receptor (GRPR) are coexpressed within a subpopulation of excitatory superficial dorsal horn neurons. These findings are the first to describe a role for NK1R interneurons in itch and extend our understanding of the complexities of spinal itch circuitry.

Disclosures: T. Sheahan: None. **C. Warwick:** None. **L. Fanien:** None. **S. Ross:** None.

Digital Abstract Session

P161. Itch

Program #/Poster #: P161.02

Topic: D.02. Somatosensation

Title: Gastrin releasing peptide receptor signaling from endosomes: a key source of itch signaling.

Authors: *D. D. JENSEN;
NYU Col. of Dent..

Abstract: Chronic pruritus is a major unmet clinical problem with one in four adults experiencing chronic pruritus in their lifetime. G-protein coupled receptors (GPCRs) are key receptors driving itch signaling and are the major therapeutic target for itch relief. The newly discovered ability of GPCRs to signal from endosomes in cells provides new challenges for understanding GPCR signaling regulation, how endosomal signaling of GPCRs contributes to disease states like chronic pruritus, and opens new targets for therapeutic development. The Gastrin releasing peptide receptor (GRPR) is a key mediator of pruritis in the spinal cord and is rapidly internalized upon activation. Yet, little is known about the molecular mechanisms regulating GRPR signaling in pruritus, if GRPR can signal from endosomes or the role of endosomal GRPR in the development of pruritus. Aims: Determine the importance of internalization and endosomal signaling of GRPR in pruritus. Methods: The endosomal inhibitors (PitStop2 & Dyngo4a) and dominant-negative forms of dynamin were used to inhibit endocytosis and trafficking of GRPR in cell lines and in mouse models for pruritus. GRPR internalization and trafficking to endosomes were quantified using BRET and fluorescent microscopy. Endosomal-mediated ERK signaling and gene expression were measured in cell lines using FRET biosensors and luciferase gene expression assays. pH-sensitive mesoporous silica nanoparticles (MSN) were used to deliver RC-3095, a GRPR antagonist, specifically to endosomes to block endosomal signaling of GRPR. Chloroquine (CQ) and deoxy-cholic acid (DCA) invoked itch responses following intrathecal injections of endosomal inhibitors were measured and neuronal activation was quantified using phospho-ERK1/2 immunofluorescence. Results: In HEK cells clathrin and dynamin disruption attenuated GRP-induced GRPR internalization and trafficking to endosomes, which blocked cytosolic and nuclear ERK signaling and the increased expression of the serum response element reporter gene. Pre-incubation of the cells with pH-sensitive nanoparticles containing GRPR antagonists also blocked the ERK signaling in the cells. CQ and DCA injections resulted in an increase in phosphor-ERK immunolabeling in spinal neurons in mice. While intrathecal injection of endosomal inhibitors attenuated scratching responses to CQ and DCA in mice. Discussion: Our results demonstrate a critical role for GRPR endosomal signaling in the transmission of itch. These results highlight the ability of endosomal released antagonist to inhibit GRPR signaling and provide a new target for developing therapeutics that block GRPR mediated pruritus.

Disclosures:

Digital Abstract Session

P161. Itch

Program #/Poster #: P161.03

Topic: D.02. Somatosensation

Support: NIH Grant NS087088
NIH Grant HL141269
Pfizer Aspire Dermatology Award to L.H.

Title: Visualizing the itch-sensing skin arbors

Authors: *Y. XING, H. R. STEELE, H. B. HILLEY, K. LAWSON, T. NIEHOFF, L. HAN;
Georgia Inst. of Technol., Atlanta, GA

Abstract: Diverse sensory neurons exhibit distinct neuronal morphologies with a variety of axon terminal arborizations subserving their functions. Due to its clinical significance, the molecular and cellular mechanisms of itch are being intensely studied. However, a complete analysis of itch-sensing terminal arborization is missing. Using a novel *MrgprC11*^{CreERT2} transgenic mouse line, we labeled a small subset of itch-sensing neurons that express multiple itch-related molecules including MrgprA3, MrgprC11, histamine receptor H1, IL-31 receptor, 5-HT receptor 1F, natriuretic precursor peptide B, and neuromedin B. By combining sparse genetic labeling and whole-mount PLAP histochemistry, we found that itch-sensing skin arbors exhibit free endings with extensive axonal branching in the superficial epidermis and large receptive fields. These results revealed the unique morphological characteristics of itch-sensing neurons and provide novel insights into the basic mechanisms of itch transmission.

Disclosures: Y. Xing: None. **H.R. Steele:** None. **H.B. Hilley:** None. **K. Lawson:** None. **T. Niehoff:** None. **L. Han:** None.

Digital Abstract Session

P161. Itch

Program #/Poster #: P161.04

Topic: D.02. Somatosensation

Support: NIH Grant NS087088
NIH Grant HL141269
Pfizer Aspire Dermatology Award

Title: Mrgprc11^{+} DRG sensory neurons mediate glabrous skin itch

Authors: *H. R. STEELE, Y. XING, Y. ZHU, H. HILLEY, K. LAWSON, Y. NHO, T. NIEHOFF, L. HAN;
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Abstract: Despite being the most common reason for visiting the dermatologist, chronic itch has very few effective treatments. Additionally, the most deliberating form of itch, glabrous skin (palms and soles) itch, has attracted virtually no attention within the field due to a lack of methodology. This is despite glabrous skin itch arising from many medical conditions (plantar and palmar psoriasis, dyshidrosis, and cholestasis). Therefore, to identify which neuronal populations mediate glabrous skin itch we examined the central and peripheral innervation pattern of three previously identified itch-sensing neurons (MrgprA3^{+}, MrgprD^{+}, and MrgprC11^{+}). We found that both MrgprD^{+} and MrgprC11^{+} sensory neurons densely innervate glabrous skin, while MrgprA3^{+} sensory neurons do so only very sparsely. Consistently, glabrous skin-innervating neurons identified by retrograde tracing showed very weak neuronal responses to MrgprA3 agonist chloroquine. These results indicate, for the first time, the potential

mechanistic differences that exist between hairy and glabrous skin itch. As MrgprC11$^+$ neurons have not previously been characterized, we used immunostaining and RNAscope *in situ* hybridization to determine that MrgprC11 is expressed in a subset of small-diameter peptidergic neurons that constitute ~18% of DRG sensory neurons, including the majority of both MrgprA3$^+$ and NPPB$^+$ itch-sensing neurons. To investigate the mechanisms of glabrous skin itch, we developed novel acute and chronic mouse behavioral assays to examine itch sensation arising from the glabrous skin of the plantar hindpaw. We found that mice exhibit specific biting behavior in response to pruritogens and licking in response to algogens. Using this assay, we demonstrated that chemogenetic activation of MrgprA3$^+$ and MrgprD$^+$ nerves in the glabrous skin do not induce biting behavior, suggesting that they do not mediate glabrous skin itch. In contrast, we found that activation of MrgprC11$^+$ neurons using both chemogenetic activation and the MrgprC11 agonist Bam8-22 induced significant dose-dependent biting behaviors in glabrous skin. Finally, we found that ablation of MrgprC11$^+$ neurons almost completely abolishes both acute (Bam8-22) and chronic glabrous skin itch. These results suggest that MrgprC11$^+$ neurons are a major mediator of glabrous skin itch. In summary, our findings reveal new avenues for future glabrous skin itch study and the development of glabrous-skin specific anti-itch therapies.

Disclosures: H.R. Steele: None. **Y. Xing:** None. **Y. Zhu:** None. **H. Hilley:** None. **K. Lawson:** None. **Y. Nho:** None. **T. Niehoff:** None. **L. Han:** None.

Digital Abstract Session

P161. Itch

Program #/Poster #: P161.05

Topic: D.02. Somatosensation

Support: NIH R01AR074062
Stanley Glaser Foundation Research Award UM SJG 2019-18

Title: The role of NPY2R$^+$ neurons in the amygdala on non-histaminergic itch

Authors: *D. PAVLENKO, T. AKIYAMA;
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Abstract: Title: The role of NPY2R$^+$ neurons in the amygdala on non-histaminergic itchA major part of the itch neural pathway is the amygdala. Previous work has shown that activating itch-responsive neurons in the amygdala causes an increase in itch- and anxiety-related behaviors. In contrast, activation of the $GABA_A$ receptor in the amygdala causes a decrease in itch-related behavior. Neuropeptide Y receptor type 2 (NPY2R) has been found primarily in the amygdala and hypothalamus. It has been categorized as a presynaptic GCPR whose activation leads to a downstream inhibition of glutamate. In the present study, we tested the role of NPY2R-expressing amygdala neurons on itch.To manipsulate the activity of NPY2R-expressing neurons, we prepared NPY2R-cre mice and injected a cre-dependent AAV into the amygdala of these mice. The cre-dependent AAV contained tdTomato and chrimsonR, which allowed us to use

light to activate NPY2R-expressing neurons and map the projection patterns of NPY2R-expressing amygdala neurons in the brain. Prior to surgery, mice had intradermal injections of the nonhistaminergic itch mediator chloroquine to see their base level scratch response to the chemical. After surgery, the intradermal injections were repeated in a light on and light off condition. When the light is on, the NPY2R neurons are activated, and there was a suppression of itch-related behavior. To further validate the role of NPY2R-expressing neurons in itch, we injected an NPY2R agonist peptide YY 3–36 (PYY3–36) into the amygdala in naïve mice and videotaped their behaviors. PYY3-36 dose-dependently elicited itch-related behavior. Based on these findings, we concluded that NPY2R-expressing amygdala neurons are involved in the regulation of itch.

Disclosures: D. Pavlenko: None. **T. Akiyama:** None.

Digital Abstract Session

P162. Pain Models

Program #/Poster #: P162.01

Topic: D.03. Somatosensation – Pain

Title: NIH HEAL Initiative: NINDS Preclinical Screening Platform for Pain (PSPP)

Authors: *S. A. WOLLER, A. TAMIZ, S. IYENGAR;
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Abstract: The National Institute of Neurological Disorders and Stroke (NINDS) aims to enhance pain management and accelerate the discovery and development of new non-addictive pain therapeutics as part of the recently launched Helping to End Addiction Long-term (HEAL) Initiative, a trans-agency effort to provide scientific solutions to the opioid crisis. With HEAL support, the NINDS Preclinical Screening Platform for Pain (PSPP) has been set up to accelerate identification of novel approaches to treat both acute and chronic pain conditions. Under NINDS direction, preclinical testing of submitted agents is performed by contract facilities on a blinded and confidential basis at no cost to the PSPP participants. Test candidates are evaluated in a suite of *in vivo* pain-related assays following *in vitro* receptor profiling, pharmacokinetic, and side-effect profile assessment. Importantly, test candidates are also evaluated in models of abuse liability. In vivo pain-related assays include models of acute to chronic pain and persistent pain mechanisms, as well as specific models of neuropathic, nociceptive and neuroplastic pain, including e.g. chemotherapy-induced neuropathic pain, osteoarthritis pain, and migraine. A key feature of the PSPP is the flexibility to continuously acquire and validate innovative new models and endpoints that more closely represent human pain conditions. Towards that end, test systems and endpoints amenable to evaluating devices can also be incorporated. PSPP provides researchers from academia and industry, in the US and internationally, an efficient, rigorous, one-stop *in vivo* screening resource to identify and profile novel non-opioid, non-addictive therapeutic candidates, including small molecules, biologics, natural products and devices for the treatment of pain. This presentation will elaborate on this novel non-opioid, non-addictive pain

therapeutic discovery and development program and its efforts to engage the drug discovery and device development community.

Disclosures: **S.A. Woller:** None. **A. Tamiz:** None. **S. Iyengar:** None.

Digital Abstract Session

P162. Pain Models

Program #/Poster #: P162.02

Topic: D.03. Somatosensation – Pain

Title: The pharmacological interaction of gabapentin and sulforaphane relief pain in a fibromyalgia like pain model.

Authors: *I. Y. ZAMORA DIAZ[1], F. J. LOPEZ MUNOZ[2], M. E. GÓNZALEZ-TRUJANO[3]; [1]CINVESTAV, CDMX, Mexico; [2]CINVESTAV, Mexico, Mexico; [3]Inst. Nacional de Psiquiatría "Ramón De la Fuente Muñiz", CDMX, Mexico

Abstract: The current management of fibromyalgia represents an important challenge for therapeutics in the control of this type of pain. Drugs of the α2-δ ligand family, characterized by blocking the activity of voltage-gated calcium channels, have shown some utility for this condition. Pregabalin belongs to this group and is one of the FDA-approved drugs for the treatment of fibromyalgia-like pain. Gabapentin, although it belongs to the family of α2-δ ligands, is a drug with less application for this condition due in part to the lack of studies that reinforce its efficacy. On the other hand, sulforaphane is an isothiocyanate derived from glucosinolate-type compounds found in cruciferous vegetables. This has been related to effects on the central nervous system as an antinociceptive, anxiolytic-antidepressant and anticonvulsant, associated in part with its modulating role in the activity of calcium channels and its neuroprotective action as an antioxidant. The objective of this study was to evaluate the antinociceptive effects of the individual administration and in combination of different doses of gabapentin and sulforaphane in two of the most important painful manifestations of fibromyalgia, using the reserpine-induced fibromyalgia pain model in rats. The antiallodynic effects were analyzed by mechanical and thermal stimulation; and the anti-hyperalgesia by mechanical stimulus. Time-course curves were made for both molecules recording behavior every 30 min and during a period time of 4 hours. The dose-response effects were obtained. Our results demonstrated that both sulforaphane and gabapentin administered individually produced significant antiallodynic and anti-hyperalgesic effects and in a dose-dependent manner in the fibromyalgia-type pain model. Additionally, it was determined that the combination of intermediate doses, but not lower doses, of both drugs facilitated antiallodynic and/or anti-hyperalgesic activities but producing the same level of effect using only 1/3 of the individual dose of each case. This study provides evidence of the potential of gabapentin and sulforaphane for the relief of fibromyalgia-type pain alone or in combination of certain doses.

Disclosures: **I.Y. Zamora Diaz:** None. **F.J. Lopez Munoz:** None. **M.E. Gónzalez-Trujano:** None.

Digital Abstract Session

P162. Pain Models

Program #/Poster #: P162.03

Topic: D.03. Somatosensation – Pain

Support: NIH Grant TL1 TR001428

Title: The heat-pain stimulus-response curve: effects of age and chronic pain

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Abstract: Aging leads to adaptations in the human somatosensory system with older individuals reporting changes in perception of odor, high and low pitch sounds, and taste. Age-related changes have also been reported in the systems that process pain with quantitative sensory testing (QST) showing that older individuals report increases in pain threshold (PTh) and decreases in pain tolerance (PTo). QST testing has improved our understanding of pain processing at the bottom and top of a range, while also providing evidence that individuals with chronic pain show increased pain sensitivity. However, conventional QST approaches tell us little about how age and chronic pain influence the calibration of the pain system across different levels of stimulus intensity. To directly address this gap in the literature, we assessed pain ratings across a heat-pain stimulus-response curve in a total of 159 participants. Participants were grouped based on age and chronic pain status; 75 healthy controls (young pain -free: 22.9 years), 25 young participants with chronic jaw pain (young pain: 35.9 years), 26 older healthy controls (old-pain-free: 66.5 years), and 33 older individuals with chronic low back pain (old pain: 66.5 years). Participants received 4 second bouts of heat stimulation to their right, volar forearm at 9 different temperatures ranging from 40°C to 48°C. Participants received each temperature twice in a pseudo-randomized order. Participants were asked to rate their pain to each stimulus on a scale from 0 to 100 (0 being no pain, 100 being intolerable pain). We used linear mixed models to quantify the relationship between temperature, pain rating, age and pain status. To further quantify associations between temperature and pain rating we ran four Pearson's Product Correlations and performed a one-way analysis of variance test using each participants R^2 value. We report 2 novel findings. First, both age and chronic pain were separately associated with a flatter slope in the heat-pain stimulus-response curve. Second, we found that age was also associated with greater variability in pain ratings across the range of temperatures. Previously reported central and peripheral pain mechanisms alone cannot account for our findings, suggesting that age and chronic pain alter the heat pain stimulus-response curve via multiple mechanisms.

Disclosures: **R.L. Ho:** None. **W. Wang:** None. **Y. Cruz-Almeida:** None. **H. Ding:** None. **Z. Huo:** None. **S. Coombes:** None.

Digital Abstract Session

P162. Pain Models

Program #/Poster #: P162.04

Topic: D.03. Somatosensation – Pain

Support: NIGMS 5U54GM104942-04
NCI 5R01CA194924 to ACD
NINDS 5R01NS092388 to RJN

Title: Dim Light at Night Exacerbates Chemotherapy-Induced Peripheral Neuropathy in Mice

Authors: *J. R. BUMGARNER, W. H. WALKER, II, J. A. LIU, H. MELENDEZ-FERNANDEZ, J. C. WALTON, C. DEVRIES, R. J. NELSON;
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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a debilitating disease associated with numerous chemotherapeutic agents used in cancer treatment. Despite its prevalent and severe effects on both patient quality of life and treatment outcome, there is currently no accepted treatment for CIPN. Considering that disruption of circadian rhythms is known to alter immune function, induce peripheral and central inflammation, alter metabolism, and even directly exacerbate pain behavior in rodents, we sought to examine the relationship between disrupted circadian rhythms and CIPN. We hypothesized that exposure to dim light at night (dLAN; 5 lux) exacerbates pain behavior associated with chemotherapy treatment. To test this hypothesis, pain behavior was assessed in female ovariectomized CFW mice before and after treatment with paclitaxel. Thermal withdrawal thresholds were tested using the hot and cold plates, and mechanical withdrawal thresholds were tested using an electric von Frey instrument. Starting on the first night of either dLAN or dark night housing conditions, mice received a 5-day i.p. treatment regimen of either paclitaxel (2 mg/kg/day) or vehicle. We observed that dLAN exposure exacerbated mechanical allodynia associated with paclitaxel treatment starting in and persisting in the third week of dLAN housing. Using qRT-PCR, we observed that paclitaxel-treated mice housed in dLAN demonstrated upregulated expression of *Il-6* in the rostral ventromedial medulla of the descending-regulatory system. Together, these data suggest harmful effects of dLAN exposure on pain behavior associated with CIPN. Future non-pharmacological therapeutic interventions for CIPN should consider mitigating circadian rhythm disruption to improve patient health and treatment outcome.

Disclosures: **J.R. Bumgarner:** None. **W.H. Walker:** None. **J.A. Liu:** None. **H. Melendez-Fernandez:** None. **J.C. Walton:** None. **C. DeVries:** None. **R.J. Nelson:** None.

Digital Abstract Session

P162. Pain Models

Program #/Poster #: P162.05

Topic: D.03. Somatosensation – Pain

Support: Department of Defense OR170276

Title: Evaluation of recombinant gene therapy with serine-histogranin and endomorphin-1 in a model of phantom limb pain in rats

Authors: A. PACHECO-SPIEWAK, A. EESWARA, F. BORJA, S. GROSS, M. HERNANDEZ, S. JERGOVA, *J. SAGEN;
Univ. of Miami, Miami, FL

Abstract: Limb amputation frequently results in emergence of phantom limb pain (PLP). This perception of pain from the missing extremity is poorly understood and current therapeutic options are marginally effective. To mimic PLP, we have developed a rodent model in which inflammation and peripheral nerve constriction injury is followed by axotomy of the sciatic nerve. This replicates complex extremity injuries that frequently occur prior to medically indicated amputation. The occurrence of prior pain has been clinically observed to predict quality and severity of subsequent PLP. Previous studies in our lab have shown alleviation of pain-like behavior induced by peripheral nerve injury or spinal cord injury by NMDA receptor antagonist serine-histogranin (SHG). Further analgesic potential was explored through combination of SHG gene with mu-opioid peptide endomorphin-1 (EM1). The current study investigates the potential of SHG and SHG/EM1 gene constructs in reducing or preventing development of PLP-like behavior. Our model aims to discover the most efficient route and best timing of delivery of the therapeutic gene constructs. Using male Sprague-Dawley rats, AAV_SHG, AAV_SHG/EM1 or AAV_GFP constructs were administered via dorsal root ganglion injection, intrathecally, or intraspinally either on the same day as the transection or one week later, with pre-amputation injury induced at 1 to 14 days prior to the transection. Autotomy onset, severity, and location were monitored daily as indicators for PLP-like behavior. Upon completion of in vivo studies, CSF and tissue were collected for neurochemical and immunocytochemical analysis. Our data shows significant attenuation in the severity and onset of the PLP-like behavior in animals treated with SHG and SHG/EM1 gene constructs compared to GFP. The most robust effect of both constructs was observed after intraganglionic injection. Comparison of SHG and SHG-EM1 outcomes showed that animals treated with SHG/EM1 displayed lower autotomy scores compared to SHG alone, suggesting that the inhibition of glutamate signaling by SHG and the simultaneous activation of mu-opioid receptor by EM1 may generate more potent PLP alleviation. Recombinant SHG and EM1 peptides were detected in the spinal tissue of treated animals. Analysis of CSF samples and immunocytochemistry showed downregulation of inflammatory mediators IL-1β and TNF-α and reduction in reactive glial markers GFAP and Iba-1 in animals treated with gene constructs compared to GFP controls. These data demonstrate the potential beneficial effect of targeted gene therapy in the prevention or attenuation of PLP.

Disclosures: **J. Sagen:** None. **A. Pacheco-Spiewak:** None. **A. Eeswara:** None. **F. Borja:** None. **S. Gross:** None. **M. Hernandez:** None. **S. Jergova:** None.

Digital Abstract Session

P162. Pain Models

Program #/Poster #: P162.06

Topic: D.03. Somatosensation – Pain

Support: VA IK2 BX003630
VA I01 BX002188

Title: Importance of the prefrontal cortex-bed nucleus of the stria terminalis pathway in visceral nociception

Authors: ***A. C. JOHNSON**[1,2], C. O. LIGON[2], B. GREENWOOD[2,1];
[1]Res. Admin. 151, VA Hlth. Care Syst., Oklahoma City, OK; [2]Physiol., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstract: *Introduction:* Corticolimbic brain circuits demonstrate abnormal activity in patients with stress-induced visceral pain disorders, such as irritable bowel syndrome (IBS), characterized by colonic hypersensitivity. The prefrontal cortex (PFC) has been shown to participate in both pain and stress modulation. We have previously found that the pathway from the central amygdala to the bed nucleus of the stria terminalis (BNST) modulates colonic sensitivity in the rat. The BNST also receives signals from the PFC, but the role of the PFC in the modulation of stress-induced colonic nociception is unknown. The hypothesis for this study was that activation of PFC-BNST pathway could induce colonic hypersensitivity. *Methods:* To test the role of PFC-BNST signaling, we infected the PFC with viral vectors to express channelrhodopsin (ChR2) or halorhodopsin (HR3.0) and implanted fiber optic cannula at the BNST in male Fischer 344 rats (n=4/group). To model a chronic stressor, a different cohort had a single micropellet (30 μg) of the stress hormone, corticosterone (CORT) or cholesterol (CHOL) control implanted in the PFC (n=8/group). Graded, isobaric colorectal distension (20-60 mmHg, 10 min) was used to assess colonic sensitivity, quantified as the number of abdominal muscle contractions in freely moving rats. In rats with opsins, colonic sensitivity was assessed with and without laser stimulation after 10 weeks to allow for opsin expression. In rats with micropellets, colonic sensitivity was measured after 8 days at the peak diffusion of the micropellet. Results were analyzed with a repeated measure two-way ANOVA with Bonferroni's post-hoc analysis (mean ± standard deviation). *Results:* We first found that inhibition of the PFC-BNST pathway in via HR3.0 did not affect colonic sensitivity in non-stressed rats (60 mmHg: 22.0 ± 2.8 vs. 21.5 ± 3.3, p=0.99). In contrast, activation of the PFC-BNST pathway with ChR2 induced colonic hypersensitivity (60 mmHg: 36.0 ± 8.8 vs. 28.5 ± 6.5, $p<0.05$. Finally, to evaluate the role of the PFC-BNST pathway to modulate stress-induced colonic hypersensitivity, we implanted CORT in the PFC and found that the rats developed colonic hypersensitivity compared to CHOL-implanted controls (60 mmHg: 37.9 ± 4.6 vs. 24.3 ± 2.7, $p<0.01$). *Conclusions:* Both ChR2

activation and CORT-induced signaling from the PFC to the BNST induced colonic hypersensitivity. These results support the role of the PFC-BNST pathway as part of a corticolimbic circuit that modulates visceral sensation in response to stress. Modulation of the PFC-BNST pathway could represent a valid target for novel therapies to treat disorders such as IBS.

Disclosures: A.C. Johnson: None. **C.O. Ligon:** None. **B. Greenwood:** None.

Digital Abstract Session

P162. Pain Models

Program #/Poster #: P162.07

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01 AA025024

Title: Female mice exhibit persistent hyperalgesia while housed with CFA-treated conspecifics or their bedding.

Authors: *Y. ZHANG, A. E. RYABININ;
Dept. of Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: It has become a well-documented observation in literature that an animal's pain experience can be altered by another's pain state depending on their social relationship. However, the neural mechanisms of such modulation of pain by social interactions is still far from understood. Our lab has previously reported that naïve mice housed in the same room with mice experiencing alcohol withdrawal-induced hyperalgesia also exhibited hypersensitivity to pain. We showed that exposure to the beddings of mice experiencing alcohol withdrawal-induced hyperalgesia was sufficient to cause hypersensitivity in co-housed naïve mice, demonstrating that olfactory signaling played an important role in the transferred pain experience. The aim of the present study was to examine whether our observation of persistent transferred pain from exposure to beddings of affected conspecifics was specific to alcohol withdrawal-induced hyperalgesia or common in other painful conditions. Female C57BL/6J mice were singly housed in separate cages. After baseline testing, one group of mice received an intra-plantar injection of CFA (CFA-primary) and were housed with a group of untreated mice (CFA-bystander) in separate cages in the same room for the rest of the testing period. Another group of naïve mice were housed in a different room (CFA-olfactory), exposed to only the bedding from CFA mice that were transferred over daily in small portions and distributed evenly outside of the cages of CFA-olfactory mice. Female mice receiving intra-plantar saline injections (Saline-primary), co-housed with Saline-primary (Saline-bystander), as well as only exposed to Saline-primary beddings (Saline-olfactory) were used as control groups. Results showed that both CFA-bystander and CFA-olfactory female mice exhibited a decrease in von Frey mechanical threshold compared to baseline. Their mechanical threshold recovered 7 days following removing CFA-primary mice or the beddings from CFA-primary mice. On the other hand, we did not detect

meaningful difference in nociceptive threshold for thermal nociception between groups in the Hargreaves test. In conclusion, we demonstrate that the olfactory stimulation from the bedding of mice experiencing CFA-induced persistent inflammatory pain contributes to development of significant allodynia in that bystander mice housed in the same room for a prolonged period. Together with our previous finding, this study provided evidence for a role of olfaction in social communication of individual's pain state.

Disclosures: Y. Zhang: None. **A.E. Ryabinin:** None.

Digital Abstract Session

P162. Pain Models

Program #/Poster #: P162.08

Topic: D.03. Somatosensation – Pain

Support: NIH Contract No 75N95019D00026
Task Order number 75N95019F00088

Title: Standardization of Preclinical Models for Pain

Authors: *M. A. VARNEY[1], E. LEAHY[1], D. BUDAC[1], E. DUGAN[1], M. URBAN[1], Q. CHANG[1], H. FERNANDES[1], J. GRESACK[1], S. A. WOLLER[2], S. IYENGAR[2], T. HANANIA[1]; [1]Psychogenics, Paramus, NJ; [2]NINDS, Rockville, MD

Abstract: Under the NIH Helping to End Addiction Long-term[SM] (HEAL) Initiative, PsychoGenics was awarded a contract to screen and profile novel therapeutics within the Preclinical Screening Platform for Pain (PSPP). The main goal of this platform is to accelerate the preclinical development of non- starting opioid, non-addictive therapies for acute and chronic pain.
In collaboration with NINDS and experts in the field, PsychoGenics evaluates assets through *in vitro* cell-based functional assays to rule out opioid receptor activity and to assess *in vitro* abuse liability, *in vivo* pharmacokinetic studies to guide the dose range and pretreatment times for the side effect profile, *in vivo* efficacy and abuse liability studies. *In vivo* efficacy studies that are used in evaluating assets include the plantar incision model and L5/L6 spinal nerve ligation model. Disease specific pain models as well as novel endpoints that may increase translation are also being evaluated and optimized. Examples include models of neuropathic pain, osteoarthritis pain, deep muscle pain and migraine. Non-evoked endpoints being developed in these models include gait, guarding and place escape avoidance paradigm (PEAP). All studies are performed in a blinded fashion in both sexes. Data are reported consistent with ARRIVE Guidelines.
This presentation will show examples of the tiered approach used to evaluate assets and the efforts to enhance novelty, rigor and reproducibility in pain research to support the NINDS PSPP program within the HEAL Initiative.

Disclosures: M.A. Varney: A. Employment/Salary (full or part-time):; Psychogenics. **E. Leahy:** A. Employment/Salary (full or part-time):; Psychogenics. **D. Budac:** A.

Employment/Salary (full or part-time):; Psychogenics. **E. Dugan:** A. Employment/Salary (full or part-time):; Psychogenics. **M. Urban:** A. Employment/Salary (full or part-time):; Psychogenics. **Q. Chang:** A. Employment/Salary (full or part-time):; Psychogenics. **H. Fernandes:** A. Employment/Salary (full or part-time):; Psychogenics. **J. Gresack:** A. Employment/Salary (full or part-time):; Psychogenics. **S.A. Woller:** A. Employment/Salary (full or part-time):; NIH. **S. Iyengar:** A. Employment/Salary (full or part-time):; NIH. **T. Hanania:** A. Employment/Salary (full or part-time):; Psychogenics.

Digital Abstract Session

P162. Pain Models

Program #/Poster #: P162.09

Topic: D.03. Somatosensation – Pain

Title: Neurological Assays performed on Dorsal Root Ganglion from Non-Human Primate and Rodent origins

Authors: L. MIRAUCOURT[1], M. ARSENAULT[3], M. ACCARDI[1], M. MAGHEZZI[1], *C. S. PERITORE[2], S. AUTHIER[1];
[1]Charles River Labs., Laval, QC, Canada; [2]Neurosci. Discovery, Charles River Labs., Wilmington, MA; [3]Médecine Vétérinaire, Univ. of Montreal, Montreal, QC, Canada

Abstract: Peripheral neurons of the dorsal root ganglia (DRG) are at the forefront of integrating and transmitting sensory information to the central nervous system. In the context of acute, chronic or neuropathic pain, DRG's are considered a primary target for treatment. One current challenge is the identification of the cellular activities most relevant to pain control. Recent advances in our understanding of the physiology and pathophysiology of DRG neurons point to voltage-gated sodium channels (Nav) as a promising target, arousing great interest in the pharmaceutical industry. Nav channels give rise to the upstroke of an action potential and are thus the ultimate checkpoint of cell excitability. Of the 10 Nav channel alpha subunits isoforms, which forms the core of the channel, a single amino acid substitution in the S5-S6 linker renders Nav1.5, Nav1.8, and Nav1.9 resistant to tetrodotoxin (TTX), making them a TTX-resistant (TTX-R) specific subgroup implicated in heart, muscle and dorsal root ganglion excitability. Furthermore, TTX-resistant Nav channel expression has been suggested to characterize selective populations of DRG neurons. Using primary cultures of DRG neurons isolated from adult non-human primates (NHP) and rodents, we performed whole cell electrophysiology using current-clamp and voltage-clamp protocols. We found that TTX-resistant Nav channels can be engaged for action potential initiation of small diameter DRG neurons (< 30 pF), but totally absent from large diameter ones (> 50 pF) in rodents. However small, as well as large, diameter DRG neuronal populations express TTX-resistant Nav channels in NHPs. This may help define species-specific context for therapeutic relevance. In addition, action potential threshold and biophysical characterization of the Nav currents from acutely isolated adult DRGs demonstrated species differences relevant to the design of *ex vivo* neuropharmacology assays.

Disclosures: **L. Miraucourt:** None. **M. Arsenault:** None. **M. Accardi:** None. **M. Maghezzi:** None. **C.S. Peritore:** None. **S. Authier:** None.

Digital Abstract Session

P163. Peripheral Mechanisms of Neuropathic Pain

Program #/Poster #: P163.01

Topic: D.03. Somatosensation – Pain

Support: NIH Grant AR047410

Title: A Free Open-Source Method of Image Cytometry in Rat Dorsal Root Ganglia with Fluorescent Immunohistochemistry

Authors: *M. B. ANDERSON;
Anat. & Cell Biol., Oklahoma State Univ. Ctr. for Hlth. Sci., Tulsa, OK

Abstract: Immunohistochemistry (IHC) is a valuable tool in clinical and biological research for evaluating proteins and other antigens in spatially bound tissue. In neuroinflammatory pain research, primary afferent neurons of the dorsal root ganglion (DRG) are studied to understand molecular signaling mechanisms involved in nociception (pain) and inflammation. Measuring IHC immunofluorescence in DRG neurons require manual hand tracing of nuclear and somatic boundaries, which is laborious, error-prone, and can require several weeks to collect the appropriate sample size with a mouse or pen-input display monitor. To overcome these limitations and increase standardization of sampling and measurement, we employed a reliable neuronal cytoplasmic reporter, exclusive to DRG neuronal soma, in a semi-automated algorithm-based approach of Image Cytometry in rat Dorsal Root Ganglion (IC-DRGs). The resulting output images are binary nuclear and somatic masks of DRG neurons, defining boundaries of measurement for CellProfiler and manually scored at 94% accurate. Herein, we successfully show a novel approach of automated image analysis for DRG neurons using a robust ImageJ/FIJI script, overcoming morphological variability and imaging artifacts native to imaging frozen tissue sections processed with fluorescent IHC.

Disclosures: **M.B. Anderson:** None.

Digital Abstract Session

P163. Peripheral Mechanisms of Neuropathic Pain

Program #/Poster #: P163.02

Topic: D.03. Somatosensation – Pain

Support: NORTE 2020 and Fundação para a Ciência e Tecnologia (project NORTE-01-0145-FEDER-028623 and PTDC/MED-NEU/28623/2017)
Fundação Grunenthal (Jovens Investigadores em Dor 2016)

Title: Dorsal root ganglion neurons assemble an axon initial segment which can initiate spontaneous activity in neuropathic pain

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Abstract: Most CNS neurons have a multipolar morphology and a specialized structure - the axon initial segment (AIS), which initiates action potentials, maintains neuronal polarity and modulates excitability *via* structural and molecular alterations. In contrast, dorsal root ganglion (DRG) neurons have a pseudounipolar morphology and it is unclear whether they possess an AIS *in vivo*. We established an *in vitro* model in which DRG neurons recapitulate their physiological morphology and assemble an AIS-like segment at the stem axon enriched in the AIS-specific protein Ankyrin-G (AnkG). This prompted us to investigate the existence of this structure *in vivo*. Strikingly, we observed that most adult myelinated DRG neurons have an AIS in the proximal stem axon, which becomes enriched in AnkG and voltage-gated sodium (Nav) channels during embryonic development. The physiological role of this segment is not clear, as action potentials are initiated in the peripheral axon terminals of DRG neurons. However, in peripheral neuropathic pain, DRG neurons develop spontaneous activity initiating in an unknown cellular compartment inside the ganglion. We decided to investigate if this newly-discovered AIS of DRG neurons is the source of such pathological activity using the chronic constriction injury (CCI) model. Immunofluorescence data show that the AIS is located in a more proximal position 4 days after CCI, and in all timepoints analyzed after injury the relative enrichment of Nav channels in the AIS with respect to the cell body is greater than in naïve animals. To investigate the contribution of the AIS and its alterations to DRG neuron excitability, we have created a detailed biophysical model of an Aδ-fiber DRG neuron based on our morphological and physiological data. Computer simulations demonstrate that the observed AIS alterations have a negligible effect on the excitability of DRG neurons, but the existence of the AIS itself facilitates the initiation of spontaneous activity. Additionally, we established a mouse model to provide a time-controlled abolition of AnkG expression in adult DRG neurons, leading to AIS disassembly. Using calcium imaging of DRG explants from these mice, we have observed that the AIS contributes to the spontaneous activity associated with CCI. Our discovery of the AIS of DRG neurons and its contribution to pathological hyperexcitability creates a new path for the development of therapeutic options for chronic pain management.

Disclosures: A.I. Nascimento: None. **L.L. Luz:** None. **F. Mar:** None. **P. Aguiar:** None. **B. Safronov:** None. **M.M. Sousa:** None.

Digital Abstract Session

P163. Peripheral Mechanisms of Neuropathic Pain

Program #/Poster #: P163.03

Topic: D.03. Somatosensation – Pain

Support: NIDA 1R21DA039621–01
Hunkele Dreaded Disease Award
Samuel and Emma Winters Foundation
Charles Henry Leach II Fund
Commonwealth Universal Research Enhancement Award
Duquesne University Inaugural Provost's Interdisciplinary Research Consortia Grant

Title: Long lasting pain relief and shift of dorsal root ganglia transcriptome in chronic nerve injury treated with nanomedicine; attenuating cyclooxygenase-2 has widespread influence on gene expression

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Abstract: Neuroinflammatory pain can transition to chronic pain through molecular mechanisms that are poorly understood. In order to discern how immune cells, neurons, and glial cells influence one another throughout this process, one strategy is to turn a specific cellular communication pathway off with targeted cyclooxygenase 2 (COX-2) inhibiting nanotherapeutic (CXB-NE) delivered directly to macrophages at the site of injury. Because this nanotherapeutic CXB-NE is specific to macrophages, we can probe the molecular cell biology and transcriptomic components that are influenced by the attenuation of the production of prostaglandin E2 (PGE2). A result of this treatment is a significant reduction in hypersensitivity and pain-like behavior. Using Chronic Constriction Injury (CCI) as well as total RNAseq, this study shows that attenuating the local production of PGE2 not only influences the transcriptome of macrophages but other cell types such as neutrophils and neurons. We also demonstrate that the changes that occur during pain relief caused by the CXB-NE nanotherapeutic affect not only the local cell types at the site of injury in the sciatic nerve, but also influences gene expression profile changes at the dorsal root ganglion (DRG). Some of the key findings reveal that critical molecular pathways active during pain relief include transducer activity, G-protein coupled receptor activity, neutrophil degranulation, and transmembrane signaling receptor activity. Increased expression of factors contributing to neuronal regeneration such as fibronectin leucine-rich transmembrane neuronal protein 3 (Flrt3) and leucine-rich repeat transmembrane neuronal proteins (LRRTM1, LRRTM2, and LRRTM3) are also enriched with CXB-NE nanotherapeutic compared to drug free nanoemulsion (DF-NE). These findings as well as others, demonstrate that the pain relief achieved by this nanotherapeutic not only influence COX2 production of PGE2, but rather have widespread influence of gene expression locally and at the corresponding DRG.

Disclosures: B.S. Deal: None. **A. Stevens:** None. **M. Saleem:** None. **L. Reynolds:** None. **J. Janjic:** None. **J.A. Pollock:** None.

Digital Abstract Session

P163. Peripheral Mechanisms of Neuropathic Pain

Program #/Poster #: P163.04

Topic: D.03. Somatosensation – Pain

Support: NIH/NINDS R01NS098426 (Cao)

Title: Contribution of peripheral changes in CD137-mediated sciatic nerve crush-induced neuropathic pain

Authors: *L. CAO, A. A. WAKLEY, H. JACOBS, E. JORDAN, E. BARTLETT;
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Abstract: We have previously shown that the costimulatory molecule CD137L is involved in the development of neuropathic pain following sciatic nerve crush (SNC). CD137L knockout (KO) mice displayed reduced sensory sensitivity and faster recovery of sensory and motor functions following SNC. CD137L was found to be mediating its effects at the spinal cord level. In this study, we sought to identify potential CD137L-mediated changes in the peripheral nervous system that could contribute to the reduced neuropathic pain-like behaviors in B6.CD137L KO mice. First, inflammation-related markers within lumbar (L3-L6) dorsal root ganglia (DRG) were examined via real-time quantitative PCR (RT-qPCR) in a time course study (days 0, 3, 7, 14, 28, and 56; n=8/group). SNC induced notable upregulation of several pro-inflammatory cytokines (including CCL2, CCL3, CCL5, and TNF-alpha) in the B6 wild type (WT) but not the CD137L KO mice, while CD137L KO mice showed notable increases in the expression of the anti-inflammatory factors IL-10 and arginase-1 (Arg-1) following SNC. Second, we assessed whether CD137L KO mice showed faster regeneration of the injured sciatic nerve. A segment of sciatic nerve containing the injury site was analyzed via whole mount immunohistochemistry in a time course study (days 0, 3, 7, 14, 28, and 56; n=4/group). The sciatic nerve segments were classified as neurofilament 200 (NF200) positive (non-nociceptive fibers), calcitonin gene-related peptide (CGRP) positive (peptidergic c-fibers), and isolectin B4 (IB4) positive (non-peptidergic c-fibers). No significant differences were observed between WT and CD137L KO mice in terms of the three types of fibers or the volume of the traumatic neuroma formed at the injury site. On the other hand, in the pin prick behavioral assay (days 0-77, n=15-17/group), both WT and CD137L KO mice showed sensory recovery from the proximal to the distal end, with CD137L KO mice recovered faster at all testing sites compared to WT mice. Altogether, a proclivity for an anti-inflammatory state within the DRG in CD137L KO mice may contribute to the reduced neuropathic pain-like behaviors in CD137L KO mice.

Disclosures: L. Cao: None. **A.A. Wakley:** None. **H. Jacobs:** None. **E. Jordan:** None. **E. Bartlett:** None.

Digital Abstract Session

P163. Peripheral Mechanisms of Neuropathic Pain

Program #/Poster #: P163.05

Topic: D.03. Somatosensation – Pain

Support: American Pain Society
Rita Allen Foundation

Title: Latent Pain Sensitization unmasked byμ-δ opioid receptor heteromer in neuropathic and inflammatory pain

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Abstract: Title: Latent Pain Sensitization unmasks μ-δ opioid heteromer in neuropathic and inflammatory painAuthors: Kufreobong E Inyang[1], Susan R George[2], Geoffroy Laumet[1]1 Department of Physiology, Michigan State University, East Lansing, MI, USA2 Department of Medicine and Pharmacology, University of Toronto, Toronto, Ontario, CanadaAbstract: Rodent models of latent pain sensitization can be used to study the episodic nature that chronic pain can exhibit. At pain remission, central sensitization is countered by the activity of endogenous opioid receptors. In animal models, latent pain sensitization can be unmasked by antagonizing opioid receptors resulting in pain relapse. Due to previous studies have focused predominantly on inflammatory pain, the presence of latent pain sensitization in animal model of chemotherapy-induced peripheral neuropathic pain and in female mice is unknown. This study investigates whether μ- and δ-OR suppress latent pain sensitization in model of chemotherapy-induced neuropathic pain in both sexes.There is significant evidence suggesting that μ-and δ-ORs form a heteromer that is capable of modulating pain sensitivity. The potential implication of the μ-δ OR heteromer in latent pain sensitization has not been fully explored due to a lack of tools to effectively modulate the heteromer. In this study, we were able to specifically target the μ-δ OR heteromer using a novel peptide to prevent its heteromerization. We then investigated whether disruption of the μ-δOR heteromer, after remission, reinstates pain hypersensitivity. At remission from cisplatin-induced neuropathic pain, antagonism of μOR and δOR reinstates pain hypersensitivity in both sexes. Disruption of the μ-δOR heteromer reinstates pain hypersensitivity in both sexes after remission from cisplatin-induced neuropathic and postsurgical pain. Taken together our findings suggest that the μ-δOR heteromer plays a crucial role in remission in various pain models and may represent a novel therapeutic target to prevent the relapse to pain and the transition to chronic pain.Acknowledgements: This study has been supported by the American Pain Society and the Rita Allen Foundation (G.L.).Competing interests: The authors except declare no competing interests.

Disclosures: K.E. Inyang: None. **G.O. Laumet:** None. **S.R. George:** None.

Digital Abstract Session

P163. Peripheral Mechanisms of Neuropathic Pain

Program #/Poster #: P163.06

Topic: D.03. Somatosensation – Pain

Title: Role of calcitonin gene related polypeptide (CGRP) in diabetic neuropathic pain model dorsal root ganglion

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Abstract: Prolonged, uncontrolled diabetes mellitus patients are prone to develop injuries to peripheral nervous system. These lead to cellular and molecular alterations throughout the nociceptive pathway. Though pro-nociceptive neuronal and non-neuronal cellular alterations in the dorsal root ganglion (DRG), have been identified the molecular mechanisms in DRG are complicated and link several signaling pathways to induce and maintain NP culminating in release of Calcitonin Gene Related Polypeptide (CGRP). Metformin is postulated to have an indirect pro-neuropathic effect via reduction of Vitamins B6 and B12 via the Adenosine Monophosphate-activated Protein Kinase (AMPK). Studies have shown that activation of AMPK reverses NP effects by negatively regulating these signaling events and decreases the excitability of dorsal root ganglion neurons via calcium channels. Thus, the rationale of this study is to understand the pathways of nociceptive pain in diabetic neuropathy and the complex interplay of the disease and its drugs as hyperglycemia may lead to a down regulation of AMPK expression. Following ARRIVE guidelines and after approval from University ethical board, 32 Balb/c male and female mice, 8 to 10 weeks age and weighing 25 to 35gms were given Alloxan (200mg/kg) to establish an animal diabetic model. These Diabetic mice were then divided into three groups. Group A were observed for three weeks for neuropathy and then given i/p normal saline for 7 days while Group B similarly observed were given i/p Metformin (200 mg/kg) for 7 days. Group C were further subdivided into C1 and C2 with C1given i/p Metformin (200 mg/kg) for initial 7 days while Group C2 were given the same days. Both mechanical and thermal hyperalgesia and allodynia were weekly recorded in each group. Mice were dissected and their DRG isolated. Immunohistochemistry was done to observe the expression of CGRP. Analysis of the data was done using MS Excel, Graph Pad Prism software version 5.0, SPSS version 22.0 and ImageJ analyzer. Neuropathic pain was validated in all the groups by significantly developed ($p<0.001$) hyperalgesia and allodynia. Earlier Metformin administration reduced the developing hyperalgesia in both Group C1 ($p< 0.01$) and C2 ($p< 0.05$) as compared to control (Group A).The IHC parameters of CGRP expression showed $p < 0.0001$ in reducing CGRP expression in DRG slides of Group C1 and Group C2 as compared to Group A (control). CGRP is one of the key neuromodulators expressed in DRG in diabetic neuropathic pain. Earlier administration of Metformin, a cAMPK activator significantly reduces the developing hyperalgesia and the up regulated CGRP expression in DRG sections of diabetic neuropathic model.

Disclosures: **Z. Huma:** None. **S. Jadoon:** None. **K. Rauf:** None.

Digital Abstract Session

P163. Peripheral Mechanisms of Neuropathic Pain

Program #/Poster #: P163.07

Topic: D.03. Somatosensation – Pain

Support: NIH R01 NS104295-01

Title: An unbiased approach to identifying changes in the gene expression profile of sensory neurons in painful diabetic neuropathy

Authors: ***D. GEORGE**, N. JAYARAJ, D. REN, R. MILLER, D. MENICHELLA; Northwestern, Chicago, IL

Abstract: Painful diabetic neuropathy (PDN) is a debilitating and intractable disease and characterized by neuropathic pain and small-fiber degeneration. Given the prevalence of the disease, there is a pressing need to identify new targets for the development of non-opioid, non-addictive, disease-modifying therapeutics for PDN. In patients with PDN, nociceptors within the dorsal root ganglion (DRG) become hyperexcitable and eventually degenerate, but the molecular mechanism underlying the phenomenon is still widely unknown. Our aim is to identify changes in the gene expression profile of the DRG neurons in PDN pathology to facilitate the discovery of novel druggable targets. Using a well-established mouse model of PDN, mice were either fed a regular diet (RD, 11% fat) or a high-fat diet (HFD, 42% fat) for 10 weeks during which these mice become obese, develop glucose intolerance, exhibit mechanical allodynia and show a decrease in the intra-epidermal nerve fiber density. We then used a single-cell RNA (scRNA-seq) sequencing approach using the 10X platform to capture the changes in the DRG in an unbiased fashion. As expected, analysis of the scRNA-seq identified both neuronal and non-neuronal clusters and several differentially expressed genes. Interestingly, we saw two closely related non-peptidergic clusters expressing a Mas-related G protein-coupled receptor (Mrgprd). While there were no differences in the expression of Mrgprd in one of the clusters (NP1 Type1), there seemed to be a significant increase in the expression of Mrgprd in a cluster we refer to as the NP1 Type2. To determine the functional relevance of the overexpression of Mrgprd, we used *in vivo* 2-photon calcium imaging with $Na_v1.8$ Cre-GCaMP6 animals fed an RD or HFD and examined whether administration of β-alanine (a known agonist of Mrgprd) in the hind paw would directly activate Mrgprd positive DRG neurons. We observed an increase in the number, as well as an increase in the magnitude of response in the HFD indicating the hyperexcitability of neurons expressing Mrgprd. Taken together, our data highlights an important role of the Mrgprd receptor in the generation and maintenance of hyperexcitability in a mouse model of PDN.

Disclosures: **D. George:** None. **N. Jayaraj:** None. **D. Ren:** None. **R. Miller:** None. **D. Menichella:** None.

Digital Abstract Session

P163. Peripheral Mechanisms of Neuropathic Pain

Program #/Poster #: P163.08

Topic: D.03. Somatosensation – Pain

Support: 5R01NS104295-02

Title: Mitochondrial Calcium Uniporter Deletion Prevents Painful Diabetic Neuropathy by Restoring Mitochondrial Morphology and Dynamics

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Abstract: Painful diabetic neuropathy (PDN) is an intractable complication affecting 25% of diabetic patients. PDN is characterized by neuropathic pain accompanied by dorsal root ganglion (DRG) nociceptor hyperexcitability, resulting in calcium overload, axonal degeneration, and loss of cutaneous innervation. However, the underlying molecular pathways responsible for these effects are unknown. Using highly stringent quantitative proteomic analyses, we found that mitochondrial proteins are differentially expressed in DRG neurons from mice with PDN caused by a high fat diet (HFD). In particular, mitochondrial fission proteins were overexpressed. Electron microscopy demonstrated fragmented mitochondrial morphology in DRG nociceptors. *In vivo* calcium imaging revealed increased calcium signaling in $Na_V1.8$-expressing DRG neurons of HFD mice. Selectively deleting the mitochondrial calcium uniporter from these neurons restored normal mitochondrial morphology and dynamics, prevented axonal degeneration, and reversed mechanical allodynia. Hence, we propose that targeting calcium entry into nociceptor mitochondria may be a promising therapeutic approach for PDN patients. Moreover, these results may illuminate other neurodegenerative diseases involving similar underlying events.

Disclosures: **N. Jayaraj:** None. **D. George:** None. **S. Hackelberg:** None. **D. Ren:** None. **S. Edaserry:** None. **C. Rathwell:** None. **R. Miller:** None. **J. Savas:** None. **R. Miller:** None. **D.M. Menichella:** None.

Digital Abstract Session

P163. Peripheral Mechanisms of Neuropathic Pain

Program #/Poster #: P163.09

Topic: D.03. Somatosensation – Pain

Support: R01-610-5210100-60054666
R01-610-5210100-60057550
P&F-610-5202000-60056119

Title: Activation of KeratinocyteGq-linked G-Protein Coupled Receptors Regulates Degeneration of Cutaneous Nerves

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Abstract: Painful diabetic neuropathy (PDN) is one of the most common complications of diabetes, affecting 25% of diabetic patients. DPN is characterized by small-fiber degeneration, which can progress to complete loss of cutaneous innervation and is accompanied by neuropathic pain. Uncovering the mechanisms underlying degeneration of cutaneous nerves in PDN remains a major challenge to finding effective and disease-modifying therapy. Keratinocytes are closely juxtaposed to cutaneous nerve terminals potentially enabling communication between keratinocytes and cutaneous afferents. Our aim is to identify mechanisms by which keratinocytes communicate with cutaneous afferents and how this signaling regulates axonal degeneration underlying neuropathic pain in PDN. In this study, we utilized a chemogenetic approach using DREADD receptors, synthetic receptors based on the structure of human muscarinic receptors that can be activated by the synthetic ligands clozapine and clozapine N-oxide (CNO). We genetically expressed stimulatory DREADD (hM3Dq) into K14 basal keratinocytes (K-14) in mice as a tool for mimicking the activation of Gq-linked G protein-coupled receptors (GPCRs) in K14-expressing basal keratinocytes. Histological characterization of the skin from mice expressing hM3Dq in K-14 positive cells revealed a clear thickening of the epidermis (as shown by H&E staining) due to K-14 expressing cell hyperproliferation (as shown by BrdU incorporation). Additionally, we observed reduced innervation of the epidermis (as shown by PGP9.5 staining), indicating that activation of K-14 Gq-linked GPCRs drives nerve fibers degeneration in the epidermis. Furthermore, transcriptional profiling of activated K-14 from the skin of K14-hM3Dq mice revealed downregulation of genes involved in neuronal survival and neurite outgrowth, including Nerve Growth Factor (NGF), artemin, a member of the Glial Cell-Derived Neurotrophic Factor (GDNF) ligand family, and Semaphorin 3D. Our results suggest that basal keratinocyte Gq-linked GPCRs could represent highly “druggable” and easily accessible targets for the development of therapeutics that by reversing axonal degeneration of cutaneous nerves in pathological conditions such as PDN could ameliorate the associated neuropathic pain.

Disclosures: A. Belmadani: None. **N. jayaraj:** None. **D. Ren:** None. **D. George:** None. **C. Rathwell:** None. **R.J. Miller:** None. **D. Menichella:** None.

Digital Abstract Session

P163. Peripheral Mechanisms of Neuropathic Pain

Program #/Poster #: P163.10

Topic: D.03. Somatosensation – Pain

Title: Differential keratinocyte expression of topical pain medication targets from patients with neuropathic pain: implications for personalized medicine

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Abstract: The overall objective of this study was to investigate potential cutaneous targets for topically applied active pharmaceutical ingredients, commonly utilized as a singularly delivered active or in combination with other actives, as a personalized medicine strategy for treating chronic pain. We set out to identify quantifiable, objective biomarkers in chronic painful skin to provide a tissue basis for directing individualized compounded topical preparations aimed to optimize the efficacy on a patient-by-patient basis. The intent of this preliminary, small-scale investigation was to demonstrate the potential for a larger proof-of-principle clinical trial of topical compounded formulations, which have seen an increase in use for various pain syndromes, including those of a neuropathic etiology, including post-herpetic neuralgia (PHN). Referencing 5 of the most common actives used in these formulations (ketamine, gabapentin, clonidine, baclofen, lidocaine), and 3 well-established cutaneous mediators (neuropeptides, cannabinoids, vanilloids), the study set out to determine if these neural signaling systems were present in epidermal keratinocytes from painful skin associated with PHN. A comprehensive immunolabeling platform was used to quantify each biomarker in skin biopsy epidermal keratinocytes taken from ipsilateral (pain) and contralateral (nonpain) dermatomes of an enriched cohort of PHN patients. Results demonstrate significant differential labeling among the cohort for each biomarker, consistent with mechanisms of action among keratinocytes. Importantly, the total biomarker panel indicates that the enriched cohort contains distinct subgroups. The heterogeneity of the cohort differences may explain studies that have not shown statistical group benefit from topically administered compounded therapies. Rather, the essential need for individual tissue biomarker evaluations is evident, particularly as a means to direct a more accurately targeted topical approach and generate positive clinical results.

Disclosures: P.J. Albrecht: None. **Y. Liu:** None. **G. Houk:** None. **B. Ruggiero:** None. **D. Banov:** None. **M. Dockum:** None. **A. Day:** None. **F. Rice:** None. **G. Bassani:** None. **M. Carvalho:** None.

Digital Abstract Session

P164. Thalamic and Cortical Processing and Descending Modulation

Program #/Poster #: P164.01

Topic: D.04. Somatosensation – Touch

Title: Heterogeneity of burst discharge in adult thalamic reticular nucleus neurons

Authors: *L. HARDING-JACKSON, J. BEATTY, C. COX;
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Abstract: Long studied for its role in epilepsies and sensory processing disorders, the thalamic reticular nucleus (TRN) is an integral regulator of sensory processing circuits between thalamus and cortex. Most, but not all TRN neurons produce action potentials (APs) in two modes: burst-discharge when hyperpolarized, and tonic-discharge when depolarized. Burst discharge varies greatly in the frequency and duration of the transient AP discharge. We investigated potential mechanisms that may account for burst frequency variation and potential functional significance of these distinct populations. Whole-cell TRN neuron recordings from adult male and female C57BL6 mice revealed a broad range of burst frequencies ranging from 4-342 Hz (n=92). In neurons with high frequency discharge (>100Hz), the burst was composed of 8±7 APs (n=54), whereas slower burst discharges (50-100 Hz) had fewer APs (3±1, n=26). In tetrodotoxin nearly all neurons (n=47/48) produce a transient low threshold spike (LTS) which underlies the burst discharge. This depolarization was blocked by low concentrations of $NiCl_2$, suggestive of the role of T-type calcium current. The amplitude of the LTS was positively correlated with the burst discharge frequency and the number of APs/burst. The small conductance calcium-activated potassium (SK) channel antagonist, Apamin, increased number of APs/burst and the LTS amplitude indicating the role of SK current in regulating burst discharge. Prior studies suggest that T-type calcium channels are distributed throughout the dendrites in neurons with high frequency burst discharge. We investigated if this was similar in neurons with lower burst discharge frequencies. Using 2-photon laser scanning microscopy and a calcium indicator, we quantified calcium signals in response to transient somatic depolarization at proximal (10-50µm), intermediate (100-150µm) and distal (200-300µm) dendritic regions. Calcium signals were smaller in slow bursting neurons at proximal and intermediate regions compared to high frequency bursting neurons. There was no significant difference at distal locations. Addition of Apamin significantly increased calcium signal in proximal and intermediate regions of the high frequency bursting neurons, but not in slow bursting neurons. These data reveal that heterogeneous TRN burst discharge frequencies may represent cell populations with different dendritic ion channel composition and distribution. With these results, we aim to understand the consequence of TRN-dependent thalamic inhibition and provide greater insight into how perturbations within the region may lead to various neurological disorders.

Disclosures: L. Harding-Jackson: None. **J. Beatty:** None. **C. Cox:** None.

Digital Abstract Session

P164. Thalamic and Cortical Processing and Descending Modulation

Program #/Poster #: P164.02

Topic: D.04. Somatosensation – Touch

Support: NIH BRAIN NINDS-R01NS104928
NIMH-U01MH106027
NINDS F31NS098691

NIH R01EY023173
NSF GRF

Title: Rapid sensory adaptation in the mouse thalamocortical circuit, and the relative contribution of thalamic state

Authors: N. C. WRIGHT[1], P. Y. BORDEN[1], Y. LIEW[1], M. F. BOLUS[1], W. M. STOY[2], C. R. FOREST[2], G. B. STANLEY[1];
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Abstract: Rapid sensory adaptation describes the interaction between the perception of a given sensory stimulus and recent stimulus history, spanning milliseconds to seconds. Despite its profound, complex, and widely-reported effects across all across sensory systems, the mechanistic basis of rapid sensory adaptation is poorly understood. A wide range of studies primarily in in-vitro and anesthetized preparations have pointed to the emergence of adaptation effects at the level of primary sensory cortex, with only modest signatures in earlier stages of processing. Yet the nature of rapid adaptation and how it shapes sensory representations during wakefulness, and thus the potential role in adaptive changes in perception, are unknown, as are the mechanisms that underlie this phenomenon. To address these knowledge gaps, we recorded spiking activity in primary somatosensory cortex (S1) and the upstream ventral posteromedial (VPm) thalamic nucleus in the vibrissa pathway of the awake mouse, and quantified responses to whisker stimuli delivered either in isolation or embedded in an adapting sensory background. We found that during wakefulness, cortical sensory responses were indeed adapted by persistent sensory stimulation; mean feature-evoked rates and synchronous spiking of S1 putative excitatory neurons were profoundly adapted, and inhibitory neurons only modestly so. Recordings in VPm and further optogenetic manipulation experiments suggest this largely reflected changes in thalamic spike timing, with little contribution from thalamocortical or intracortical synaptic depression. A network model supports this conclusion, and further suggests that robust feedforward inhibition serves to dampen excitatory firing in the adapted condition. Taken together, these results suggest that cortical adaptation results from changes in the timing of thalamic input, and the way in which this differentially impacts cortical excitation and feedforward inhibition, pointing to a prominent role of thalamic gating in rapid adaptation of primary sensory cortex.

Disclosures: **N.C. Wright:** None. **P.Y. Borden:** None. **Y. Liew:** None. **M.F. Bolus:** None. **W.M. Stoy:** None. **C.R. Forest:** None. **G.B. Stanley:** None.

Digital Abstract Session

P164. Thalamic and Cortical Processing and Descending Modulation

Program #/Poster #: P164.03

Topic: D.03. Somatosensation – Pain

Support: NIH R01AR074062

Stanley Glaser Foundation Research Award UM SJG 2019-18

Title: Role of PBN projecting CeA^{pdyn} amygdala neurons in nociception

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Abstract: The role of the amygdala in the processing of pain and itch has been studied. A recent study revealed that the parabrachial nucleus (PBN) projecting central nucleus (CeA) amygdala neurons regulate pain. The neuronal subpopulations in the CeA include prodynorphin (Pdyn)-expressing neurons. In this study, we investigated whether the optogenetic stimulation of Pdyn+ amygdala neurons and PBN projecting CeA^{pdyn} amygdala neurons altered pain and itch sensitivity in mice. To modulate the activity of Pdyn+ amygdala neurons, we used Pdyn-cre transgenic mice. An AAV encoding a Cre-dependent fast opsin (ChrimsonR) fused to the red fluorescent protein (rAAV5/Syn-FLEX-ChrimsonR-tdTomato; Addgene) was bilaterally injected into the CeA. The injection volume was 0.25 µl, injected over 1 min using a glass needle and plunger. An optic fiber (200 µm diameter) was implanted directly above each injection site or PBN and fixed to the skull with dental cement. Mice were allowed three weeks to recover from surgery before behavior testing. For scratching behavior test, histamine (50 µg/10 µl; Sigma-Aldrich) or chloroquine diphosphate salt (100 µg/10 µl; Sigma-Aldrich) was injected intradermally into the shaved rostral back skin. Behavior was video recorded for 30 min. For optogenetic stimulation, light pulses were delivered at constant intensity and frequency (3 mW, 2 Hz) via an LED driver connected to a waveform generator. Optogenetic stimulation of Pdyn+ amygdala neurons or PBN projecting CeA^{pdyn} amygdala neurons inhibited histamine- and chloroquine-evoked scratching. Additionally, optogenetic stimulation of Pdyn+ amygdala neurons suppressed the number of Fos positive neurons increased by intradermal injection of chloroquine. For thermal pain sensitivity, paw withdrawal latencies were measured by Hargreaves plantar test. Hindpaw heat withdrawal latency was increased by optogenetic stimulation of Pdyn+ amygdala neurons and PBN projecting CeA^{pdyn} amygdala neurons. For mechanical pain sensitivity, paw withdrawal thresholds were determined by von Frey filament test. Optogenetic stimulation of PBN projecting CeA^{pdyn} amygdala neurons, but not Pdyn+ amygdala neurons, increased the mechanical withdrawal threshold. Our data indicate that PBN projecting CeA^{pdyn} amygdala neurons regulate nociception.

Disclosures: H. Funahashi: None. **T. Akiyama:** None. **D. Pavlenko:** None. **K. Sakai:** None.

Digital Abstract Session

P164. Thalamic and Cortical Processing and Descending Modulation

Program #/Poster #: P164.04

Topic: D.03. Somatosensation – Pain

Support: NHMRC APP1121376

Title: Thalamocortical control of vagal sensory processes

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Abstract: The nodose and jugular vagal ganglia supply sensory innervation to the airways and lungs. Jugular vagal airway sensory neurons wire into a brainstem circuit with ascending projections into the submedius thalamic nucleus (SubM) and ventrolateral orbital cortex (VLO), regions known to regulate the endogenous analgesia system. Here we investigate whether the SubM-VLO circuit exerts descending regulation over airway vagal reflexes in male and female rats using a range of neuroanatomical tracing, reflex physiology and chemogenetic techniques. Anterograde and retrograde neuroanatomical tracing confirmed connectivity of the SubM and VLO. Laryngeal stimulation in anesthetized rats reduced respiration, a reflex that was potently inhibited by activation of SubM. Conversely, inhibition of SubM potentiated laryngeal reflex responses, while prior lesions of VLO abolished the effects of SubM stimulation. In conscious rats, selective chemogenetic activation of SubM neurons specifically projecting to VLO significantly inhibited respiratory responses evoked by inhalation of the nociceptor stimulant capsaicin. Jugular vagal inputs to SubM via the medullary paratrigeminal nucleus were confirmed using anterograde transsynaptic conditional herpes viral tracing. Respiratory responses evoked by microinjections of capsaicin into the paratrigeminal nucleus were significantly attenuated by SubM stimulation, whereas those evoked via the nucleus of the solitary tract were unaltered. These data suggest jugular vagal sensory pathways input to a nociceptive thalamocortical circuit capable of regulating jugular sensory processing in the medulla. This circuit organization suggests an intersection between vagal sensory pathways and the endogenous analgesia system, potentially important for understanding vagal sensory processing in health and mechanisms of hypersensitivity in disease.

Disclosures: A.E. McGovern: None. **T. Bautista:** None. **A. Verberne:** None. **M.W. Trewella:** None. **M. Farrell:** None. **S.B. Mazzone:** None.

Digital Abstract Session

P164. Thalamic and Cortical Processing and Descending Modulation

Program #/Poster #: P164.05

Topic: D.04. Somatosensation – Touch

Support: NIH 1ZIAAT000033-03

Title: A-fiber sensation strongly contributes to pleasantness of deep pressure and CT-fiber mediated pleasant touch

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Abstract: C-tactile (CT) afferents are unmyelinated sensory afferents in hairy skin with a maximal response to slow, gentle stroking. Firing of CT afferents strongly correlates with ratings of touch pleasantness, leading to the labelling of the CT pathway as a social-affective sensory pathway (Loken, Wessberg et al. 2009). Patients with AB deafferentation and consequent loss of light touch sensation still perceive slow gentle stroking as pleasant, corroborating its C-fiber origin (Olausson, Lamarre et al. 2002). Recently, however, ablation of the spinothalamic pathway (by which C-fiber sensations ascend to the thalamus) was found to impair all C-fiber mediated sensations of pain, temperature, and itch *without* any effect on pleasant touch perception (Marshall, Sharma et al. 2019). This finding suggests that integration of A and CT fiber input occurs prior to its projection to the thalamus. However, it remains unclear how A fiber sensory input contributes to CT-associated pleasant sensation. In addition, the reliance of pleasant pressure perceived in deeper tissues on A or C fibers is unknown. We conducted pressure nerve blocks in 5 healthy human volunteers (3 males and 2 females, ages 21-25) to eliminate A fiber input and tested the pleasantness of gentle stroking and deep pressure before appreciable loss of C-fiber function. Our findings show that once A-fiber sensation is eliminated, the perceived intensity and pleasantness of *both* gentle stroking and deep pressure are nearly abolished. In contrast, intensity and pleasantness of stroking and pressure are maintained in the unaffected arm. These findings demonstrate for the first time that perceiving the pleasantness of deep pressure critically depends on A fibers. In addition, A-fiber input appears to play a strong role in the pleasantness of CT-optimal gentle brushing. We extend the conclusion of Marshall and colleagues to suggest that in typical individuals, both A and C sensory afferents are important contributors to CT pleasant sensation.

Disclosures: L.K. Case: None. **N.J. Madian:** None. **M.V. McCall:** None. **M. Bradson:** None. **J. Liljencrantz:** None. **A.T. Chesler:** None. **M.C. Bushnell:** None.

Digital Abstract Session

P164. Thalamic and Cortical Processing and Descending Modulation

Program #/Poster #: P164.06

Topic: D.03. Somatosensation – Pain

Support: CIHR
Mayday Fund

Title: The potential clinical utility of cuff-pressure versus heat-based paradigms to measure conditioned pain modulation: an exploratory study in healthy individuals and those with chronic pain

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Abstract: Background: Conditioned pain modulation (CPM) is a physiological measure thought to reflect an individual's endogenous pain modulation system. CPM refers to the reduction of pain evoked by a painful test stimulus (TS) applied to one body region due to a painful conditioning stimulus (CS) applied to another region. CPM varies across individuals and provides insight into chronic pain pathophysiology. There is growing evidence that CPM may help predict individual pain treatment outcome. However, paradigm variabilities and practical issues have impeded widespread clinical adoption of CPM assessment. The aim of this exploratory study was to compare two different CPM paradigms in healthy controls (HCs) and those with chronic pain. **Methods:** All participants provided informed consent to REB approved protocols. CPM was evaluated in 11 people with chronic pain and 9 HCs. Each participant underwent two CPM paradigms: one used heat stimuli (Q-Sense, Medoc Inc) and the other used pressure cuff stimuli (Nocitech). Stimulus intensities were individually set to evoke pain intensity rated at ~50/100. The TS were applied before and during a CS applied to a contralateral limb. The CPM effect was calculated as a %change of pain evoked by the TS with vs without the CS, with negative scores indicating pain inhibition. **Results:** The pressure paradigm required less time to administer than the heat paradigm. In the chronic pain group, most individuals exhibited inhibitory CPM using either the heat (*N*= 5 CPM<0%, 3 CPM=0%, 3 CPM>0%) or pressure (*N*= 7 CPM<0%, 1 CPM=0%, 3 CPM>0%) paradigms. Heat- and pressure- evoked CPM was correlated (r=0.48) in those with chronic pain. In contrast, in the HC group, CPM evoked by pressure stimuli (-52%+/-39%) was more pronounced than from heat stimuli (0.5%+/-32%). Most HCs (7 of 9) did not exhibit inhibitory CPM using the heat paradigm (CPM 0-43%) but did exhibit strong inhibitory CPM evoked by the pressure paradigm (CPM -20% to -90%). **Conclusion:** These findings highlight that compared to heat-based, a pressure-based paradigm can assess CPM in chronic pain with the advantages of producing consistent findings to the literature in HCs, using a simpler system, a shorter test time and lower cost; important factors for both research and clinical use.

Disclosures: R. El-Sayed: None. **C. Fauchon:** None. **L.B. Kisler:** None. **J.A. Kim:** None. **S. Firouzian:** None. **N.R. Osborne:** None. **K.D. Davis:** None.

Digital Abstract Session

P164. Thalamic and Cortical Processing and Descending Modulation

Program #/Poster #: P164.07

Topic: D.04. Somatosensation – Touch

Support: NIH Grant U01 NS103470

Title: Circuit-targeted fMRI of brain-wide inputs to posterior medial thalamic nucleus

Authors: *N. LI, S. GHOSH, M. SCHWALM, W. ZHENG, A. JASANOFF;
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Abstract: The posterior medial thalamic nucleus (POm) plays critical roles in modulating somatosensory perception, motor behavior, and well-learned sensorimotor habits. The circuit mechanisms underlying such modulatory functions involve pathways from both cortical and subcortical brain regions, but the process whereby these disparate inputs combine to influence POm function in sensory perception is poorly understood. Here we study the integration of brain-wide inputs to POm during somatosensory processing using a novel neural activity indicator called NOSTIC, which transduces neuronal calcium signals into fMRI responses. NOSTIC is targeted to POm inputs using a retrograde trans-synaptic herpes viral vector, enabling selective functional imaging of monosynaptic POm afferents. Our results reveal several clustered POm input regions engaged by stimulation of the rat forepaw. The activated POm input regions are located not only in the primary somatosensory cortex and secondary somatosensory cortex but also in additional deep brain regions, such as superior colliculus and zona incerta. Our findings suggest mechanisms by which multisensory information impinges on somatosensory processing and provide a basis for further investigation of the subcortical pathways involved in thalamic modulatory roles.

Disclosures: N. Li: None. **S. Ghosh:** None. **M. Schwalm:** None. **W. Zheng:** None. **A. Jasanoff:** None.

Digital Abstract Session

P165. Central Mechanisms of Neuropathic Pain

Program #/Poster #: P165.01

Topic: D.03. Somatosensation – Pain

Support: McGill start up fund

Title: Single-cell RNA sequencing defines distinct populations of microglia after peripheral nerve injury

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Abstract: Microgliosis in the spinal dorsal horn caused by peripheral nerve injury contributes to the development of neuropathic pain. Interestingly, microglia inhibition reverses mechanical allodynia caused after peripheral nerve injury only in male mice. Microglia from the brain present transcriptional sex-differences as well as distinct subpopulations in health and disease. To further study the potential mechanisms by which microglia promote pain, and to investigate

changes in their transcriptional states, we performed microglia single-cell RNA-seq (scRNA-seq) following spared nerve injury (SNI) in male and female mice at different phases of pain development. We collected the lumbar spinal cord of male and female naive C57BL/6 mice as well as mice of both sexes at day 3 (developmental phase), day 14 (early chronic phase), and 5 months (chronic phase) post-SNI and sham controls. One biological sample consisted of four pooled lumbar segments. Unsupervised clustering analysis of single-cell RNA sequencing data revealed eleven distinct microglia clusters, with uniquely expressed genes identified in seven of them. Differentially expressed genes (DEGs) were found across time points and between both sexes, with a higher number of DEGs present in male mice. Gene ontology enrichment analysis pointed to clusters involved in proliferation, immune response, and signal transduction. Microglia belonging to each cluster were present in both SNI and sham groups. However, males showed a higher proportion of microglia belonging to clusters with high expression of proliferation-related genes when compared to female mice. To confirm differences in proliferating microglia, we labeled cells with Ki67, a proliferation marker, and Iba1, a microglia marker. Quantification of Ki67-positive microglia in the dorsal horn spinal cord three days post-SNI showed a significantly higher number of proliferating microglia in males as compared to females (68.85 ± 5.89, and 46.6 ± 1.83, respectively, $p<0.011$), with no differences in total microglia (82.81 ± 5.52 in males, and 76.41 ± 1.84 in females, $p=0.313$). We also identified a male-specific microglia cluster with an inflammatory profile restricted to the early time point. Our findings are consistent with previous studies showing that male microglia display stronger inflammatory responses as compared to female microglia. The results of this study have the potential to contribute to the identification of subsets of microglia involved in the development and establishment of chronic pain, as well as providing evidence for sex-specific responses of microglia to peripheral nerve injury.

Disclosures: **S. Tansley:** None. **S. Uttam:** None. **A. Ureña Guzmán:** None. **M. Yaqubi:** None. **A. Khoutorsky:** None. **L. Healy:** None. **J.S. Mogil:** None.

Digital Abstract Session

P165. Central Mechanisms of Neuropathic Pain

Program #/Poster #: P165.02

Topic: D.03. Somatosensation – Pain

Support: NIH R01DA34749
NIH R01DA047089
NIH R01DA047157
NIH R01DA047157-03S1

Title: Spinal cMyc and SIRT3 plays important role in HIV gp120 with morphine-induced neuropathic pain.

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Abstract: Opiate use disorder and human immunodeficiency virus (HIV) infection are two major public health problems. Chronic morphine use up-regulates HIV entry into immune cells. Emerging clinical data suggests that repeated use of opioid pain medications can increase neuropathic pain (NP) in people living with HIV. Thus, it is significant to elucidate the exact mechanisms of chronic pain. HIV gp120 increases the expression of transcription factor c-Myc in mesangial cell proliferation. Sirtuins 3 (Sirt3) deacetylates MnSOD and thereby activates it for scavenging $mtO_2^{\bullet-}$. In the present study, we investigated the role of cMyc and Sirt3 in a HIV coat glycoprotein gp120 with morphine (gp120/M)-induced neuropathic pain state. HIV-related neuropathic pain was induced by repeated administration of intrathecal recombinant HIV-1 gp120 and morphine. Mechanical allodynia was assessed using von Frey filaments. Protein expression of spinal cMyc, EZH2, and SIRT3 was examined using Western blots. Intrathecal administration of antisense oligonucleotides against cMyc, GSK126 (EZH2 inhibitor), mito-Tempol (Mito-T, a $mtO_2^{\bullet-}$ scavenger) or recombinant Sirt3 (rSIRT3) was given intrathecally. The image of $mtO_2^{\bullet-}$ in the spinal dorsal horn was examined using MitoSox Red (mitochondrial superoxide indicator) image assay. HIV-gp120 with morphine induced mechanical allodynia lasting for over 3 weeks. HIV-gp120/M upregulated the expression of cMyc, EZH2, and down-regulated Sirt3 in the spinal dorsal horn. Intrathecal administration of antisense oligonucleotides against cMyc, GSK126 (EZH2 inhibitor), Mito-T, or recombinant Sirt3 (rSIRT3) reduced mechanical allodynia in the gp120/M neuropathic pain model. Using ChIP assay, **the enrichment of** H3K27me3 binding to SIRT3 gene promoter region was increased in gp120/M treated rats compared to that in control group. These results suggest that spinal transcriptional factor cMyc, epigenetic writer EZH2, and Sirt3 play an important role in the HIV gp120-related neuropathic pain state, and provide evidence for a novel approach to treating chronic pain due to HIV/opioid.

Disclosures: S. Hao: None. **X. Zhu:** None. **K. Hayashi:** None. **H. Yi:** None. **J. Gu:** None. **K. Takahashi:** None. **Y. Kashiwagi:** None. **S. Liu:** None.

Digital Abstract Session

P166. Treatments For Persistent Pain

Program #/Poster #: P166.01

Topic: D.03. Somatosensation – Pain

Support: Boston Scientific

Title: Computational modeling predicts dorsal columns are involved in fast-acting sub-perception spinal cord stimulation (SCS)

Authors: *J. E. GILBERT[1], N. D. TITUS[1], T. C. ZHANG[3], R. ESTELLER[3], W. M. GRILL[2]; [1]Biomed. Engin., [2]Biomed. Engineering, Electrical and Computer Engineering, Neurobiology, and Neurosurg., Duke Univ., Durham, NC; [3]Neuromodulation Res. and Advanced Concepts, Boston Scientific Neuromodulation, Valencia, CA

Abstract: Sub-perception SCS has emerged as a successful therapy for chronic neuropathic pain that provides therapeutic efficacy, but unlike conventional SCS does not elicit paresthesia. Sub-perception SCS at high frequencies (1-10 kHz) typically has a slow (hours – days) onset of analgesia, but recent studies of sub-perception SCS at lower frequencies (≤150Hz) demonstrated pain relief with a more rapid onset (minutes) following placement corresponding to paresthesia-pain overlap and titration of the pulse width and frequency. Although some sub-perception SCS mechanisms of action may involve dorsal horn effects, ≤150Hz sub-perception SCS may also engage dorsal column (DC) axons, given that the electrode placement and analgesic timing are consistent with conventional SCS, which activates DC axons. We quantified how SCS parameters (placement, pulse width, frequency, and amplitude) influenced activation of DC axons and subsequent neural responses in the dorsal horn using an integrated computational model. We calculated potentials generated by a bipolar SCS electrode in the rat spinal cord using a finite element model and applied the potentials to an axon model tuned to match measured DC axon responses. The outputs of the DC axon models were inputs to a validated network model of the spinal dorsal horn that includes representations of center and surround peripheral receptive fields. We measured the firing rate of the model center receptive field wide-dynamic range (WDR) projection neuron as a proxy for pain. SCS applied at amplitudes as low as 60% of the predicted sensory threshold with appropriately titrated pulse widths activated model DC axons and subsequently reduced WDR firing rates across all tested frequencies. However, WDR firing increased with SCS amplitude, creating a non-monotonic response curve with maximal suppression at 75-85% of the sensory threshold. Minimizing WDR firing rate required spatially targeting stimulation to include surround receptive fields. While all tested frequencies generated reductions in model WDR firing rates, the greatest reductions occurred between 50Hz and 90Hz. High frequency (1.2kHz and 10kHz) SCS produced small and transient reductions in model WDR firing rates suggesting different underlying mechanisms of action. Our results support the hypothesis that sub-kHz, sub-perception SCS generates rapid analgesia by activating a small number of dorsal column axons. Furthermore, our results demonstrate the importance of spatial targeting and appropriate selection of amplitude and pulse width for maximum suppression of model WDR neuron firing rate.

Disclosures: J.E. Gilbert: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Boston Scientific. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Boston Scientific. **W.M. Grill:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Boston Scientific. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding

diversified mutual funds); Boston Scientific. F. Consulting Fees (e.g., advisory boards); Boston Scientific. **N.D. Titus:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Boston Scientific. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Boston Scientific. **T.C. Zhang:** A. Employment/Salary (full or part-time):; Boston Scientific. **R. Esteller:** A. Employment/Salary (full or part-time):; Boston Scientific.

Digital Abstract Session

P166. Treatments For Persistent Pain

Program #/Poster #: P166.02

Topic: D.03. Somatosensation – Pain

Support: Institutional Support from the University of Arizona

Title: Terpenes Found in Cannabis sativa are Potential Novel Therapeutics for Chemotherapy-Induced Neuropathic Pain

Authors: *R. HECKSEL, A. KERESZTES, J. M. STREICHER;
Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: *Cannabis sativa* has been cultivated and used both medically and recreationally for thousands of years. However, there continues to be a lack of literature on some potentially therapeutic compounds found in the cannabis plant called terpenes. Terpenes are a class of organic chemicals that give plants, including cannabis, their distinct odors and flavors. Anecdotally, terpenes are thought to enhance the cognitive and therapeutic effects caused by certain cannabis strains; however there is very little, if any, empirical evidence for this claim. We previously found that some of the major terpenes found in *Cannabis sativa*, namely α-humulene, β-pinene, geraniol, and linalool, induce tetrad effects (analgesia, hypothermia, hypolocomotion and catalepsy) at high doses in mice. These tetrad behaviors could be blocked by cannabinoid receptor type 1 (CB1) and adenosine A2a antagonists, suggesting CB1 and A2a receptor mediated mechanism. In our current study, we sought to extend these findings to determine if these terpenes could be novel treatments for neuropathic pain using a mouse model of chemotherapy-induced peripheral neuropathy (CIPN). We also investigated the rewarding or aversive effects of terpenes using a Conditioned Place Preference (CPP) mouse model. Our results indicate that acute terpene administration in CIPN induced efficacious anti-nociception and dose-dependently induced analgesic tolerance upon chronic treatment. CPP results indicated significant aversive behavior for animals treated with α-humulene, β-pinene, and β-caryophyllene. Interestingly, administration of geraniol and linalool produced neither aversion nor preference. These findings suggest that geraniol and linalool could be novel efficacious therapies for CIPN with no aversive or rewarding side effects. Seeking a mechanism for these results, we used an *in vitro* model of BV-2 microglia cells expressing an NFκB-luciferase

reporter stimulated with lipopolysaccharide. This model demonstrated dose-dependent decreases in NFκB activation for all terpenes with full blockade by α-humulene, β-pinene, and geraniol, suggesting these to be the most efficacious anti-inflammatory agents. Using CHO cells expressing a human CB1 receptor, we also found that all terpenes produced rimonabant-sensitive CB1 activation, suggesting that all terpenes act as CB1 agonists. Anti-inflammatory and CB1 activity could thus be mechanisms for the anti-nociception observed in CIPN, which will be tested in future studies. Translationally, this research could help identify specific cannabis terpenes beneficial for medical *Cannabis* strains used to treat pain or inflammatory conditions.

Disclosures: R. Hecksel: None. **J.M. Streicher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity stake in Botanical Results, LLC, a company that makes cannabidiol products. **A. Keresztes:** None.

Digital Abstract Session

P166. Treatments For Persistent Pain

Program #/Poster #: P166.03

Topic: D.03. Somatosensation – Pain

Support: NCCIH R00-AT009466
NCCIH F32-AT006949

Title: Widespread pressure conferred by a weighted blanket reduces chronic pain in adults with high trait anxiety

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Abstract: Deep pressure is an affective touch stimulus that has been found to increase pleasantness ratings and decrease anxiety. Recent work has also demonstrated analgesic effects of deep pressure on experimentally evoked pain. However, the effect of deep pressure on *chronic* pain and the mechanisms underlying its pain-relieving effects are unknown. Emerging evidence suggests that weighted blankets (WB) – blankets sewn with weighted materials to provide deep widespread pressure to the body – reduce anxiety and insomnia, factors known to influence pain. We hypothesized that wearing a WB would reduce ratings of chronic pain, mediated by improvements in anxiety and sleep. In a remote, smartphone-based ecological momentary assessment (EMA) study, 93 adults with chronic pain diagnoses (79% female; age $M = 43.54$ $SD = 12.32$) were randomized (stratified by sex) to wear a heavily WB ($n = 49$; 15 pounds) or a lightly (placebo control) WB ($n = 44$; 5 pounds) once for 15-minutes and then overnight for seven days. Participants were blinded to the manipulation of blanket weight. Participants rated their initial expectation for pain relief from their blanket and made daily ratings of pain, anxiety, and sleep quality on visual analog scales. Chronic pain, trait anxiety, and sleep disturbance were

assessed from before wearing the WB to after the week-long intervention using validated questionnaires. After controlling for trait anxiety, we obtained a significant time x group interaction (p = .04, Cohen's f = .18) demonstrating reductions in chronic pain from before to after the intervention in the heavily WB group relative to the lightly WB group. This effect was maintained after controlling for expected pain relief (p = .04, Cohen's f = .19). Post-hoc analyses revealed that individuals reporting high trait anxiety obtained greater pain reductions from the heavily WB than the lightly WB (p < .01, Cohen's f = .56), even after controlling for expected pain relief (p < .01, Cohen's f = .60). In contrast, there were no significant differences in pain reductions between the two blankets for individuals low in trait anxiety (p = .16). Blanket-related pain reductions were not mediated by reported changes in anxiety or sleep, suggesting direct sensory effects on pain perception. We propose effects of sensory gating and/or changes in interoceptive sensibility as candidate mechanisms to be investigated in future studies. Overall, these results demonstrate that widespread deep pressure from a WB may be an effective and low-cost therapeutic tool for highly anxious adults living with chronic pain.

Disclosures: J.N. Baumgartner: None. **D. Quintana:** None. **L. Leija:** None. **L. Case:** None.

Digital Abstract Session

P166. Treatments For Persistent Pain

Program #/Poster #: P166.04

Topic: D.03. Somatosensation – Pain

Support: Department of Defense PR182408

Title: Recombinant GABAergic progenitors releasing conopeptide MVIIA alleviate hypersensitivity in rat models of neuropathic pain

Authors: *B. MARIN, M. HERNANDEZ, J. SAGEN, S. JERGOVA;
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Abstract: Chronic pain is a debilitating condition affecting quality of life. Treatment of chronic pain is clinically challenging due to the development of tolerance and addiction to analgesic drugs, such as opioids. Recombinant cell-based therapy provides a new avenue for the management of chronic pain because it can continually produce and secrete analgesic substances at local pain sites in the spinal cord. One of the hallmarks of neuropathic pain is a reduction of GABAergic inhibitory signaling in the spinal cord that leads to exaggerated hyperexcitability of spinal dorsal horn neurons. Transplantation of GABAergic neuronal cells may restore the inhibitory potential in the spinal cord and replace dysfunctional interneurons. Grafted cells may also release analgesic peptides by means of genetic engineering to further enhance the benefits of cell-therapy approach. The omega-conopeptide, MVIIA, an N-type Ca2+ channel blocker, was one of the earliest tested treatments for severe pain in animal models and is in clinical use marketed as FDA approved Prialt (Ziconotide). However, due to its poor penetration through the blood brain barrier it must be delivered intrathecally. The goal of this project is to develop

transplantable recombinant GABAergic cells releasing MVIIA that can alleviate pain-like behavior following peripheral and spinal cord injury. Rat models of chronic constriction of the sciatic nerve for peripheral neuropathic pain and spinal clip compression for spinal cord injury were used to induce chronic pain. Animals were tested weekly for the presence of tactile, cold and heat hypersensitivity. Rat embryonic E14 neuronal progenitors were harvested from medial ganglionic eminence and transfected with p_lenti_MVIIA. Their viability, proneuronal phenotype, and the ability to release recombinant MVIIA were confirmed via immunostaining and FLISA analysis. Recombinant, naive cells or saline were intraspinally injected into animals when signs of chronic pain were detected. An analgesic effect of both recombinant and naive grafts was observed in both pain models compared to saline injected animals, with more robust effect in the recombinant graft group, with hypersensitivity in recombinant graft groups showing progressive decline over several weeks. Conditioned place preference with gabapentin revealed significant reduction of ongoing pain in both graft groups, with stronger effect in the recombinant graft group. Inflammatory biomarkers Iba-1, IL1-β, and TNF-α were reduced in treated animals compared to controls. Our results demonstrate the enhanced beneficial effect of targeted recombinant cell-based therapy in the management of chronic pain.

Disclosures: B. Marin: None. **M. Hernandez:** None. **J. Sagen:** None. **S. Jergova:** None.

Digital Abstract Session

P166. Treatments For Persistent Pain

Program #/Poster #: P166.05

Topic: D.03. Somatosensation – Pain

Support: Department of Defense SC170295

Title: Identification of cannabinoid receptor ligands within the Conus venom.

Authors: *S. JERGOVA[1], K. LIEBMAN[1], N. CHANDNANI[1], S. C. GROSS[1], J. IMPERIAL[2], B. M. OLIVERA[2], J. SAGEN[1];
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Abstract: Chronic neuropathic pain is a clinically challenging condition insufficiently controlled by current pharmacotherapy. Cannabinoid (CB) receptors have been recently recognized as valuable targets for various clinical indications. Several preclinical studies showed strong analgesic effects of CB1 receptor agonists. However, most of the drugs used therapeutically that interact with CB receptors are derived from cannabis and due to their psychoactive components are not be widely used for pain management. Therefore, selective ligands of CB receptors that do not induce side effects are of clinical interest. The venom of marine snail genus Conus represents a natural source of various peptides with potent antinociceptive activity. We have previously screened several Conus venoms for their ability to induce CB1 receptor internalization using HEK293 cells expressing CB1 receptor. The most potent samples were further fractionated by HPLC and screened in vitro and in vivo. C. Textile venom samples were selected as the best

candidate to search for CB1 receptor ligand. The aim of this study was to identify the CB1 ligand(s) withing the C. Tex HPLC venom fractions that can be further developed for pain therapies, and to conduct similar screening to identify CB2 receptor ligands. HEK 293 cells expressing CB1 or CB2 receptors were incubated with venom fractions, CP55,940 or WIN55,212-2 as positive controls and confirmed by selective CB1 and CB2 antagonists. Internalization of CB receptor was evaluated by immunofluorescence. Analgesic effects of the most potent subfractions were evaluated in the formalin model and peripheral and central pain via intrathecal delivery using Alzet pumps for chronic infusion. After each set of in vitro and in vivo evaluations, the most potent samples were further fractionated and evaluated. Immunoprecipitation was conducted to isolate possible CB1 receptor ligand from the C. Tex subfraction and the sample was analyzed by mass spectrometry. Results showed C. Geo venom fractions were the most potent inducing CB2 receptor internalization. Their effect was reduced by proteolytic enzymes, suggesting a peptidergic component. The selected C. Tex subfraction showed analgesic effects in animal models and stability when administered via intrathecal pumps for 4 weeks. The search for CB1 receptor ligand was successful in identifying the possible sequence of the active fraction. These findings indicate that CB-active conopeptide derivatives have potential for development as a chronic pain therapy.

Disclosures: **S. Jergova:** None. **K. Liebman:** None. **N. Chandnani:** None. **S.C. Gross:** None. **B.M. Olivera:** None. **J. Imperial:** None. **J. Sagen:** None.

Digital Abstract Session

P166. Treatments For Persistent Pain

Program #/Poster #: P166.06

Topic: D.03. Somatosensation – Pain

Support: IZKF TN1-1/IA 532001
IZKF TN1-5/IA 532005
IZKF TN1-6/IA 532006
IZKF TN1-9/IA 532009
IZKF TN1-2/IA 532002
Nanion SyncroPatch 384i Research Grant
DFG LA 2740/3-1

Title: High-throughput electrophysiology approach to identify personalized medicine for pain patients with sodium channel mutations

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[4]Uniklink RWTH Aachen, Department of Neurology, Germany; [5]RWTH Aachen Univ., Aachen, Germany; [6]Nanion Technologies GmbH, Munich, Germany

Abstract: Pain syndromes such as small fiber neuropathy (SFN) can be linked to mutations in voltage-gated sodium channels (Na_vs). Several Na_V mutations are described, and many were shown by manual patch-clamping to display gain-of-function characteristics. In some very rare individual cases, approved drugs were identified that specifically interact with the mutations and make the channel more sensitive e.g. for a specific local anesthetic. Such rare constellations can support the patients' clinical treatment , which is often insufficient, leaving the patient with severe untreated symptoms over years.Within the framework of the Sodium Channel Network Aachen (SCN^{Aachen}) we aim to (i) identify mutation-induced patient-specific Na_V gating changes, and (ii) systematically screen for US/EU-approved drugs, which specifically target mutation-induced Na_V malfunctions in a favorable manner. To this end, we use stable cell lines and HEK/ND7 cells transiently expressing Nav1.7 and Nav1.8 variants to comprehensively characterize mutation-induced functional changes in activation and inactivation gating and to screen a compound library of approved drugs on the SyncroPatch, a high-throughput electrophysiology robot. In addition to several known Na_v mutations, we will characterize variants identified in a cohort of SFN patients at the RWTH Aachen University Hospital with respect to their biophysical effects, temperature dependence and pharmacological profiles. We will present the results of this extensive work and thus provide a high-throughput approach for the identification of personalized treatment options for pain patients.

Disclosures: R. Hausmann: None. **R. Bott:** None. **A. Kalia:** None. **J. Körner:** None. **A. Erickson:** None. **R. Rolke:** None. **M. Dohrn:** None. **B. Namer:** None. **A. Maier:** None. **M. Häusler:** None. **M. Hampl:** A. Employment/Salary (full or part-time):; Salary from Nanion. **I. Rinke-Weiß:** A. Employment/Salary (full or part-time):; Sa. **A. Obergrussberger:** A. Employment/Salary (full or part-time):; Salary from Nanion. **I. Kurth:** None. **G. Schmalzing:** None. **A. Lampert:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research contract with Hoffmann La Roche. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Nanion SyncroPatch 384i Research Grant.

Digital Abstract Session

P166. Treatments For Persistent Pain

Program #/Poster #: P166.07

Topic: D.03. Somatosensation – Pain

Support: National Institutes of Health NS102722
National Institutes of Health DE026806
National Institutes of Health DK118971
National Institutes of Health DE029951
Department of Defense W81XWH1810431

Cancer Australia and Cure Cancer GNT1157171
ARC DE180100418

Title: Genetic deletion of neuronal F2rl1 gene and pharmacological inhibition of protease-activated receptor-2 attenuates oral cancer nociception in mice.

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Abstract: Oral squamous cell carcinoma (SCC) secretes proteases into the cancer microenvironment. These secreted proteases cleave protease-activated receptor-2 (PAR_2) which in turn initiate nociceptive signaling. PAR_2 is upregulated in the lingual nerve of patients with tongue cancer (oral SCC). We hypothesize that genetic deletion of *F2rl1* (the gene that codes for PAR_2), or pharmacological inhibition of PAR_2, attenuates oral cancer nociception. To test this hypothesis, we undertook experiments with *Par2 Nav1.8* knockout mice (the *F2rl1* gene is knocked out in $Na_v1.8$-positive neurons); wild type C57BL/6J mice were used as a control. Over the course of 16 weeks, 100 µg/mL of the carcinogen 4-Nitroquinoline 1-oxide (4NQO) was administered in the mouse drinking water to induce oral SCC. Simultaneously, mice were trained weekly in a dolognawmeter®, a validated operant assay of orofacial nociception. The dolognawmeter® assay was performed once a week for 28 weeks to quantify orofacial dysfunction secondary to oral cancer nociception. In a second experiment, *F2rl1* RNAi was injected into the tongue of wild type mice with 4NQO-induced oral SCC. In a third experiment, human oral SCC (HSC-3) supernatant was injected into the paw of *Par2 Nav1.8* and wild type mice with and without administration of the PAR_2 antagonist GB88. We observed attenuated nociception at 28 weeks in *Par2 Nav1.8* mice with 4NQO-induced oral SCC [83.4 ± 173.8 (%) in *Par2 Nav1.8* versus 221.2 ± 238.7 (%) in wild type]. Similarly, we found attenuated cancer nociception 28 weeks after the first administration of 4NQO in mice in which the *F2rl1* gene was genetically deleted through RNAi [271.6 ± 448.5 (%) in *F2rl1* RNAi versus 580.9 ± 391.6 (%) in random RNAi]. *Par2 Nav1.8* mice exhibited attenuated nociception in the paw secondary to HSC-3 supernatant injection at 1, 3, and 6 hour(s) post-injection [0.3 ± 0.1 (g) in *Par2 Nav1.8* versus 0.1 ± 0.05 (g) in wild type at 6 hours]. Pharmacological inhibition of PAR_2 by GB88 attenuated nociception in the paw following injection of HSC-3 supernatant in wild type mice at 1, 3, 6, and 12 hour(s) post-injection [0.4 ± 0.1 (g) in GB88 versus 0.05 ± 0.05 (g) in control at 6 hours]. We infer from these findings that PAR_2 is a central component of the nociceptive signaling mechanism responsible for oral SCC pain. Our findings may open new avenues for the development of genetic or pharmacological approaches for treating oral cancer pain by targeting PAR_2.

Disclosures: **N.H. Tu:** None. **K. Inoue:** None. **D.H. Kim:** None. **H.D. Tran:** None. **Y. Taguchi:** None. **J.C. Dolan:** None. **S. Yamano:** None. **L.E. Edgington-Mitchel:** None. **N.W. Bunnett:** None. **B.L. Schmidt:** None.

Digital Abstract Session

P167. Pain Models: Physiology

Program #/Poster #: P167.01

Topic: D.03. Somatosensation – Pain

Support: BBSRC (BB/R00823X/1)
MRC (MR/L019248/1)
MRC (G0901269)

Title: Early life pain experience alters pain behaviour and pain related activity in the somatosensory and the medial prefrontal cortices in adulthood

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Abstract: Background and Aims: Early life painful experiences have long-lasting effects upon adult behavioural pain sensitivity but it is not known whether cortical activity related to pain perception is also affected. Here we recorded pain related neural activity in the somatosensory cortex (S1) and the medial prefrontal cortex (mPFC) in awake adult rats, following early life skin incision. We hypothesised that functional connectivity between these two cortical areas would be altered following neonatal skin injury, resulting in altered pain perception. Methods: Simultaneous pain behaviour and electrophysiological local field potential activity was recorded in S1 and mPFC in awake male Sprague-Dawley rats. Data was collected at baseline (BL) and at 0 (2hrs), 1, 4 and 10 days after unilateral hindpaw skin incision in a control group and a 'primed' group (exposed to skin incision at postnatal day 3) (n=8-10 per group). Behavioural pain sensitivity (paw withdrawal threshold, PWT) and S1-mPFC functional connectivity (phase locking value, PLV) were measured following electronic von Frey (vF) stimulation of the hindpaw. Experiments were regulated by the Home Office Animal (Scientific Procedures) Act, 1986. Results: Primed rats displayed heightened behavioural pain sensitivity to mechanical von Frey (vF) stimulation of the hindpaw following adult skin injury compared to controls (2 way ANOVA, $P < 0.001$). Skin injury also elicited an increase in mPFC-S1 PLV in the theta range, relative to baseline, in both groups on the D0 (permutation t-test, mean difference between BL and D0: primed= 0.11 [95%CI 0.05, 0.20], p=0.01; control=0.14 [95%CI 0.05, 0.25], p=0.03). The adult skin injury evoked increase in theta mPFC-S1 PLV was correlated with behavioural pain sensitivity in both groups (PLV increase inversely related to PWT, $R^2 =0.26$, P=0.003) indicating that the PLV is a measure of pain related cortical connectivity. Importantly, the theta mPFC-S1 PLV increase was significantly prolonged in primed rats compared to controls, and still present at day 4 post injury (permutation t-test, mean difference between BL and D4: Primed =0.13 [95%CI 0.04, 0.23], p=0.01; control =0.15 [95%CI -0.03, 0.19], p=0.15). Conclusion: Painful experience in early life has a significant effect on both behavioural pain sensitivity and the function of pain related cortical circuits in adults. Long-lasting changes in pain related functional S1 and mPFC connectivity follow postnatal skin incision, supporting the hypothesis that brain circuits underlying pain perception are altered.

Disclosures: **P. Chang:** None. **L. Fabrizi:** None. **M. Fitzgerald:** None.

Digital Abstract Session

P167. Pain Models: Physiology

Program #/Poster #: P167.02

Topic: D.03. Somatosensation – Pain

Support: John R. Evans Leaders Fund grant from the Canada Foundation for Innovation
Discovery Grant from the Natural Sciences and Engineering Research Council of Canada
Early Career Research Grant from the International Association for the Study of Pain
Early Career Investigator Pain Research Grant from the Canadian Pain Society and Pfizer Canada
Project/Operating Grants from the Canadian Institutes of Health Research

Title: A neuronal mechanism of spinal hyperexcitability is sexually dimorphic in rodent and human models of pathological pain

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Abstract: Clinical and epidemiological evidence suggest a sex difference in the mechanisms underlying chronic pain, with female chronic pain patients outnumbering their male counterparts by 2:1. Despite this, the neurobiological underpinnings of sexually dimorphic pain signaling remain unclear in rodents and are virtually unexplored in human preclinical models. Within the nociceptive system, the superficial dorsal horn (SDH) is a critical site of pain modulation. Pathological pain arises when there is an imbalance between excitation and inhibition in neurons of the SDH. We have recently characterized a pathological pain pathway in male rat and human SDH neurons where brain-derived neurotrophic factor (BDNF) mediates a loss of inhibition through the downregulation of chloride co-transporter 2 (KCC2), driving downregulation of the phosphatase $STEP_{61}$ and a subsequent potentiation of GluN2B-containing N-methyl D-aspartate receptors (NMDARs) (Dedek, Xu et al., Brain, 2019). Here, we investigated whether molecular mechanisms of spinal hyperexcitability and pathological pain are conserved between sexes in both rodents and humans. Our models of pathological pain included *ex vivo* BDNF pain pathology models in rodent and human spinal cord and the *in vivo* complete Freund's adjuvant (CFA) rodent model of inflammatory pain. For the human tissue translational pain models, we collected human spinal cord samples from male and female organ donors 1 to 3 hours post-aortic cross-clamping. We paired patch-clamp electrophysiological recordings of synaptic NMDAR responses with *ex vivo* pharmacology, biochemical approaches, and behavioral testing. In

contrast to male rodents, we find that in female rats, NMDAR responses at lamina I SDH synapses are not potentiated by spinal cord *ex vivo* treatment with BDNF nor in the *in vivo* CFA model of inflammatory pain. Parallel biochemical evidence suggests that active $STEP_{61}$ and KCC2 are not downregulated and active Fyn and GluN2B are not upregulated at SDH synapses in female rodent (CFA inflammatory pain and *ex vivo* BDNF) as well as human (*ex vivo* BDNF) models of pathological pain. Electrophysiological recordings and biochemical investigations in ovariectomized rats suggest hormonal mediation of this sex difference. We conclude that neuronal mechanisms of SDH hyperexcitability are sexually dimorphic in both rats and humans, with a BDNF/$STEP_{61}$/Fyn-dependent potentiation of GluN2B-containing NMDARs in males but not females. This sexual divergence in underpinning neurobiological mechanisms of chronic pain has profound implications for the development of novel pain therapeutics.

Disclosures: **A. Dedek:** None. **J. Xu:** None. **C.M. Kandegedara:** None. **J.A. Landrigan:** None. **C. Dedek:** None. **P.J. Lombroso:** None. **E.C. Tsai:** None. **M.E. Hildebrand:** None.

Digital Abstract Session

P167. Pain Models: Physiology

Program #/Poster #: P167.03

Topic: D.03. Somatosensation – Pain

Support: DoD Grant MR141214

Title: Persistent pain after traumatic brain injury: pain symptoms, sensory signs, neuropsychological and psychosocial impact

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Abstract: Persistent pain develops in approximately 50% of people who experience a traumatic brain injury (TBI), yet effective treatment is still lacking mainly because pain symptoms, sensory signs, psychosocial and neuropsychological factors associated with this condition have not been fully characterized. For this, we assessed and compared these factors in people with TBI without persistent pain (N=23, age: 29.74 ± 7.90), with TBI-related persistent pain (N=26, age: 34.35 ± 11.10) and healthy controls (N=38, age 28.68 ± 7.54). Pain was characterized using the International SCI Basic Pain Dataset version 2 modified for TBI and the neuropathic pain diagnostic questionnaire DN4. Sensory function was measured in three standard sites during a quantitative sensory testing (QST). Neuropsychological tests and questionnaires evaluated cognitive and emotional functions, and the multidimensional pain inventory (MPI) determined the impact of pain in people's life. Participants with TBI-related pain reported mean pain intensity of 7.65 ± 1.71 (0-10 scale) during the last 7 days and 92.3% of these participants had more than one pain problem commonly located in the head (76%), shoulders and neck (67%),

and back (43%) with a neuropathic nature in 42.31% of the participants (DN4 total score ≥3 out of 7). Kruskal-Wallis (DF=2, Bonferroni-adjusted *P*) test was used to determine differences between groups. As predicted, the pain group presented significant higher anxiety (Beck's anxiety inventory, *H*=26.399, *P*=0.000, *P*=0.000), higher depression (Beck's depression inventory, *H*=26.102, *P*=0.004, *P*=0.000), and lower total recall (Hopkins Verbal Learning Test, *H*=9.448, *P*=0.029, *P*=0.17) compared to the TBI without pain and AB control groups correspondingly. QST lower graphesthesia scores (*H*=8.954, *P*=0.010), forehead lower cool (*H*=7.008, *P*=0.026), lower warm (*H*=6.323, *P*=0.038) and higher vibration (*H*=10.670, *P*=0.004); and right medial calf higher vibration (*H*=6.427, *P*=0.034) detection thresholds were found between the pain group and AB control group suggesting abnormal sensory function. Additionally, MPI scores (0-6 scale) of pain severity (3.76 ± 1.41), life interference (3.64 ± 1.71) and affective distress (3.09 ± 1.45) indicate the negative impact of the persistent pain in the participants, although most of them feel support (4.21 ± 1.77) and life control (4.27 ± 1.17). Collectively, our findings contribute to our understanding of the sensory, neuropsychological and psychosocial factors associated with the TBI-related persistent pain. Nevertheless, future work is required to fully characterize the nature of the pain and the mechanisms undergoing this condition.

Disclosures: L.E. Robayo: None. **V. Govind:** None. **L. Fleming:** A. Employment/Salary (full or part-time):; Miami Project to Cure Paralysis. **D. Nilanjana:** None. **E. Widerstrom-noga:** None.

Digital Abstract Session

P168. Nociceptors

Program #/Poster #: P168.01

Topic: D.03. Somatosensation – Pain

Support: NIH grant NS102722
NIH grant DE026806
NIH grant DK118971
NIH grant DE029951
Department of Defense W81XWH1810431

Title: Oral squamous cell carcinoma supernatant sensitizes TRPV4 in trigeminal ganglia neurons through PAR_2

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Abstract: Pain is the worst symptom experienced by oral cancer patients. The transient receptor potential vanilloid 4 (TRPV4) ion channel may play a role in mechanical stimulus-induced oral cancer nociception. In this study, we asked the question whether oral cancer cells release mediators that activate PAR_2 which then sensitize TRPV4 channels in trigeminal ganglia (TG) neurons. We collected supernatant from a cell line of human oral squamous cell carcinoma (HSC-3). We then incubated TG neurons in this supernatant. Using calcium imaging, we measured calcium transient response to TRPV4 agonist GSK1016790A in TG neurons treated with either supernatant or vehicle (DMEM media). We found that HSC-3 supernatant treatment yielded a greater percentage of small and medium-sized TG neurons that responded to GSK 1016790A in C57BL/6J mice. Calcium transient amplitudes in HSC-3 supernatant treated neurons were greater than those in neurons treated with DMEM media.
We utilized *Par2*$^{-/-}$ mice to determine the role of PAR_2 in HSC-3 supernatant induced TRPV4 sensitization. We found that the percentage of neurons responding to GSK 1016790A after treatment with HSC-3 supernatant was similar to the percentage responding to DMEM media. HSC-3 supernatant sensitization of TRPV4 was diminished in TG neurons with PAR_2 deletion. From our findings we infer that HSC-3 supernatant sensitizes TRPV4 in TG neurons and that PAR_2 is required for sensitization.

Treatment	TG responded to GSK (%)		Δf/f (mean ± SEM)	
	C57BL/6J	*Par2*$^{-/-}$	C57BL/6J	*Par2*$^{-/-}$
DMEM	13.93	5.45	0.40 ± 0.03	0.27 ± 0.02
HSC-3	29.11	6.43	0.77 ± 0.12*	0.37 ± 0.14

*$P < 0.05$, vs. DMEM media (unpaired student's *t*-test)

Disclosures: **E.W. Chen:** None. **S. Nicholson:** None. **K. Inoue:** None. **L. Edgington-Mitchell:** None. **N. Bunnett:** None. **B.L. Schmidt:** None.

Digital Abstract Session

P168. Nociceptors

Program #/Poster #: P168.02

Topic: D.03. Somatosensation – Pain

Support: NIH R01 NS115209
NIH F31 NS117087

Title: Excitatory chloride physiology discriminately encodes noxious cold in a multimodal sensory neuron

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Abstract: *Drosophila* larvae detect both noxious cold and innocuous touch using Class III (CIII) dendritic arborization neurons, a class of sensory neuron which tiles the larval barrier epidermis. The ability to differentiate between harmful and innocuous stimuli is key to organism survival, but is also relevant to human health, as the inability to discriminate between painful and non-painful stimuli underlies many neuropathic pain conditions. It is therefore important to elucidate generalizable mechanisms by which multimodal pain sensors (nociceptors) operate. CIII nociceptors are thought to function via a high-low filter, whereby high levels of activation drive cold-associated behaviors (primarily full-body contraction; CT), and low levels of activation drive a complex suite of touch-associated behaviors. Both modalities make use of Transient Receptor Potential channels, but it remains unknown what other molecular factors contribute to the activity required to selectively drive cold-evoked behaviors. Transcriptomic analysis of CIII neurons reveals enriched expression of TMEM16/anoctamin family genes, which often function as calcium-activated chloride channels. Loss-of-function disruptions of the anoctamins *subdued* and *CG15270* result in a reduced capacity to CT, and reduced cold-evoked CIII activity, but not reduced touch sensitivity, suggesting a modality-specific and excitatory function. Although Cl^- currents in archetypal neurons are hyperpolarizing, genetic, *in vivo* manipulations of Cl^- physiology (via knockdown and overexpression of SLC12 channels) and *ex vivo* manipulations of extracellular [Cl^-] indicate that CIII Cl^- currents are depolarizing. Consistent with this, Cl^- optogenetics, which are more often inhibitory, instead activate CIII neurons, and can be used to initiate CT behavior *sans* cold. Moreover, overexpression of the SLC12 *ncc69* (related to *NKCC1/2*) results in nociceptive sensitization and increased spontaneous CIII activity, a genotype-phenotype relationship consistent with some types of neuropathic pain. These results collectively indicate a conserved, excitatory role for SLC12 and TMEM16 channels in animal sensory neurons, and present a genetically-tractable system in which to study mechanisms relevant to neuropathic pain.

Disclosures: N.J. Himmel: None. **A. Sakurai:** None. **J.M. Letcher:** None. **M.N. Benson:** None. **T.R. Gray:** None. **D.N. Cox:** None.

Digital Abstract Session

P168. Nociceptors

Program #/Poster #: P168.03

Topic: D.03. Somatosensation – Pain

Support: NIH R01DE026806

Title: Cancer cell-derived exosomes mediate oral cancer pain

Authors: *Z. A. DUBEYKOVSKAYA, T. H. NGUYEN, B. L. SCHMIDT, D. G. ALBERTSON;
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Abstract: Oral cancer patients experience pain at the site of the cancer prior to treatment. Pain is initiated and maintained in the tumor microenvironment by mediators that sensitize primary afferent nociceptors. Anti-nociceptive therapy with opioids does not adequately manage pain. Patients no longer experience pain after removal of the tumor. Intraplantar injection of conditioned media from HSC-3 (RRID:CVCL_1288) cancer cells induces mechanical allodynia and thermal hypersensitivity, while conditioned media from noncancer (dysplasia) DOK (RRID:CVCL_1180) cells do not evoke nociception. Conditioned media contain soluble substances and exosomes. Exosomes are 30-150 nm extracellular vesicles. They are formed within the endosomal system as intralumenal vesicles of multivesicular bodies and are released into the extracellular space. Here, we investigated whether exosomes derived from nociceptive cancer cells conditioned media mediate mechanical and thermal nociception in a mouse model. **Methods**. We isolated exosomes from conditioned media of cancer cell lines HSC-3 and OSC-20 and noncancer DOK cells by differential centrifugation. Morphology, size and purity of exosomes were validated by transmission electron microscopy, nanoparticle tracking analysis and western blot. We separated exosome-positive and exosome-depleted fractions from conditioned media by size-exclusion chromatography. Exosome-positive and exosome-depleted fractions were combined to generate a reconstituted fraction. We injected cell line media DMEM, conditioned media, the exosome-depleted and the reconstituted fractions into the right hind paws of male mice (n=5 per group). Paw withdrawal was measured in response to mechanical (von Frey filaments) and thermal stimulation. **Results**: Intraplantar injection of HSC-3 cell conditioned media induced nociception. Depletion of exosomes from HSC-3 cell conditioned media reduced or abolished mechanical allodynia and thermal hypersensitivity. Nociceptive behaviors were restored in response to the conditioned media reconstituted with the HSC-3 exosome fraction, but not with DOK cells-derived exosomes. By contrast, nociceptive behavior was not observed when mice received intraplantar injections of DOK cell conditioned media, DOK cell conditioned media depleted of exosomes or exosome depleted media reconstituted with the DOK cells exosomes. Nociceptive responses were induced when the DOK conditioned media was reconstituted with HSC-3 cells exosomes. **Conclusions:** Oral cancer pain mediators are released in exosomes.

Disclosures: Z.A. Dubeykovskaya: None. **T.H. Nguyen:** None. **B.L. Schmidt:** None. **D.G. Albertson:** None.

Digital Abstract Session

P168. Nociceptors

Program #/Poster #: P168.04

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01 NS087033

Title: The calcium sensor stim1 senses pain, not itch

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Abstract: Stromal interaction molecule 1 (STIM1) is an endoplasmic reticulum (ER) calcium sensor that can sense a calcium drop in ER and reacts to extracellular stimuli. STIM1 is expressed and functional in nociceptive neurons in the dorsal root ganglion (DRG). We wonder whether sensory STIM1 can sense peripheral stimuli such as alogenic agents and pruritogens. Here we show that peripheral inhibition of STIM1 or deletion of STIM1 in sensory neurons (SN-STIM1 knockout) significantly reduces noxious mechanical-, cold-, capsaicin-, AITC- and bradykinin-induced nociception. Activation of STIM1 by thapsgargin (an ER Ca^{2+}-ATPase inhibitor) triggers spontaneous nociceptive behavior and induces pain hypersensitivity, which is significantly attenuated in SN-STIM1 knockout (KO) mice. These effects are confirmed by L3/4 DRGs STIM1 knockdown mice. Interestingly, pruritogens (histamine (His), the peptide Ser-Leu-Ile-Gly-Arg-Leu (SLIGRL) or chloroquine (CQ)-induced itch behavior is not altered in SN-STIM1 KO mice. Moreover, activation of STIM1 induces pain but not itch via inhibition of potassium currents and increase in neuronal excitability. Consistently, capsaicin-, AITC- or bradykinin-induced calcium entry is significantly decreased while CQ- or His-induced calcium influx is intact in SN-STIM1 KO neurons. Activation of TRPV1 triggers STIM1 translocation and SOC entry, suggesting a novel link between STIM1 and pain targets. Strikingly, STIM1 is expressed and functional in most TRPV1+ neurons but is not functional in His- responding neurons. Our findings reveal that STIM1 distinguishes pain and itch, two distinct sensory modalities.

Disclosures: **D. Wei:** None. **I. Zhang:** None. **F. Wang:** None. **H. Zhao:** None. **D. Li:** None. **T. Rohacs:** None. **Y. Tao:** None. **H. Hu:** None.

Digital Abstract Session

P168. Nociceptors

Program #/Poster #: P168.05

Topic: D.03. Somatosensation – Pain

Support: NIH R01 NS115209
NIH F31 NS117087

Title: Evolutionarily ancient roles for TRPA1 in cold-evoked nociceptive behavior

Authors: ***J. M. LETCHER**, N. J. HIMMEL, A. SAKURAI, M. HOLGUIN-LOPEZ, D. N. COX;
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Abstract: Thermosensory nociception alerts organisms to potential environmental dangers, thereby serving as a protective mechanism for driving adaptive behavioral responses to safeguard against incipient damage. Transient receptor potential (TRP) channels play a key role in thermosensation and can be activated directly or indirectly by changes in temperature. However, less is known regarding how TRPs mechanistically function in regulating noxious cold detection and whether there is molecular conservation across phyla. TRPA1 has a functional role in chemical sensing that predates the protostome-deuterostome split (>550 mya), yet it remains unknown whether TRPA1 thermal sensing is equally ancient. Protostome, and many deuterostome, TRPA1s have been associated with high-temperature sensing, while some deuterostome TRPA1s (including those of mice and humans) have been identified as noxious cold sensitive. Among invertebrates, *C. elegans* TRPA1 is implicated in detecting cool (sub-noxious) temperatures, however, it is unknown if this holds for noxious cold. Conversely, in *Drosophila* TRPA1 is known to function in class IV (CIV) polymodal nociceptor neurons for mechanical and high heat nociception. Our previous studies identified class III (CIII) multidendritic sensory neurons as cold nociceptors that are required for noxious cold-evoked contraction (CT) behavior in larvae. We further demonstrate via CIII-specific neurogenomic studies that TRPA1 is enriched in these neurons, suggesting it may contribute to noxious cold sensing. Analyses of multiple TRPA1 whole-animal mutants revealed severe impairment of cold-evoked CT behavior, which we likewise observed with CIII-specific TRPA1 knockdown. Behavioral studies of TRPA1 mutations revealed isoform-specific requirements for noxious cold sensing that are distinct from isoforms linked to noxious heat sensing or UV sensing via CIV neurons. Electrophysiological recordings demonstrate that TRPA1 is required in CIII neurons for cold-evoked electrical activity. TRPA1 appears to function in cold-evoked sensory transduction, however physiological and behavioral defects are independent of alterations in CIII neuronal morphology. CIII-specific expression of TRPA1 rescued nocifensive behavior in whole animal TRPA1 mutants. Further, behavior deficits were also rescued by ectopic expression of human TRPA1. Collectively, these findings shed new light on evolutionarily ancient properties of TRPA1 in thermal sensing and raises interesting questions regarding the molecular and mechanistic underpinnings of TRPA1 polymodality.

Disclosures: J.M. Letcher: None. **N.J. Himmel:** None. **A. Sakurai:** None. **M. Holguin-Lopez:** None. **D.N. Cox:** None.

Digital Abstract Session

P169. Spinal Circuits

Program #/Poster #: P169.01

Topic: D.02. Somatosensation

Support: DFG Research Fellowship FI 2367/1-1
NIH MINDS 1R21NS120665-01

Title: Tools for efficient 3D cell and projection analysis and atlas registration of whole mouse spinal cord

Authors: *F. FIEDERLING, L. A. HAMMOND, C. A. MASON, J. DODD;
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Abstract: The spinal cord (SC) contains a remarkable and complex variety of interconnected cells that relay and process somatosensory information and execute movement. Classic research on the embryonic and adult SC has identified major classes of neurons, based on embryonic markers, morphology and position within the SC. But recent advances in transcriptional profiling have revealed that the extent of spinal cell diversity far exceeds previous assumptions.
To achieve better characterization of spinal cell types and the detailed connectivity between cells that form functional circuits, it is necessary to map the spatial relations of neurons and projections. To date, however, unbiased approaches to map positional information in the context of the whole SC are severely limited by a lack of tools for efficient data collection and analysis. Moreover, the absence of a standardized 3D reference atlas for the mouse SC complicates anatomical mapping of data and comparison between samples and results across the field.
Here we present tools for practicable analysis of labelled cells and projections in whole mouse SC in the context of a novel 3D anatomical atlas. We have designed soluble, 3D-printed, 'Spine Racks' that guide oriented and parallel embedding of serial tissue segments of the entire SC within a single block for synchronous cryo-sectioning. Spine Racks precisely position tissue pieces within each block section array, permitting automated imaging of slides and rostro-caudal sorting of section images. Additionally, we have developed "SpinalJ", a user-friendly plugIn for ImageJ, to register section images and to map the reconstructed data to a prototype 3D reference atlas. SpinalJ further combines various tools for manual and automated cell and projection analysis, as well as for data visualization.
Together, these tools capacitate high-throughput comparative analyses of neurons and their projections in whole SC.

Disclosures: F. Fiederling: None. **L.A. Hammond:** None. **C.A. Mason:** None. **J. Dodd:** None.

Digital Abstract Session

P169. Spinal Circuits

Program #/Poster #: P169.02

Topic: D.02. Somatosensation

Support: NIH Grant R21AT010517 (to WRR)
NIH Grant K01AT005935 (to WRR)
NIH Grant U19AAT004137 (to Joel G. Pickar)

Title: Effects of thrust magnitude and duration of spinal manipulation on immediate muscle spindle response in an animal model.

Authors: *C. R. LIMA[1], A. LAW[2], P. LI[1], W. REED[1];
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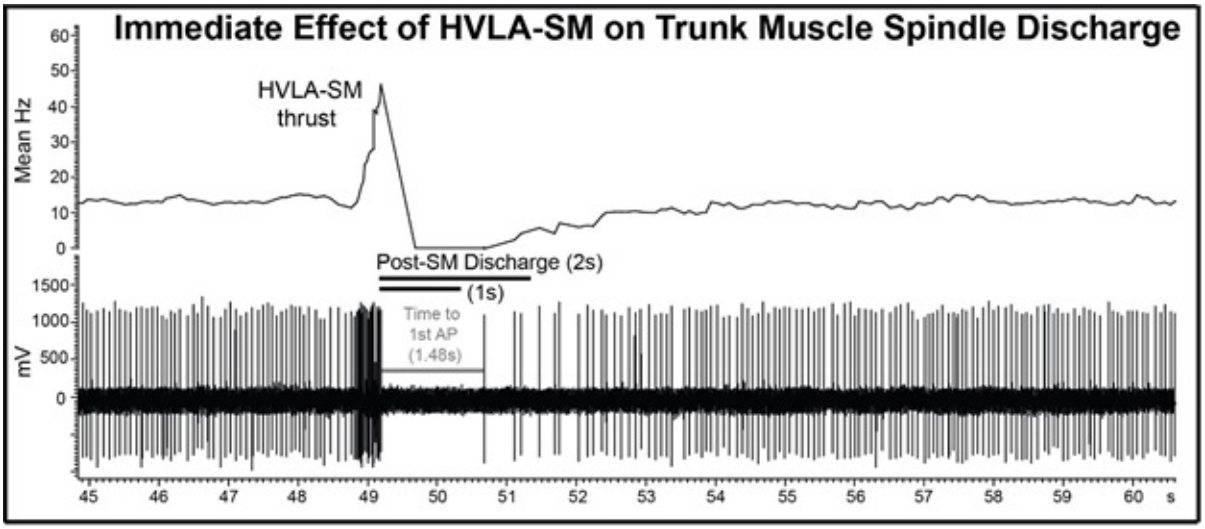
Abstract: Rationale: High velocity low amplitude spinal manipulation (HVLA-SM) is a non-pharmacological approach commonly used by chiropractors, physical therapists and osteopaths to treat acute and chronic back pain, but little is known about the relationship between HVLA-SM biomechanical characteristics and its subsequent neurophysiological effects. **Objective:** To characterize trunk muscle spindle responses immediately after high velocity, low amplitude (HVLA-SM) delivered at various thrust magnitudes and thrust durations.**Methods:** Secondary analysis from multiple studies involving anesthetized adult male/female cats (n = 70; 2.3-6.0 kg) receiving L6 HVLA-SM. Muscle spindle afferent recordings were obtained from L6 dorsal rootlets prior to, during, and immediately after HVLA-SM. L6 HVLA-SM was delivered posterior-to-anterior using a feedback motor with peak thrust magnitudes of 25, 55, 85% of cat body weight (BW) and thrust durations of 25, 50, 75, 100, 150, 200, and 250ms. Time to 1st action potential (AP; ms) and muscle spindle discharge frequency during 1 and 2s post-HVLA-SM (Hz) were determined.**Results:** A significant association between HVLA-SM thrust magnitude and immediate muscle spindle response was found ($p < 0.001$). For non-control thrust magnitude pairwise comparisons (25%, 55%, 85%BW), 55%BW thrust magnitude had the most consistent impact on post-HVLA-SM discharge outcomes particularly at a thrust duration of 50ms (False Discovery Rate < 0.05). No significant association was found between thrust duration and immediate post-HVLA-SM muscle spindle response ($p > 0.05$).**Conclusion:** HVLA-SM thrust magnitudes delivered at 55%BW are more likely to impact immediate (≤ 2s) post-HVLA-SM muscle spindle response, particularly when HVLA-SM is delivered at shorter thrust durations (≤ 50ms).

Figure 1. An example recording of a trunk muscle spindle response during an L6 HVLA-SM. The immediate post-HVLA spindle response outcomes (time to 1st action potential, 1s post-HVLA-SM activity, and 2s post-HVLA-SM) that were measured in this study are illustrated.



Disclosures: C.R. Lima: None. **A. Law:** None. **W. Reed:** None. **P. Li:** None.

Digital Abstract Session

P169. Spinal Circuits

Program #/Poster #: P169.03

Topic: D.02. Somatosensation

Title: Spinal cord reflexes in a brain iron-deficient model of restless legs syndrome: Role of dopamine and adenosine receptor modulators

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Abstract: Restless Legs Syndrome (RLS) is a sensorimotor disorder that severely disrupts sleep. RLS patients regularly present with a condition known as brain iron deficiency (BID), and BID is commonly associated with altered dopamine (DA) and adenosine (ADE) neurotransmission. We here tested if a diet-induced brain iron-deficient animal model mimics the increased spinal cord excitability reported in other RLS animal models, and the responsiveness of this model to treatment with DA and ADE receptor modulators.
Following previously established protocols, newly-weaned C57Bl/6 mice were separated into two cohorts that were fed control iron (CTRL ID, ~ 48 ppm Fe, 8 males and 8 females) and two cohorts that were fed iron-reduced diets (ID, 4-6 ppm Fe, 10 males and 10 females). Blood analyses after 10 weeks revealed that the ID diet did not induce an anemic phenotype. To assess spinal excitability, we measured thermal pain reflex (TPRs) withdrawal latencies, starting at one week after diet exposures. At 3 weeks on their respective diets, both male and female ID cohorts showed significantly lower TPR latencies than their respective CTRL ID cohorts, and this difference remained stable over time. Based on the current standard treatment for patients with RLS, all cohorts were then administered DA D3 receptor (D3R) agonist (pramipexole, PPX, 0.5 mg/kg, i.p.). PPX did not significantly alter spinal reflex latencies but led to strongly increased locomotor and rearing activities in both ID cohorts. Next, we tested the effects of the DA D1 receptor (D1R) antagonist ecopipam (SCH 39166, 0.5 mg/kg, i.p.). SCH 39166 had no effect on TPRs in CTRL ID animals, but it rescued the hyperexcitable phenotype in male ID animals and slightly improved the outcome in female ID animals. Finally, we tested in week 9 the effects of the ADE A1 receptor (A1R) agonist N6-cyclpentyladenosine (CPA, 1 mg/kg, i.p.) and the ADE receptor antagonist caffeine (50 mg/kg, i.p.). CPA increased TPR latencies in the ID cohort, while caffeine reduced them in CTRL ID animals.
Together, our data show that diet-induced iron-deficiency leads to a decrease in TPR latencies and that, in this model, increased spinal cord excitability is unresponsive to D3R modulation but is responsive to D1R and A1R receptor modulation. As RLS patients present with BID and are responsive to D3R-based treatment in the clinic, these findings suggest that D3R effect in the clinic is not congruent with the ID animal model. Instead, our data point to the possibility that ID-induced changes in reflex excitability may be linked to an altered D1R/A1R system in the spinal cord.

Disclosures: S. Woods: None. **S. Clemens:** None.

Digital Abstract Session

P169. Spinal Circuits

Program #/Poster #: P169.04

Topic: D.02. Somatosensation

Support: New Investigator Award from the Arizona Biomedical Research Commission (#ADHS18-198875)

Title: Identifying the Spinal Cord Neuronal Circuit through which Heat Shock Protein 90 Regulates ERK MAPK Signaling and Opioid Anti-Nociception

Authors: *B. ROMAN[1], K. GABRIEL[2], J. M. STREICHER[2];
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Abstract: Heat shock protein 90 (Hsp90) has been studied and recognized as a vital regulator of protein biochemistry; recent studies from our lab have shown that Hsp90 is also a crucial regulator of opioid-induced pain relief through several molecular mechanisms in the nervous system. Through past testing, Hsp90 inhibitors like 17-AAG have shown to affect anti-nociception oppositely when applied to either the brain or spinal cord. When 17-AAG was injected in the spinal cord, anti-nociception was enhanced rather than reduced like in the brains of mice. Our previous work further found that signaling proteins including ERK and RSK1/2 mediate the enhanced anti-nociceptive effects of Hsp90 inhibition in spinal cord. Though this circuit is normally suppressed through the Hsp90 protein, by inhibiting it with 17-AAG and applying a co-treatment of the opioid agonist morphine, the circuit is activated to promote the enhanced anti-nociception seen in mice. Through the same study, and utilizing immunohistochemistry analysis of the spinal cords, phosphorylated ERK was found to overlap with the dendrite marker MAP2. Though not a complete overlap, the imaging suggests that activation of the ERK mechanism, and thus the RSK mechanism and anti-nociception, occurs in the post synaptic dendrites, located in the dorsal horn of the spinal cord. Uncovering the neuronal circuit in which these molecular mechanisms take place will bring more insight into the organization of Hsp90 and Mu opioid receptors (MOR) in the spinal cord. Our proposed study will identify spinal cord neuronal circuits that mediate anti-nociception when Hsp90 proteins are inhibited. We report on our progress using double-labeling of Hsp90 or phospho-ERK with different neuronal markers to place these signaling events in a circuit context; pre- vs. post-synaptic, ascending vs. descending modulation, interneurons vs. projection neurons, and similar circuit analysis. These markers include synapsin I or synaptophysin to determine pre-synaptic localization; PSD95 or SHANK3 to determine post-synaptic localization; if pre-synaptic localization is observed, alpha2 adrenergic receptor (descending modulation) or IB4 (ascending modulation); if post-synaptic localization is observed, NK1 or CGRP. Through these studies we aim to illuminate a novel neuronal circuit through which Hsp90 promotes enhanced opioid pain relief.

Disclosures: K. Gabriel: None. **J.M. Streicher:** None.

Digital Abstract Session

P169. Spinal Circuits

Program #/Poster #: P169.05

Topic: D.02. Somatosensation

Support: NIH 1R01DC018060-01

Title: Determination of the proprioceptive organs of the rat larynx

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Abstract: Introduction: The larynx is a group of muscles, cartilages, and mucosa in the upper airway that plays a vital role in respiration, airway protection, and phonation. These functions rely on the precise opening, closing, and vibration of the vocal folds (VF), key structures within the larynx, which in turn are controlled by the intrinsic laryngeal muscles (ILM). Exquisite ILM coordination is required for proper VF movement, which often entails near-instantaneous microadjustments. Any defect in ILM coordination can result in defective VF movement and subsequent functional impairment, such as the dysfunctional voice observed laryngeal dystonia. Such precise coordination requires a well-calibrated proprioceptive apparatus. However, controversy remains over whether canonical proprioceptive organs - muscle spindles and Golgi tendon organs - are present in the intrinsic laryngeal muscles. In this exploratory work, we seek to identify the proprioceptive organs of the larynx.
Methods: Sixty-two Sprague-Dawley rats were distributed across 5 age groups post-birth: P3, P8, P11, P14-15, and adult. Larynxes were sectioned and immunostained for VGLUT1, a marker of primary afferent sensory nerve endings used to identify muscle spindles in other muscle groups, and beta-tubulin III to identify and visualize laryngeal sensory structures. S46, GNAT3, PLCβ, S100b, and CGRP were used as corroborative markers to classify identified sensory structures. Extrinsic laryngeal muscle and leg muscles were used as positive controls.
Results: Using VGLUT1 staining, muscle spindles were rarely observed in the ILM and were identified only in the lateral thyroarytenoid muscle of three P8 animals. S46 staining demonstrated no muscle spindles in the ILM. In contrast, rich staining for VGLUT1, beta-tubulin III, and S100 was observed in the laryngeal mucosa overlying the arytenoid cartilages in all laryngeal specimens. In addition, VGLUT1-positive intramuscular receptor-like entities were observed in nearly all ILM samples. Finally, VGLUT1 was not found to colocalize with CGRP, GNAT3, PLCβ, ruling out nociceptive and gustatory functions for VGLUT1-positive structures.
Conclusion: The study findings suggest rat ILM rarely contain muscle spindles. The larynx may exhibit a non-canonical proprioceptive mechanism comprised of VGLUT1-positive nerve endings in the arytenoid mucosa and intramuscular receptors. A future study of laryngeal proprioception will further define the afferent arm identified here, providing insights required to advance treatment for VF disorders such as spasmodic dysphonia.

Disclosures: **I. Hernandez-Morato:** None. **V.X. Yu:** None. **S. Brenner-Morton:** None. **M. Pitman:** None.

Digital Abstract Session

P169. Spinal Circuits

Program #/Poster #: P169.06

Topic: D.02. Somatosensation

Title: Heat shock protein 90 inhibition in spinal cord enhances opioid anti-nociception by suppressing AMPK signaling

Authors: ***K. GABRIEL**;
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Abstract: Opioid misuse is a prevailing problem for chronic pain treatment that is accompanied by sideeffects such as constipation, tolerance, and addiction. Mu opioid receptor (MOR) signalmodulation has been heavily studied in an attempted to optimize opioids and to decrease theirside effects. Our lab has demonstrated an important role for the chaperone protein Heat shockprotein 90 (Hsp90) in regulating ERK MAPK signaling and pain relief downstream of the MOR.When 17-AAG, an Hsp90 inhibitor, is administered to the spinal cord (intrathecally, IT), there isan augmentation in morphine induced antinociception in mice. This signifies that Hsp90 acts torepress ERK MAPK signaling to reduce antinociception in the spinal cord. This led us to performquantitative proteomic analysis on the spinal cords of male and female CD-1 mice treated with0.5 nmol 17-AAG, in order to elucidate the signaling network that activates opioid anti-nociceptionin the spinal cord. Network analysis of this data revealed that 5'-AMP-activated protein kinasesubunit beta-1 (AMPK) was downregulated by 1.4-fold. Previous studies have implicated AMPKactivation as a target of interest in the opioid field because it seems to reduce or preventpathological pain. This result suggested that a reduction in AMPK activity could lead to enhancedopioid signaling and pain relief. We first tested this in female mice using the AMPK agonist AICAR(100 nmol, IT), which we found decreased 0.5 nmol 17-AAG's enhanced morphine (3.2 mg/kg,subcutaneous, SC) antinociception in the 52°C tail flick test, with no effect on the vehicle/morphinegroup. In contrast, AICAR not only decreased 17-AAG's enhanced antinociception, it alsodecreased morphine's effect in the vehicle group in male mice. Conversely, when using the AMPKinhibitor dorsomorphin (20 nmol, IT), we were able to see an enhancement of morphineantinociception greater than 17-AAG alone in female mice. Our data supports our hypothesis thatHsp90 inhibition in spinal cord enhances opioid pain relief by decreasing AMPK signaling. Thisleads to the enhanced opioid antinociception, which can be reversed when an AMPK agonist isadministered. We have thus elucidated a key molecular mechanism by which Hsp90 is regulatingopioid signaling in the spinal cord, which helps us stablish the basic science foundation of howHsp90 inhibition leads to enhanced opioid antinociception. We have also discovered a novelAMPK signal transduction role in the spinal cord downstream of acute opioid treatment that variesbetween male and female mice.

Disclosures: K. Gabriel: None.

Digital Abstract Session

P169. Spinal Circuits

Program #/Poster #: P169.07

Topic: D.03. Somatosensation – Pain

Support: The Alice and Joseph E. Brooks Funds
The Blavatnik Biomedical Accelerator Fund
NIH grant NS097344
NIH grant AT011447
NIH grant AR063772
NIH grant NS096705
the Bertarelli Foundation

Title: Parallel ascending spinal pathways for affective touch and pain

Authors: *S. CHOI[1], J. HACHISUKA[2], M. A. BRETT[1], A. R. MAGEE[1], H. R. KOERBER[2], S. E. ROSS[2], D. D. GINTY[1];
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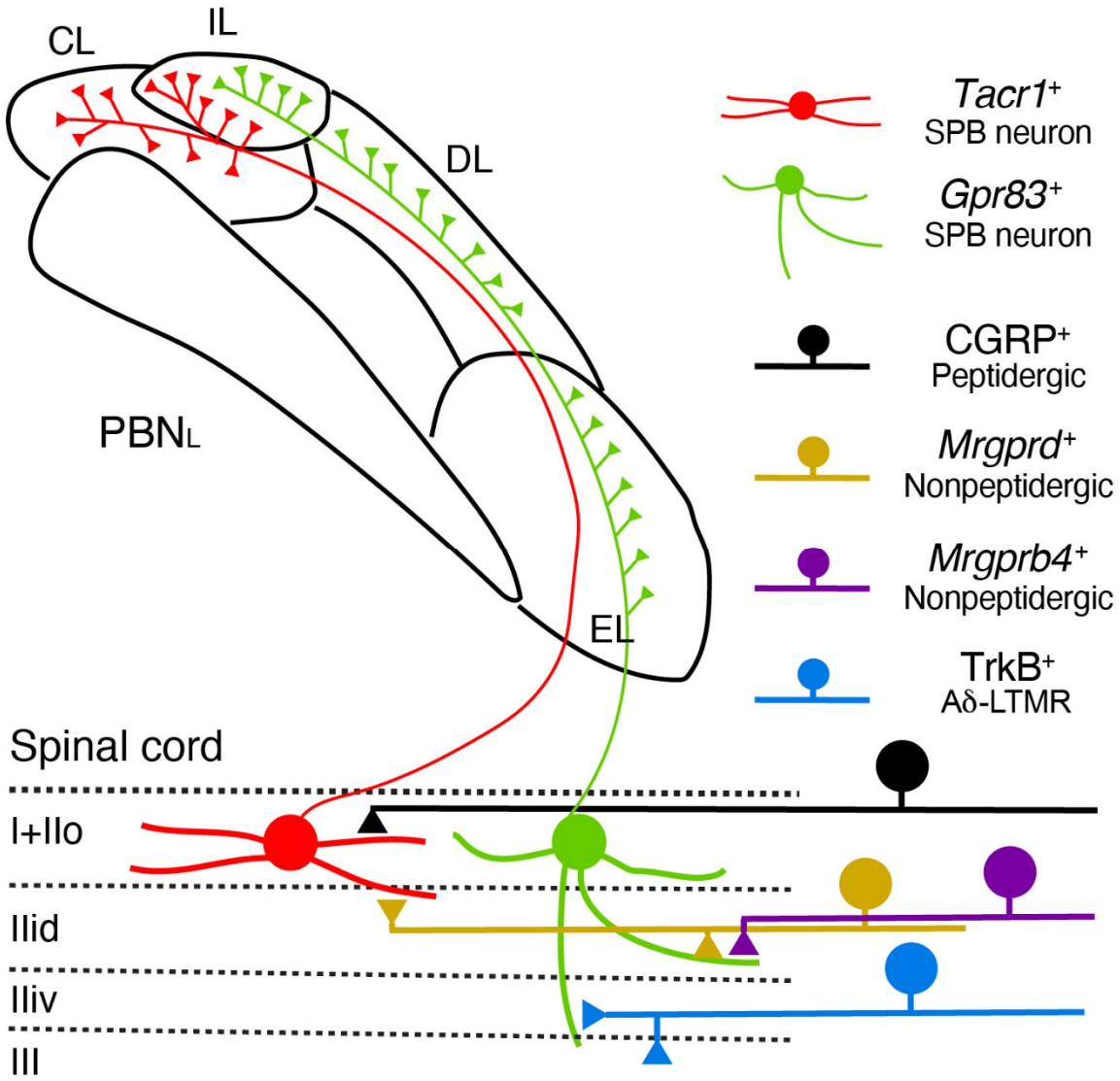
Abstract: Each day we experience myriad somatosensory stimuli: hugs from loved ones, warm showers, a mosquito bite, and sore muscles after a workout. These tactile, thermal, itch, and nociceptive signals are detected by peripheral sensory neuron terminals distributed throughout our body, propagated into the spinal cord, and then transmitted to the brain through ascending spinal pathways. Primary sensory neurons that detect a wide range of somatosensory stimuli have been identified and characterized. In contrast, very little is known about how peripheral signals are integrated and processed within the spinal cord and conveyed to the brain to generate somatosensory perception and behavioral responses. We tackled this question by developing new mouse genetic tools to define projection neuron (PN) subsets of the anterolateral pathway, a major ascending spinal cord pathway, and combining these new tools with advanced anatomical, physiological, and behavioral approaches. We found that *Gpr83*$^+$ PNs, a newly identified subset of spinal cord output neurons, and *Tacr1*$^+$ PNs are largely non-overlapping populations that innervate distinct sets of subnuclei within the lateral parabrachial nucleus (PBN_L) of the pons in a zonally segregated manner. In addition, *Gpr83*$^+$ PNs are highly sensitive to cutaneous mechanical stimuli, receive strong synaptic inputs from primary mechanosensory neurons, and convey tactile information bilaterally to the PBN_L in a non-topographically organized manner. Remarkably, *Gpr83*$^+$ mechanosensory limb of the anterolateral pathway controls behaviors associated with different hedonic values (appetitive or aversive) in a scalable manner. Our study reveals a dedicated spinal cord output pathway that conveys affective touch signals to the brain as well as parallel ascending circuit modules that cooperate to convey tactile, thermal and noxious cutaneous signals from the spinal cord to the brain. Furthermore, our study provides an

insight into the new therapeutic opportunities for developing treatments for neurological disorders associated with pain and affective touch.



Disclosures: **S. Choi:** None. **J. Hachisuka:** None. **M.A. Brett:** None. **A.R. Magee:** None. **H.R. Koerber:** None. **S.E. Ross:** None. **D.D. Ginty:** None.

Digital Abstract Session

P169. Spinal Circuits

Program #/Poster #: P169.08

Topic: D.09. Multisensory Integration

Support: Conacyt: CF1311312, ZFL 1001233.

Title: Effect of the avulsion of the lumbar ventral root on locomotion and afferent information of the spinal cord in the domestic rabbit

Authors: Z. FLORES-LOZADA[1], D. SÁNCHEZ[2], M. MARTÍNEZ-GÓMEZ[4], I. JÍMENEZ ESTRADA[5], R. ZEMPOALTECA RAMÍREZ[3], ***D. L. CORONA QUINTANILLA**[3];
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Abstract: In the spinal cord there are sensory-motor regulation nerve circuits that actively participate in maintaining posture or performing locomotion. In the spinal cord, specifically at the lumbosacral levels (L6-S2) are integrated somatic and visceral reflexes. After of spinal cord injury produced a muscular deterioration and the unrest in the sensation of balance. These injuries compress, stretch or break (avulsion) the roots of the spinal cord causing sensory, motor and autonomic dysfunctions. The separation of the nerve roots occurs as a result of the traction force on the spinal cord, when this injury occurs in the anterior root is called ventral root avulsion (VRA). The studies that exist on this injury have determined the poor survival of motor neurons. A first approach was to characterize the afferent information of the lumbosacral region during the electro-stimulation of five lumbosacral nerves in intact rabbits. For this purpose, 4 virgin rabbits of the Chinchilla breed (6 months old) were used. The females were anesthetized with 20% urethane (diluted in distilled water) and placed in a dorsal position to make a sagittal incision in the dorsal-caudal skin from segment L5 to S3. Under a stereomicroscope, a longitudinal incision was made in the dura mater to expose the dorsal roots. Each one of the roots was placed on a silver electrode to record the DRPs. Five nerves were identified from the lower left extremity. Electrical stimuli of variable intensity and voltage were applied to each of the nerves. In addition, to observe the changes produced by VRA, 4 virgin rabbits divided in 2 groups (n=2) were used: a) Sham and b) VRA. All the rabbits were anesthetized with ketamine and sodium pentobarbital. A sagittal incision was made in the dorsal skin from segment L5 to L7, the vertebral segments were discovered to locate the right ventral root of the medullary segment L6 under a stereomicroscope. The Sham group was only located the roots and sutured again. Meanwhile, the roots of the VRA group were located and with the help of an avulsion hook, one of the ventral roots of the right medullary segment L6 was broken. The results obtained show that at level S1, for the sciatic nerve, there is a greater amplitude and quantity of components (3 to 4); for the pudendal nerve the number of components decreases, showing records of only one or two waves. This may be due to the fact that the sciatic nerve is a large nerve and the amplitude of the DRPs is related to the degree of facilitation of axon excitability at the site of stimulus.

Disclosures: Z. Flores-Lozada: None. **D. Sánchez:** None. **M. Martínez-Gómez:** None. **I. Jímenez Estrada:** None. **D.L. Corona Quintanilla:** None. **R. Zempoalteca Ramírez:** None.

Digital Abstract Session

P170. Inflammatory Pain

Program #/Poster #: P170.01

Topic: D.03. Somatosensation – Pain

Support: NIH Grant 5R01AR069951

Title: The impact of neural activity on immune cell profile in the mouse colon

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[2]Dept. of Neurobio., [1]Univ. of Pittsburgh, Pittsburgh, PA; [3]Dept Neurobio., Univ. of Pittsburgh Dept. of Neurobio., Pittsburgh, PA

Abstract: Pain detecting sensory neurons are now recognized as contributors to the body's immune response under normal physiological and pathological conditions. In the colon, dysregulation of this communication is thought to contribute to the chronic and recurring inflammatory conditions of the gut evident in inflammatory bowel disorders (Crohn's and colitis). In this exploratory study we are using optogenetic strategies to probe how nerve activity impacts immune cell populations in the distal colon. Recent studies using pharmacological agonism and chemical ablation strategies have demonstrated the capacity of visceral nociceptors to sense bacteria and modulate the inflammatory response. These approaches provide new but limited insight into whether sensory afferents have a direct or indirect role in these processes. To address this issue, we are using mice that express the light-activated channelrhodopsin (ChR2) protein under control of the TRPV1 promoter. Targeted optogenetic activation of sensory afferents that innervate the distal colon is done *in situ* using a fiberoptic probe inserted into the distal colon. Colon tissue is then isolated from stimulated mice and analyzed using immunolabeling of sectioned and whole mount colon tissue to determine the relative number and location of MHCII+ antigen-presenting cells and CD3+ T cells in the colon tissue. Comparison of three groups of mice is being done: Group 1 mice express ChR2 in TRPV1+ neurons and receive optogenetic stimulation, Group 2 mice that express ChR2 in TRPV1+ neurons and but do not receive optogenetic stimulation and Group 3 mice are control mice that do not have ChR2 expression. Mice utilized for these experiments were on a C57/Bl6 background and were between 8-24 weeks old. Both male and female mice were included for analysis, but sex differences were not assessed. Mice were stimulated for two 30 min sessions over 6 hours prior to tissue collection. Initial results indicate that light stimulation of TRPV1+ colon afferents decreases the number of MHCII+ cells in the lamina propria of the colon with little or no change in CD3+ cell number. These studies were supported by NIH grant 5R01AR069951.

Disclosures: A.Y. Epouhe: None. **K.M. Albers:** None. **B.M. Davis:** None.

Digital Abstract Session

P170. Inflammatory Pain

Program #/Poster #: P170.02

Topic: D.03. Somatosensation – Pain

Support: NIH Grant DK124955
NIH Grant OT2-OD-023859

Title: The role of ExPAN to myenteric neuron communication in colon dysmotility following inflammation

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Abstract: Colitis is thought to lead to colon dysmotility by altering the intrinsic and extrinsic neural circuits of the enteric nervous system. The colon receives extrinsic primary afferent neuron (ExPAN) input from thoracolumbar (TL; T10-L2) and lumbosacral spinal levels (LS; L5-S1) and the nodose ganglion. ExPAN processes course throughout the myenteric ganglia of the enteric nervous system, where data suggests that they can have local effector functions through the release of glutamate and/or neuropeptides (e.g. CGRP, substance P) or act indirectly through autonomic reflexes. However, there is little known on how different ExPANs modulate myenteric activity, nor how this may change in inflammatory states. To determine the effects of inflammation on colon motility patterns, we recorded colonic migrating motor complexes (CMMC) in isolated colon preparations. To model colitis, we used 3% dextran sodium sulfate (DSS) for 5 days or vehicle treated male and female mice. To visualize activity in myenteric neurons, we used the calcium indicator GCaMP. We first established if colitis changes baseline myenteric neuron activity in an isolated colon preparation. Next, we used an *ex vivo* colon, pelvic nerve, and L6 dorsal and ventral root preparation to determine how colitis modulates LS ExPAN and/or parasympathetic efferent communication to myenteric neurons.This model of colitis caused a 21% increase in the frequency (p=0.03) and 20% increase in the speed of CMMC (p=0.02). Interestingly, we did not find changes in the percentage of myenteric neurons with spontaneous or evoked activity in isolated colons, suggesting that dysmotility after colitis may be due to changes in extrinsic populations that are also known to regulate motility (e.g. ExPANs, sympathetic, or parasympathetic input). To test this, we used our *ex vivo* preparation to electrically stimulate either the L6 dorsal or ventral root to activate the LS ExPANs or parasympathetic efferents, respectively. Previous work from our lab has shown ventral root stimulation activates myenteric neurons, but dorsal root stimulation does not activate myenteric neurons unless the spinal cord remains intact. We confirmed this result in our vehicle treated animals and tested whether ExPANs may be able to directly activate these cells after colitis. DSS treatment did not cause myenteric activation in response to dorsal root stimulation. Furthermore, we found no change in the percentage of responsive myenteric neurons to ventral root

stimulation after colitis. This suggests that the LS level may not be involved in the dysmotility seen after colitis leaving the possibility that a different neuraxis level (TL or nodose) may be involved.

Disclosures: **K.A. Meerschaert:** None. **K. Smith-Edwards:** None. **K.M. Albers:** None. **B.M. Davis:** None.

Digital Abstract Session

P170. Inflammatory Pain

Program #/Poster #: P170.03

Topic: D.03. Somatosensation – Pain

Support: NIH NIAMS Grant AR056019

Title: Uncovering the role of sleep and exercise in the transition from acute to persistent pain

Authors: D. M. KLYNE[1], B. A. HILLIARD[2], M. Y. HARRIS[2], M. AMIN[2], C. TESTA[2], L. J. HOBSON[2], G. E. CRUZ[2], P. W. HODGES[1], *M. F. BARBE[2];
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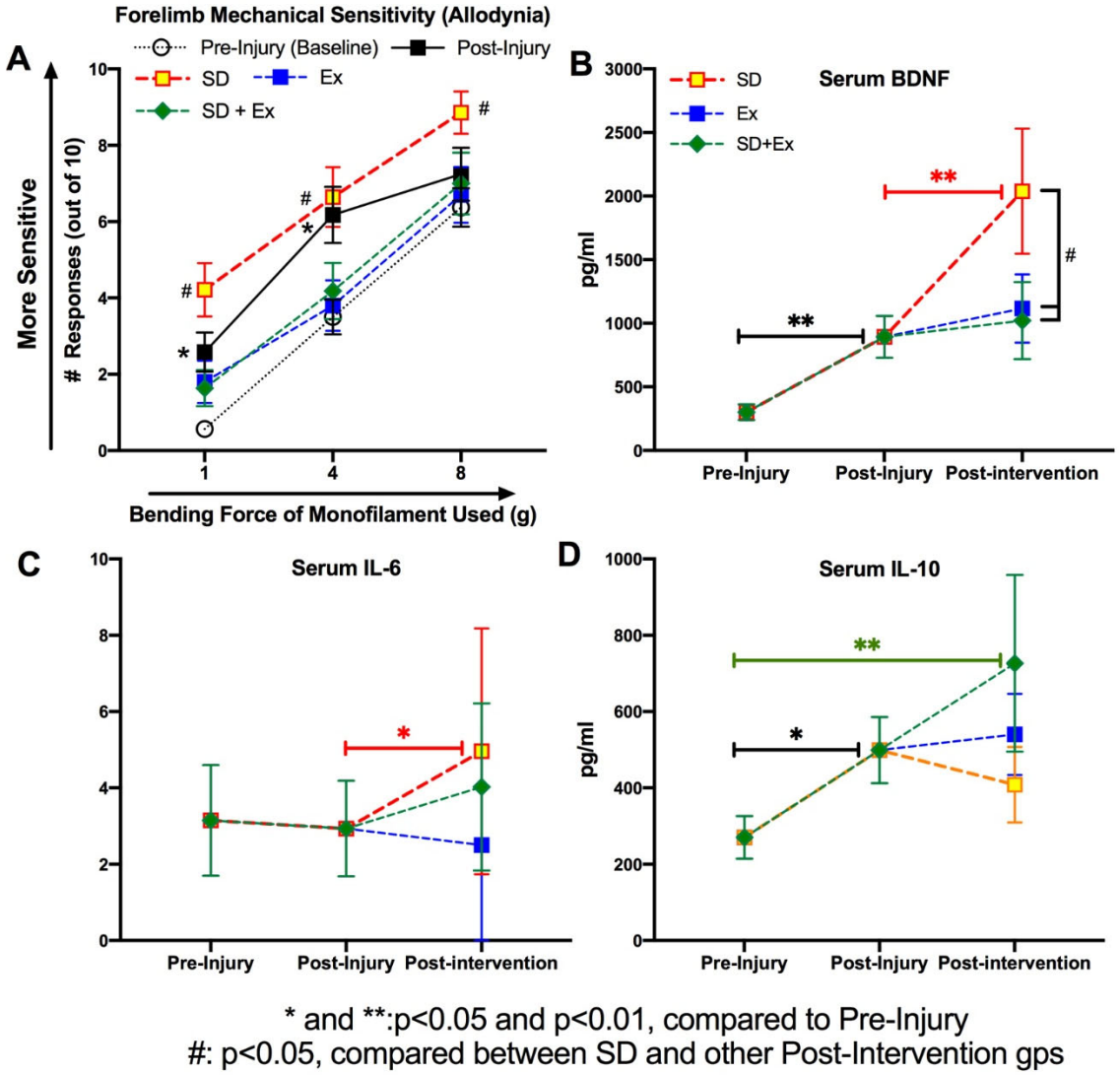
Abstract: Emerging evidence suggests that sleep is not only affected by pain, but also influences pain, with poor sleep increasing pain. The question remains, however, whether poor sleep contributes to pain chronicity. Exercise is thought to evoke pain relief via inflammation-reducing mechanisms, similar to those attributed to “healthy” sleep. This raises a further question as to whether exercise could protect against the negative effects of poor sleep. This study aimed to test these questions in a clinically relevant rat model of acute overuse-induced nerve injury. Twenty-five young adult female Sprague-Dawley rats performed a high-repetition high force lever-pulling task for 4 weeks. Rats were then split into 3 groups and exposed for 3 weeks to either: (1) voluntary exercise via access to a running wheel (from 1800-600, 5 d/wk, Ex), (2) sleep disturbance (from 600-1800, 4 random d/wk, SD), or (3) both (Ex+SD). Forepaw mechanical sensitivity was assessed by monofilament testing methods. Tail vein blood draws was performed, and serum assayed for inflammatory cytokines, brain-derived neurotrophic factor (BDNF) and corticosterone. Data were longitudinally compared between groups using mixed-effects models with repeated measures, followed by Tukey post-hoc tests (means and SEM are presented). Post-injury exercise was associated with forepaw hypersensitivity; this sensitivity improved with exercise with and without sleep disturbance, but worsened with sleep disturbance alone (Fig. 1A). With respect to serum biomarkers (Fig. 1B-D), sleep disturbance enhanced BDNF and interleukin-6 (IL-6), but suppressed the potent anti-inflammatory interleukin-10 (IL-10), relative to exercise with and without sleep disturbance. Corticosterone decreased with each intervention. The findings provide preliminary evidence for a role of poor sleep in the transition from acute to

chronic pain, and the potential for exercise to counter these sleep-induced effects. Changes in BDNF and inflammation may be a common underlying mechanism for these relationships.



Disclosures: **D.M. Klyne:** None. **B.A. Hilliard:** None. **M.Y. Harris:** None. **M. Amin:** None. **C. Testa:** None. **L.J. Hobson:** None. **G.E. Cruz:** None. **P.W. Hodges:** None. **M.F. Barbe:** None.

Digital Abstract Session

P170. Inflammatory Pain

Program #/Poster #: P170.04

Topic: D.03. Somatosensation – Pain

Support: NIH R01 DA041529

Title: Early life pain exposure alters fever response to adult immune challenge

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Abstract: Infants born prematurely are significantly more likely to be admitted to the Neonatal Intensive Care Unit (NICU) where they experience upwards of 10-18 painful procedures each day, often with no anesthesia or analgesia. Pre-clinical studies have shown that early exposure to pain disrupts the normal development of the CNS in multiple ways that persist into adulthood. Similar findings are reported clinically as well. The present study aims to explore the effects of neonatal injury on the response to an immune challenge in adulthood. Male and female rats were exposed to a short-term inflammatory insult induced by intraplantar administration of 1% carrageenan (CGN) on the day of birth (P0). In adulthood (P60-P90), rats were implanted with Thermicron iButtons to monitor core body temperature; 14 days later, rats were injected with lipopolysaccharide (LPS) to elicit an immune response. Animals were sacrificed after 24 hours or at their peak fever point and brain tissue collected for immunohistochemical analysis of VGAT, VGLUT, Fos and prostaglandin receptor within the median preoptic area (MnPO), a critical CNS site for the fever response. Analysis of LPS-induced febrile response at 24 hours show a trend in both males and females exposed to early life pain to have higher peak fever temperature than controls. Fever duration was also longer in pain exposed females. Immunohistological analysis of MnPO tissue is currently in progress. Together, these studies may elucidate a mechanism through which children experiencing unresolved pain during the perinatal period show an increased severity of sickness behavior and attenuated immune signaling.

Disclosures: **M.G. Gomez:** None. **C. Searles:** None. **L. Hanus:** None. **A.Z. Murphy:** None.

Digital Abstract Session

P171. Pain Imaging and Perception

Program #/Poster #: P171.01

Topic: D.03. Somatosensation – Pain

Support: NIH/NINDS K23 NS114178
Young Investigator Grant from the “Brain and Behavior Research Foundation”

Title: Validation of a custom-built thermal grill to generate the perception of pain in healthy human subjects

Authors: *R. M. CASTON, T. S. DAVIS, E. H. SMITH, J. D. ROLSTON;
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Abstract: The thermal grill illusion (TGI) is an experimental pain model consisting of interlaced warm and cool bars. The temperature difference between the bars increases the perceived illusory pain. However, bars that are too cold or hot conflates the pain generated solely from the

bar arrangement with thermal pain. Temperature perception also varies among individuals. To account for individual variation and maximize temperature difference between bars without causing thermal pain, we built a custom-thermal grill. We validated the illusion with a rigorous psychophysical paradigm. **Methods**: We recruited 20 healthy human participants between the ages of 18-65. We incorporated a tree-structured classification algorithm into our thermal grill software to determine each subject's hot pain threshold (HPT) and cold pain threshold (CPT). Thresholds were used to generate three temperature settings: 1) all the bars set 1°C < HPT, 2) all the bars 1°C > CPT, and 3) alternating warm and cool (TGI). Categories were randomly presented 15 +/- 0.4 times per subject for 10 seconds each. Subjects rated discomfort, pain, hot, and cold on a sliding scale from 0-10 (none to worst). Subjects were classified as responders if their TGI discomfort rating was different than both the cool and warm stimuli (Tukey-Kramer post hoc pairwise comparison, a=0.05). **Results**: Across all responders, a Kruskal-Wallis H test showed a difference in discomfort and for pain between the TGI, cool, and warm settings (discomfort: $X^2(2) = 19.14$, $p < 0.005$, mean rank score of 27.18 for TGI, 13.77 for cool, and 10.05 for warm; pain: $X^2(2) = 14.04$, $p < 0.005$, mean rank score of 23 for TGI, 14 for cool, and 14 for warm). Using a Tukey-Kramer post hoc pairwise comparison on the mean ranks (a=0.05), we found that for discomfort: the TGI was significantly different from cool ($p < 0.01$) and from warm ($p < 0.001$); similarly, for pain: the TGI was significantly different from cool ($p < 0.01$) and from warm ($p < 0.01$). The normalized pain rating for cool and warm was 0 +/- 0 and 1.03 +/- 0.38 for the TGI. We found that 11 subjects experienced the TGI. **Conclusion**: Here we report the validation of our novel thermal grill and psychophysical paradigm. All software and hardware schematics are open source and freely available to promote this classic psychological illusion's reproducibility. Our responder rate is slightly lower than previous reports, which may be due to our stringent responder criteria or because our protocol eliminates the conflating effects of thermal pain shown by none of our responders reporting warm or cool as painful. Our custom-built thermal grill and software can be used as an experimental paradigm to study human subjects' pain processing.

Disclosures: R.M. Caston: None. **J.D. Rolston:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NINDS K23 NS113178. **E.H. Smith:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Young Investigator Grant from the. **T.S. Davis:** None.

Digital Abstract Session

P171. Pain Imaging and Perception

Program #/Poster #: P171.02

Topic: D.03. Somatosensation – Pain

Support: CIHR Operating Grant MOP130555
CIHR Doctoral Research Award GSD164127

Title: Abnormal brainstem trigeminal fiber microstructure as a key feature of treatment non-response across trigeminal neuralgia subtypes: a DTI study

Authors: *S. TOHYAMA[1], M. R. WALKER[1], J. Y. ZHANG[1], J. C. CHENG[2], M. HODAIE[3]; [1]Krembil Res. Inst., Toronto, ON, Canada; [2]Stony Brook Univ. Sch. of Med., Stony Brook, NY; [3]Neurosurgery, Toronto Western Hosp., Univ. of Toronto, Toronto, ON, Canada

Abstract: Background & Aims: Trigeminal neuralgia (TN) is a highly disabling chronic neuropathic facial pain condition. TN is classified into several different subtypes based on the etiology and pathogenesis, including classical TN (CTN), TN secondary to multiple sclerosis (MS-TN), and TN associated with a solitary pontine lesion (SPL-TN). While the differing treatment response rates across the subtypes of TN is well-established, the rationale behind this spectrum is not well understood. Using diffusion tensor imaging (DTI), we aimed to determine whether alterations in the brainstem trigeminal fibers are associated with surgical response in TN and whether the degree of microstructural alterations predicts the likelihood of response across subtypes of TN. **Methods**: We retrospectively studied 98 TN patients (39 males and 59 females, mean age +/- SD: 58.3 +/- 13.8 years), consisting of 61 CTN, 26 MS-TN, and 11 SPL-TN patients. All patients underwent neurosurgical treatment for TN and based on clinical follow-up data, were identified as responders or non-responders. Responders were those that achieved greater than 75% pain relief at 12 months post-treatment. Each patient underwent a 3 Tesla MRI session before treatment to acquire a T1-weighted anatomical scan and a 60 directions diffusion-weighted imaging scan. Diffusivity metrics of fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD) were obtained from the proximal brainstem segment of the trigeminal nerve pathway. Age- and sex-matched healthy individuals and the contralateral, unaffected side served as controls. **Results**: Overall, treatment non-responders (n=47) showed significantly decreased FA and AD, and increased RD in the brainstem trigeminal fiber microstructure, compared to responders (n=51) and healthy controls (n=50). There was also a statistically significant difference between the subtypes of TN non-responders for FA, MD, and RD on the affected side. SPL-TN non-responders (n=10) demonstrated the greatest degree of microstructural alterations, characterized by decreased FA, and increased MD and RD, followed by MS-TN non-responders (n=15), and CTN non-responders (n=22). No significant diffusivity differences were observed for the responder subgroups of CTN (n=39) and MS-TN (n=11). **Conclusions**: We find that abnormal brainstem trigeminal fiber microstructure is a key feature of treatment non-response in TN. Furthermore, the degree (or extent) of these alterations predicts the likelihood of surgical response across the different types of TN. Thus, DTI may serve as an important tool in the clinical setting to aid treatment decision making.

Disclosures: S. Tohyama: None. **M.R. Walker:** None. **J.Y. Zhang:** None. **J.C. Cheng:** None. **M. Hodaie:** None.

Digital Abstract Session

P171. Pain Imaging and Perception

Program #/Poster #: P171.03

Topic: D.03. Somatosensation – Pain

Support: DFG PL 321/10-2
DFG PL 321/11-2
Studienstiftung des Deutschen Volkes

Title: Modulating Brain Rhythms of Pain using Transcranial Alternating Current Stimulation (tACS)

Authors: E. MAY[1], V. D. HOHN[1], M. M. NICKEL[1], L. TIEMANN[1], C. GIL AVILA[1], H. HEITMANN[1], P. SAUSENG[2], *M. PLONER[1];
[1]TU Muenchen, Munich, Germany; [2]LMU, Munich, Germany

Abstract: Pain protects the body. However, pain can also occur for longer periods without serving protective functions. Such chronic pain conditions are associated with substantial changes of brain structure and function and are difficult to treat. Thus, a better understanding of the underlying brain mechanisms and new approaches for the treatment of pain are urgently needed. Here, we therefore investigated a causal role of oscillatory brain activity for pain and explored the potential of transcranial alternating current stimulation (tACS) as a new treatment approach for pain. To this end, we investigated whether tACS can modulate pain and pain-related autonomic activity in 29 healthy human participants using a tonic heat pain paradigm as an experimental model of chronic pain. In 6 recording sessions, participants received tACS over prefrontal or somatosensory cortices at alpha or gamma frequencies or sham tACS. During tACS, pain ratings and autonomic responses were collected. Using the present setup, tACS did not modulate pain intensity, the stability of pain ratings or the translation of the noxious stimulus into pain. Likewise, tACS did not change autonomic responses. Bayesian statistics further indicated a lack of tACS effects in most conditions. The only exception was alpha tACS over somatosensory cortex where evidence for tACS effects was inconclusive. Taken together, the present study did not find significant tACS effects on tonic experimental pain in healthy human participants. However, considering the conceptual plausibility of using tACS to modulate pain and the urgent need for novel pain treatments, further tACS studies are warranted. Based on the present and previous findings, such studies might apply refined stimulation protocols targeting alpha oscillations in somatosensory cortices.

Disclosures: **E. May:** None. **V.D. Hohn:** None. **M.M. Nickel:** None. **L. Tiemann:** None. **C. Gil Avila:** None. **H. Heitmann:** None. **P. Sauseng:** None. **M. Ploner:** None.

Digital Abstract Session

P171. Pain Imaging and Perception

Program #/Poster #: P171.04

Topic: D.03. Somatosensation – Pain

Support: R01 DK099611
T32HD057850

Title: Reduced left hippocampal volume and decreased hippocampal n-acetylaspartate post acute stress in a mouse model of early life stress: an magnetic resonance study

Authors: *A. D. BRAKE[1], X. YANG[1], C.-Y. LEE[2], P. LEE[2,3], P. KESELMAN[2], O. ELLER[1], I.-Y. CHOI[2,4], J. HARRIS[2,1], J. CHRISTIANSON[1];
[1]Dept. of Anat. and Cell Biol., [2]Hoglund Biomed. Imaging Ctr., [3]Radiology, [4]Neurol., Univ. Of Kansas Med. Ctr., Kansas City, KS

Abstract: Early life stress (ELS) exposure is a significant risk factor for developing chronic pain syndromes, psychiatric disorders including depression and anxiety, and metabolic disorders later in life. Dysregulation of the hypothalamic pituitary adrenal (HPA) axis has been shown to contribute to these outcomes. The hippocampus is negative limbic regulator of the HPA axis and is sensitive to increased glucocorticoids during early development. We have previously described that ELS in mice, in the form of neonatal maternal separation (NMS), results in suppressed hippocampal neurogenesis, dysregulated corticosterone production, metabolic syndrome, and urogenital hypersensitivity. This study aimed to determine whether NMS mice have decreased hippocampal volumes using magnetic resonance imaging (MRI), similar to what has been observed in patients with depression and/or a history of ELS. We also measured the spectral contents of the hippocampus prior to and after an acute stress exposure (water avoidance stress) using magnetic resonance spectroscopy (MRS). In order to determine if metabolic changes contribute to these observations, body weight was correlated with hippocampal spectral contents after WAS. NMS was performed by separating entire litters for 3 hours/day from postnatal day (P) 1 to P21. At nine months of age, body weight and composition of naïve (n=5) and NMS (n=9) female mice was assessed using qMRI. Mice then underwent MRI and MRS both prior to and 1 day after a 1h exposure to water avoidance stress (WAS). Whole brain volumetric analysis was performed using Voxel Base Morphometry. MRS was analyzed in a single voxel over the right hippocampus and MRS analysis was performed with LCModel software. The left hippocampus of NMS mice was 0.038 mm^3 smaller compared to naïve mice ($p<0.005$). No significant differences in hippocampal spectral contents were observed between naïve and NMS mice prior to WAS. However, at 1 day after WAS, NMS mice showed significantly lower N-acetyl-aspartate (NAA), pCreatine, and pCholine, and a trend toward decreased glutathione levels, compared to naïve mice. NMS mice showed a trend toward increased body weight and body fat percentage compared to naïve mice. Interestingly, a significant negative correlation was observed between body weight and pCreatine content post-WAS in NMS mice, as well as a positive correlation between body weight and Glutamine for both naïve and NMS mice.

Together, these data suggest that NMS in mice reduces left hippocampal volume and disrupts stress-induced neurochemical responses in adulthood, which may also be related to whole body metabolic changes.

Disclosures: **A.D. Brake:** None. **X. Yang:** None. **C. Lee:** None. **P. Lee:** None. **P. Keselman:** None. **O. Eller:** None. **I. Choi:** None. **J. Harris:** None. **J. Christianson:** None.

Digital Abstract Session

P171. Pain Imaging and Perception

Program #/Poster #: P171.05

Topic: D.03. Somatosensation – Pain

Support: NIH-NIDCR grant DE0027808 (JYR)
NIH-NIA grant AG053783 (JYR)

Title: Pain modulatory network is influenced by sex and age in a healthy state and during osteoarthritis progression in rats

Authors: ***J. DA SILVA**[1,2], Y. ZHANG[1], A. TOFIGHBAKHSH[1], D. SEMINOWICZ[1], J. Y. RO[1];
[1]Neural and Pain Sci., Univ. of Maryland Baltimore, Baltimore, MD; [2]Dept. of Psychiatry, Johns Hopkins Univ., Baltimore, MD

Abstract: Pain modulatory network is influenced by sex and age in a healthy state and during osteoarthritis progression in rats.Joyce T. Da Silva [1,2]*, Youping Zhang[1], Amir Tofighbakhsh[1], David A. Seminowicz[1x], Jin Y. Ro[1x].
[1] Department of Neural and Pain Sciences, School of Dentistry, University of Maryland Baltimore, Baltimore, United States of America. [2] Department of Psychiatry, School of Medicine, Johns Hopkins University, Baltimore, United States of America.[X] Senior authors.
Old age and female sex are risk factors for the development of osteoarthritis (OA) and chronic pain. We investigated the effects of sex and age on pain modulatory networks in a healthy state and during OA progression. We used functional MRI to determine the effects of sex and age on periaqueductal gray functional connectivity (PAG FC) in a healthy state (pre-OA) and during the early and late phases of monosodium iodoacetate (MIA)-induced OA in male and female Fischer 344 rats (young, 3-6 mo / old, 20-24 mo). For functional MRI, rats were scanned using a Bruker 7T MRI (TR = 1500 ms, in plane resolution = 400 μm, slice thickness 1 mm, acquisition time: 15 minutes) and under isoflurane anesthesia ≤ 1.5%. We then examined how sex and age affect longitudinal changes in PAG FC in OA. In a healthy state, females exhibited more widespread PAG FC than males, and this effect was exaggerated with aging. Young males had moderate PAG FC changes during the early phase but recruited additional brain regions, including the rostral anterior cingulate cortex (ACC), during the late phase. Young females exhibited widespread PAG FC in the early phase, which includes connections to insula, caudal ACC, and nucleus accumbens (NAc). Older groups had strong PAG FC with fewer regions in the early

phase, but they recruited additional brain regions, including NAc, in the late phase. Overall, our findings show that PAG FC is modulated by sex and age in a healthy state. A widespread PAG network in the early phase of OA pain may contribute to the transition from acute to chronic OA pain and the increased risk of developing chronic pain for females. Enhanced PAG FC with the reward system may represent a potential mechanism underlying chronic OA pain in elderly patients.

Disclosures: J. Da Silva: None. **Y. Zhang:** None. **A. Tofighbakhsh:** None. **D. Seminowicz:** None. **J.Y. Ro:** None.

Digital Abstract Session

P171. Pain Imaging and Perception

Program #/Poster #: P171.06

Topic: D.03. Somatosensation – Pain

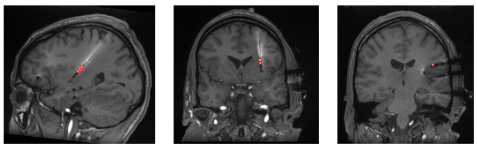
Support: FNRS (Belgium)

Title: Modulations of ongoing oscillations exerted by sustained painful heat and non-painful cold stimuli recorded from the human insula using intracerebral EEG

Authors: *G. LIBERATI[1], D. MULDERS[1], L. LEBRUN[1], A. COURTIN[1], S. FERRAO SANTOS[2], J.-G. RIBEIRO VAZ[2], C. RAFTOPOULOS[2], A. MOURAUX[1];
[1]Univ. catholique de Louvain, Brussels, Belgium; [2]St. Luc Univ. Hosp., Brussels, Belgium

Abstract: The human insula is a target for spinothalamic input, but there is still no consensus on its role in pain perception. Using intracerebral electroencephalography (iEEG), we recently observed that sustained periodic warm nociceptive stimuli selectively modulated theta- and alpha-band ongoing oscillations recorded from the insula. These modulations, however, are not necessarily pain-specific, as they could be simply related to the activation of the spinothalamic system, and/or to the conveyance of thermal information. The aim of the present study was to disentangle these aspects by comparing the effects of sustained periodic painful heat stimulation and sustained periodic non-painful cold stimulation on insular ongoing oscillatory activity.
6 patients undergoing iEEG (34 insular contacts) participated in the experiment (Fig. 1). Each patient received 2 blocks of sustained periodic stimuli (painful heat and non-painful cold stimuli; frequency: 0.2 Hz). The order of the blocks was randomized across participants.
Whereas both heat and cold stimuli elicited an increase of EEG power at the frequency of stimulation (0.2 Hz), only heat stimuli exerted a 0.2 Hz modulation of ongoing oscillations in the theta frequency band (Fig. 2). These findings suggest that the modulation of theta oscillations exerted by painful heat stimuli is not merely related to the activation of the spinothalamic system and/or to the conveyance of thermal information, but could be more strongly related to pain perception.

Patient 1



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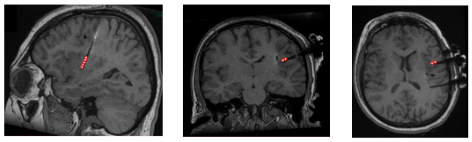

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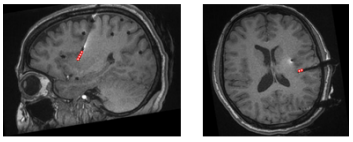

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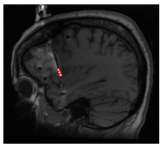

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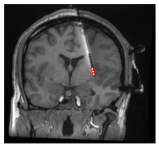

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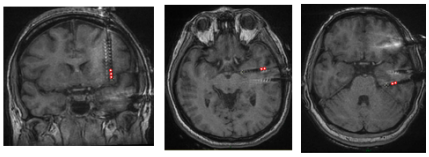

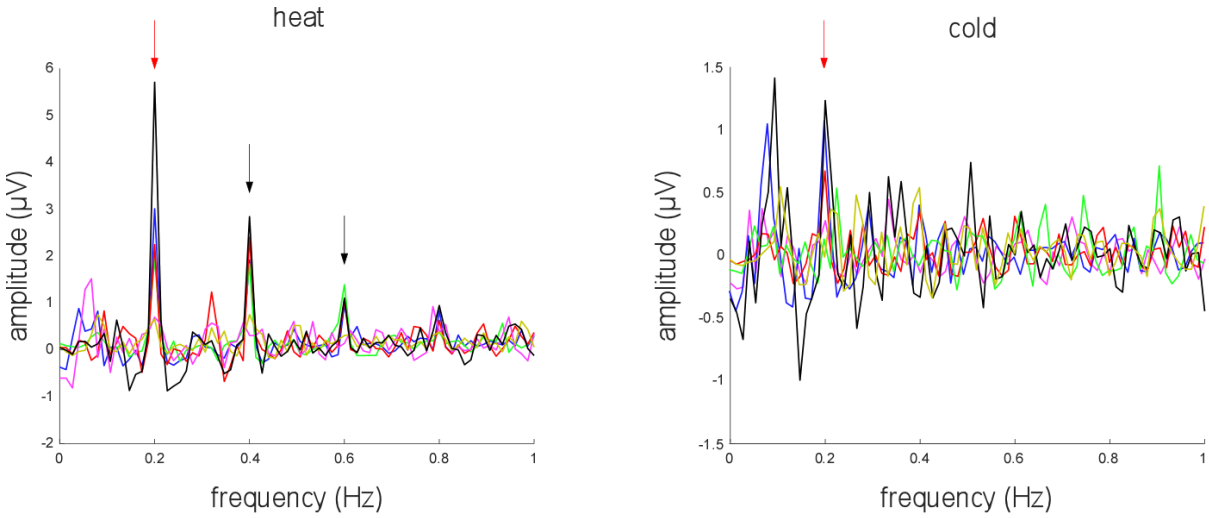

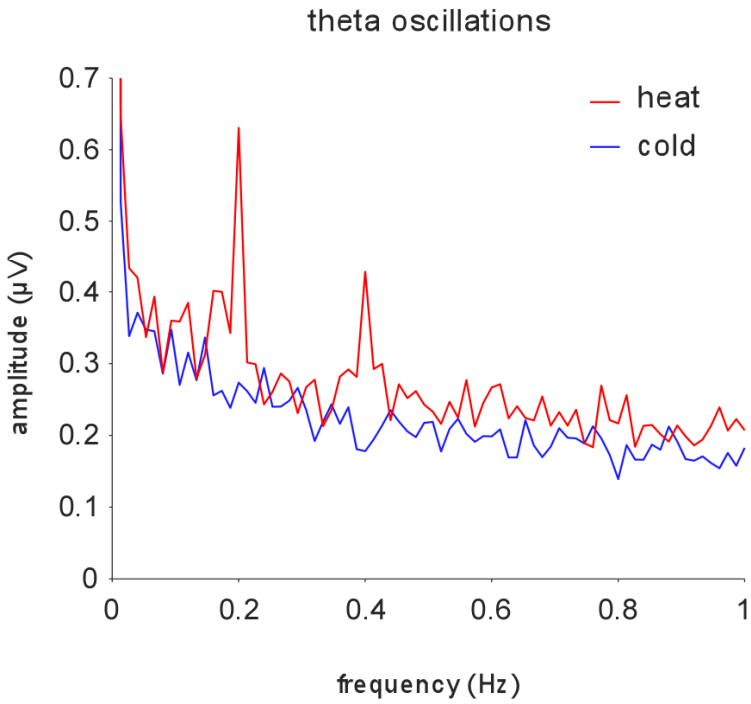

Disclosures: **G. Liberati:** None. **D. Mulders:** None. **L. Lebrun:** None. **A. Courtin:** None. **S. Ferrao Santos:** None. **J. Ribeiro Vaz:** None. **C. Raftopoulos:** None. **A. Mouraux:** None.

Digital Abstract Session

P172. Opioids

Program #/Poster #: P172.01

Topic: D.03. Somatosensation – Pain

Support: Arizona Biomedical Research Commission New Investigator Award #ADHS18-198875

Title: The Hsp90 Isoform-Selective Inhibitor KUNG65 Enhances Opioid Anti-Nociception while Reducing Tolerance

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Abstract: Opioids are the gold standard for pain management, however, adverse side effects like respiratory depression and addiction limit their viability. One approach to improve opioid therapy focuses on a dose-reduction strategy by amplifying opioid analgesia without boosting side effects, so that less opioid can be given. To this end, we've focused on Heat shock protein 90 (Hsp90), a central protein regulator in the cell; ongoing studies from our lab have shown that inhibition of Hsp90 in the spinal cord improves morphine anti-nociceptive potency by 2-3 fold in acute and chronic pain while reducing tolerance, rescuing established tolerance, and not changing reward and constipation, enabling a dose-reduction strategy. However, our results also showed that non-selective Hsp90 inhibitors given systemically (intravenous, IV) have the opposite effect, blocking opioid pain relief. Seeking a means to surmount this roadblock, we determined which Hsp90 isoforms regulate opioid pain relief in brain and spinal cord in mice using selective small molecule inhibitors and CRISPR/Cas9 gene editing. We found that Hsp90α alone regulated opioid signaling and pain relief in the brain. However, in the spinal cord,we found that the isoforms Hsp90α, Hsp90β, and Grp94all regulated opioid signaling and pain relief. This led to our hypothesis that targeting spinal cord Hsp90 with isoform-selective inhibitors given IV could boost opioid pain relief without altering side effects, enabling a dose-reduction strategy. We tested this hypothesis with the novel Grp94-selective inhibitor KUNG65, given at a 1mg/kg dose IV in male and female CD-1 mice, followed by a 24hr treatment time, then analysis of opioid anti-nociception and side effects. We found that systemic (IV) KUNG65 treatment resulted in a 1.9 fold increase in morphine potency to relieve tail flick pain, consistent with our earlier studies injecting inhibitor directly into spinal cord. We also found that KUNG65 boosted morphine efficacy in paw incision pain, with dose/response analysis in progress. Additionally, we found that KUNG65 injection could rescue established morphine tolerance in the tail flick assay, again as we found for direct spinal cord injection. These results support our hypothesis that isoform-selective Hsp90 inhibitors can selectively engage Hsp90 in the spinal cord when given systemically, resulting in improved opioid anti-nociception and side effects. These results strongly suggest that Hsp90 isoform-selective inhibitors could be a powerful new tool to improve opioid therapy through a dose-reduction strategy, and further show that this effect can be achieved through a translationally relevant dosing route.

Disclosures: J.M. Streicher: None. **S. Mishra:** None. **B.S.J. Blagg:** None.

Digital Abstract Session

P172. Opioids

Program #/Poster #: P172.02

Topic: D.03. Somatosensation – Pain

Support: NIH R01DA041529

Title: The impact of advanced age and sex on Mu Opioid Receptor signaling in the midbrain periaqueductal gray: implications on analgesia

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Abstract: Chronic pain is exceedingly prevalent in individuals over 65 years of age, but is under-managed in this population due in large part to a dearth of knowledge regarding the impact of age on opioid efficacy for chronic pain management in the elderly. We have previously shown that advanced age and sex alter morphine modulation of persistent inflammatory pain (induced by intraplantar administration of Complete Freund's adjuvant (CFA)), such that morphine potency is highest in adult male rats (2mos), with EC_{50} values 2-fold higher in aged males (18mos) and females regardless of age. Age induced changes in morphine EC_{50} values were accompanied by a reduction in mu opioid receptor (MOR) expression in the ventrolateral periaqueductal gray (vlPAG), a CNS region critical in pain modulation. The present studies further explore the impact of sex and age on opioid signaling within the PAG. MOR affinity, availability, and G-protein activation were assessed using radioligand binding assays and GTPγS assays in vlPAG tissue from adult and aged, male and female rats collected 72h following CFA administration. Regulation of opioid induced G-protein signaling was assessed using RNAscope to analyze mRNA expression of Regulator of G-Protein Signaling (RGS) family members RGS4 and RGS9-2. We find that aged males and females (adult and aged) exhibit reduced vlPAG MOR binding potential (radioligand binding assay) and reduced G-protein activation efficiency (GTPγS assay) compared to adult males, suggesting that age- and sex- induced alterations in MOR machinery contribute to reduced opioid potency. Our analyses of RGS mRNA revealed increased expression of RGS4 and RGS9-2 in the vlPAG of aged animals compared to adults, indicating that MOR signaling is subject to greater negative regulation in the aged vlPAG. The observed age-related reductions in vlPAG MOR expression, MOR agonist binding, and opioid induced G-protein activation, along with the observed increase in vlPAG RGS4 and RGS9-2 have significant implications in pain management in the aged population. Our novel findings elucidate several mechanisms responsible for reduced morphine potency in aged animals, and identify potential therapeutic targets to improve pain management in the elderly.

Disclosures: E.F. Fullerton: None. **M.C. Karom:** None. **M. Rubaharan:** None. **J.M. Streicher:** None. **A.Z. Murphy:** None.

Digital Abstract Session

P172. Opioids

Program #/Poster #: P172.03

Topic: D.03. Somatosensation – Pain

Title: The Src kinase signaling pathway: a potential regulator of intermittent fasting-induced enhancement in morphine antinociception

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Abstract: The opioid epidemic is a public health crisis in the United States, marked by high levels of abuse and poor quality of life for chronic pain patients requiring continuous use of opioids. In an effort to find a non-pharmacological alternative or adjunct therapy to opioid medication, we previously showed that daily intermittent fasting (IF) in mice improved morphine efficacy in acute and chronic pain, while mitigating reward, tolerance and constipation. The present study aimed to characterize the molecular mechanism by which IF enhances morphine-induced antinociception. Male and female CD-1 mice were subjected to 7 days of IF or *ad libitum* control (AL), with daily 18-hour periods of fasting, followed by 6-hour periods of feasting on standard chow. Spinal cords were harvested from male and female mice, and protein extraction was performed. Using quantitative proteomic analysis, we found that the expression levels of 654 proteins were significantly altered in IF mice compared to AL controls. In the present study, we chose to examine the role of Src Kinase Signaling Inhibitor 1 (SRCIN1) in modulating IF effects. SRCIN1 protein expression was downregulated 32% by IF, suggesting an increase in Src signaling. We thus subjected male and female CD-1 mice to 7 days of the aforementioned IF regimen, then treated them with the Src kinase inhibitor Src-1I (10 nmol, intrathecal injection) or Vehicle, 10 minutes, followed by morphine (3.2 mg/kg, subcutaneous injection), and assessed antinociception using the thermal tail flick assay. The previously seen IF-induced increase in thermal latency was eliminated by Src inhibition in male mice, and this effect was less pronounced in females. These data suggest that the antinociceptive effects of IF are promoted by Src kinase signaling, with a potential sex difference in the molecular mechanism of IF. Further discoveries in this mechanism could result in the development of improved pain management therapies, as well as uncover novel molecular circuits linking IF to changes in opioid antinociception.

Disclosures: F. Hanak: None. **P. Langlais:** None. **J.M. Streicher:** None.

Digital Abstract Session

P172. Opioids

Program #/Poster #: P172.04

Topic: D.03. Somatosensation – Pain

Support: NIH K01 DA042902

Title: Loss of SUR1 subtype K_{ATP} channels alters antinociception and locomotor activity after opioid administration

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Abstract: There are several classes of opioids targeting the mu opioid receptor, each with various functional selectivity of downstream pathways including K_{ATP} channels. As part of an on-going investigation into differences between synthetic, semi-synthetic, and non-synthetic opioids and their downstream effects on potassium channel signaling, we hypothesize there are differences in K_{ATP} channel activation between buprenorphine, fentanyl, and [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO). To test this hypothesis, male and female mice lacking the SUR1-K_{ATP} channel subunit (SUR1 KO) were behaviorally tested against wild-type (SUR1 WT) using modified Hargreaves and von Frey methods to measure thermal and mechanical antinociception, respectively. Latency and force measurements were taken before and after drug injection (DAMGO, 10 mg/kg; fentanyl, 0.25 mg/kg; buprenorphine, 5.83 mg/kg) at 3 min., 15 min., 30 min., 45 min., 60 min., and 120 min., post subcutaneous injection. Potassium channel flux was measured using a fluorescence intensity plate reader in cultured primary dorsal root ganglia and dorsal horn cells in SUR1 KO and WT mice. In addition, we also tested if loss of SUR1 would affect locomotor activity of mice administered buprenorphine, fentanyl, or DAMGO at the same doses tested for thermal/mechanical antinociception. Mice with a global loss of SUR1 have significantly attenuated thermal and mechanical paw withdrawal latencies/forces compared to SUR1 WT mice. Potassium flux decreased in after fentanyl and buprenorphine exposure in cultured dorsal root ganglia and spinal cord neurons from SUR1 KO mice compared to WT mice. Hyperlocomotion was significantly attenuated in SUR1 KO mice after buprenorphine administration, similar to morphine, but was not affected after fentanyl or DAMGO administration. We conclude that there are differences in K_{ATP} channel dependent activity between fentanyl, buprenorphine, and DAMGO from our behavioral assays and fluorescence intensity data. Future experiments will possibly investigate the K_{ATP} channel contribution of analgesia in the central nervous system, utilizing tissue cultures from higher order brain regions

Disclosures: A.H. Klein: None. **G. Sakamaki:** None. **K. Johnson:** None. **M. Mesinger:** None. **E. Hmu:** None.

Digital Abstract Session

P172. Opioids

Program #/Poster #: P172.05

Topic: D.03. Somatosensation – Pain

Support: NIH Grant K01DA042902

Purdue University College of Pharmacy

Title: Intrathecal Knockdown of Adenylyl Cyclase 1 Attenuates Morphine Tolerance and Withdrawal in Mice

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Abstract: Opioids are clinically used to alleviate pain, accomplishing analgesia by inhibiting neuronal signal transmission. Chronic opioid use for pain management leads to tolerance and dosage escalation, potentially increasing substance dependence. Opioid tolerance and opioid induced hyperalgesia during repeated opioid administration and chronic pain are associated with upregulation of adenylyl cyclase activity. The objective of this study was to test the hypothesis that a reduction in adenylyl cyclase 1 (AC1) activity would attenuate morphine tolerance and hypersensitivity, and inflammatory pain. Mice were subjected to twice daily treatments of saline, 15 mg/kg morphine, for 5 days. Trigeminal ganglia, dorsal root ganglia, sciatic nerves, spinal cord, and brain stem were collected from mice in each treatment group for Quantitative PCR. mRNA expression of adenylyl cyclase (Adcy) 1, 2, 3, 5, 6, 8; Epac 1 (Rapgef 3) and Epac 2 (Rapgef 4); and PKA subunits A and B (Prkaca and Prkacb) were compared to controls. Adcy1 was upregulated in the spinal cord, dorsal root ganglia, and sciatic nerves of morphine tolerant mice. Short-hairpin RNA (shRNA) gene knockdown of Adcy1 in the spinal cord and dorsal root ganglia was accomplished using a lumbar injection of an associated adenovirus viral vector (AAV9-GFP-U6-m-Adcy1-shRNA) and negative controls (AAV9-GFP-U6-m-scrambl-shRNA). Behavioral testing such as open field testing, rotarod testing, burrowing, thermal and mechanical paw withdrawal latencies were tested after injection. Morphine tolerance (15mg/kg, sc, 5 days and 10-40mg/kg escalation over 4 days) and opioid-induced hypersenstivity were also assessed after inoculation. Lumbar intrathecal administration of a vector incorporating short-hairpin RNA against Adcy1 did not affect baseline parameters such as open field testing, rotarod testing, and burrowing testing, nor did it affect C-fiber compound action potentials. Pharmacological inhibition of AC1, also attenuated morphine induced hypersensitivity. Morphine tolerance and withdrawal were attenuated in Adcy1 shRNA mice compared to control vector mice. Gene knockdown of Adcy1 decreases morphine tolerance and opioid-induced hypersensitivity, which could form the basis for novel therapeutics in the future.

Disclosures: **A. Doucette:** None. **K. Johnson:** None. **V. Watts:** None. **A.H. Klein:** None.

Digital Abstract Session

P172. Opioids

Program #/Poster #: P172.06

Topic: D.03. Somatosensation – Pain

Support: Health Research Council of New Zealand

NIH Grant DA018151
NIH Grant GM001385

Title: Investigating the role of G-protein bias in the antinociceptive and side effect profiles of two novel mu opioid receptor agonists kurkinorin and kurkinol.

Authors: A. F. ALDER[1], S. KASKA[2], D. LUO[2], T. E. PRISINZANO[2], ***B. M. KIVELL**[1]; [1]Victoria Univ. of Wellington, Wellington, New Zealand; [2]Univ. of Kentucky, Lexington, KY

Abstract: Current pain medications that activate mu-opioid receptors (MOPr) have high abuse potential and overdose risk due to respiratory depressive effects. One mechanism that has been proposed to generate safer MOPr agonists for the treatment of pain is the development of G-protein biased agonists. Here we evaluate two novel MOPr agonists with different G-protein signalling bias properties, in preclinical models of pain and side-effects to determine whether G-protein bias correlates to improved side effects. We found that both kurkinorin (a weak G-protein biased MOPr agonist), and kurkinol (a strong G-protein biased MOPr agonist) had a similar duration of action to morphine in the tail-withdrawal and hotplate anti-nociceptive assays in male and female C57BL/6J mice. We also show that G-protein bias correlated to reduced tolerance in the tail-withdrawal assay of acute nociception. Furthermore, the antinociceptive potency and tolerance of kurkinorin and morphine were reduced in male β-arrestin2 knockout mice in the tail-flick anti-nociceptive assay, confirming that anti-nociceptive tolerance is β-arrestin2 dependent in this assay. In contrast, in the chemotherapy-induced model of neuropathic pain, tolerance to kurkinol and kurkinorin developed at the same rate as morphine. The strong G-protein biased MOPr agonist Kurkinol induced similar respiratory depression effects in whole-body plethysmography tests as morphine. Kurkinol, but not kurkinorin induced constipation in assays of small intestine transit time and faecal accumulation. Kurkinol showed increased motor coordination impairment compared to the less bias kurkinorin in the accelerating rotarod assay. Moreover, the respiratory depressive and constipation effects of kurkinol were not reversed in β-arrestin2 knockout mice, indicating that both respiratory depression and gastrointestinal effects of MOPr agonists act via G-protein signalling pathways. In conclusion, we have identified that kurkinorin, a weakly G-protein biased MOPr agonist induces antinociception with reduced respiratory and gastrointestinal side effects, whereas kurkinol, the highly selective G-protein biased MOPr agonist induced potent antinociceptive effects with a worse side effect profile, indicating an inverse relationship between G-protein bias and side effect profile.

Disclosures: B.M. Kivell: None. **A.F. Alder:** None. **D. Luo:** None. **S. Kaska:** None. **T.E. Prisinzano:** None.

Digital Abstract Session

P172. Opioids

Program #/Poster #: P172.07

Topic: D.03. Somatosensation – Pain

Support: 20200490

Title: Antinociceptive synergistic interaction between palmitoylethanolamide and morphine in the mouse formalin test

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Abstract: Pharmacological synergism has been used to obtain higher efficacy at drug concentrations at which side effects are minimal. In this study, the pharmacological antinociceptive interaction between *N-palmitoylethanolamide* (PEA) and morphine was investigated. Individual concentration-response curves of PEA (0.1-100 μg/paw) and morphine (0.3-56.2 μg/paw) were evaluated in mice in which nociception was induced intraplantarly with 2% formalin. Different nociceptive responses counted flinches and licking behavior. Thus, we establish two EC_{50} according to the behavior analyzed; in flinches, behavior PEA generated EC_{50}= 11.61 ± 2.13 μg/paw and morphine produce EC_{50} = 3.96 ± 0.2 μg/paw. However, in licking behavior PEA generated EC_{50} = 29.39 ± 4.2 μg/paw and morphine produce EC_{50} = 10.94 ± 0.2 μg/paw. Flinching behavior was used to determine the experimental combinations according to the isobolographic method in a fixed 1:1 ratio. The isobologram demonstrates that the combinations investigated in this study produced a synergistic interaction; flinching experimental values (Z_{exp}= 2.72 ± 0.17 μg/paw) were significantly smaller than those calculated theoretically (Z_{add}= 7.78 ± 1.07 μg/paw) and licking experimental values (Z_{exp}= 6.28 ± 0.89 μg/paw) were significantly smaller than those calculated theoretically (Z_{add}= 19.71 ± 2.13 μg/paw). The antinociceptive mechanisms of the PEA and morphine combination involve PPAR-α receptors. These findings suggest that PEA and morphine could be interesting to treat inflammatory pain.

Disclosures: M. Deciga-Campos: None. **F.J. López-Muñoz:** None.

Digital Abstract Session

P173. Non-Opioid Treatments

Program #/Poster #: P173.01

Topic: D.03. Somatosensation – Pain

Support: US Department of Defense W81XWH-11-1-0672 Grant# 10669042
Lisa Dean Moseley Foundation LDMF1709JC
Cleveland Clinic Anesthesiology Institute Interval Research Fund

Title: Comparative analysis of human mesenchymal stem cells from bone marrow, adipose and umbilical cord as sources of anti-inflammation therapy

Authors: *Q. FAN, C. AKESSON, J. LI, J. CHENG;
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Abstract: Objective: Human mesenchymal stem cells (hMSCs) have come to the forefront as a potential treatment for chronic pain and opioid tolerance due to their trophic and immunomodulatory properties. hMSCs are commonly sourced from the bone marrow (BM), adipose (AD), and umbilical cord (UC) tissues. Studies have suggested that hMSCs isolated from different sources may have different immunomodulatory properties and gene expression profiles. In order to compare hMSCs from the three sources, we developed a transwell co-culture system to study the interactions between hMSCs and murine microglia cell line BV2 cells. Methods: BV2 cells were seeded and cultured in high-glucose DMEM supplemented with 10% FBS. After reaching 80%-90% confluency, BV2 were incubated with lipopolysaccharide (LPS) at 100ng/ml and 1000ng/ml separately for durations ranging from 6-24 hours. Real-time PCR was performed on total RNA extracted from the activated BV2 to measure the transcriptional expression levels of TNF-α, IL-1β, and IL-6. Then, BV2 cells were treated with LPS (1000ng/ml) for 6 hours, washed out, and co-cultured with the 3 types of hMSCs separately in a transwell plate for 24 hours. Real-time PCR was then performed on total RNA extracted from both BV2 and hMSCs to measure the transcriptional expression levels of TNF-α, IL-1β, and IL-6 in BV2, and TGF-β1, TGF-β2, and CX3CL1 in the 3 sources of hMSCs. Results: 1) LPS exposure increased robust BV2 cell transcriptional expression levels of TNF-α, IL-1β, and IL-6, dose-dependently and time-dependently, by as much as 80- (TNF-α), 7000- (IL-1β) and 18,000-fold (IL-6). 2) Co-culture with 3 sources of hMSCs dramatically reduced transcriptional expression levels of TNF-α and IL-6, but not IL-1β in BV2 cells. 3) Incubating 3 sources of hMSCs with activated microglia did not affect the hMSCs' transcriptional expression level of TGF-β. 4) Incubating BM and AD hMSCs with activated microglia resulted in a dramatic increase of the hMSCs' transcriptional expression level of CX3CL1. This increase in CX3CL1 expression was not seen in UC hMSCs. Conclusion: These results suggest that the therapeutic effects that hMSCs confer *in vivo* are likely mediated by immune cells, such as microglia within the nervous system, and that hMSCs from difference sources may have different immunomodulatory properties and therapeutic potentials.

Disclosures: Q. Fan: None. **C. Akesson:** None. **J. Li:** None. **J. Cheng:** None.

Digital Abstract Session

P173. Non-Opioid Treatments

Program #/Poster #: P173.02

Topic: D.03. Somatosensation – Pain

Support: Stanley Medical Research Institute grants 03-484 and 06T-797
NIH NIMH/FIC/NCCAM grant R21MH095644
Contract # HHSN-271-2013-00017-C and HHSN-271-2018-00023-C

Title: Pain modulating ethnobotanicals in Peruvian traditional medicine: a potential source of non-opioid therapeutics

Authors: *C. GALLO[1], G. POLETTI[1], R. ROJAS[1], J. ALBÁN[2], A. VAISBERG[1];
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Abstract: Chronic pain affects several million of individuals around the world being a leading cause of burden of disease and years lived with disability. This because of the lack of adequate relief provided by current therapies, which show low efficacy, development of tolerance, and dose-limiting toxicities. Opioids, particularly, are highly addictive and its dependence causes a large societal burden. Despite this, few novel non-opioid molecules targeting chronic pain have been identified in the past decade.Rescuing traditional medicine practices is one of the possible avenues to discover novel pharmacological approaches for clinical conditions like chronic pain. We have a repository of 1315 extracts from an equal number of plant collections corresponding to 350 species from 117 different plant families used in Peruvian traditional medicine. The collections were done in several localities and geographic regions of Peru.A total of 105 collections from this repository are used for the modulation of pain. Eighty-three of them were analyzed for cytotoxic effects; most (78%) showed very low or no cytotoxicity. Thirty-nine of these extracts were screened in binding and/or functional tests for 64 G-protein coupled receptors (GPCRs) and neurotransmitter transporters in the NIMH Psychoactive Drug Screening Program (PDSP) - University of North Carolina, Chapel Hill (UNC). From these 39 extracts, 11 showed non-significant binding to the mu, kappa or delta opioid receptors, and another 11 of them showed binding to the kappa or delta, but not to the mu opioid receptor.These preliminary data confirm the potential of Peruvian ethnobotanicals in the efforts directed to the discovery of novel non-opioid or less toxic and better tolerated therapeutics for pain disorders.

Disclosures: C. Gallo: None. **G. Poletti:** None. **R. Rojas:** None. **J. Albán:** None. **A. Vaisberg:** None.

Digital Abstract Session

P173. Non-Opioid Treatments

Program #/Poster #: P173.03

Topic: D.03. Somatosensation – Pain

Support: GM075770

Title: Evaluation of adverse side effects of a novel glycinergic analgesic for treatment of chronic pain

Authors: *J. CAPOROSO, L. JIANG, T. S. TILLMAN, P. TANG, Y. XU;
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Abstract: Glycine receptors (GlyRs) are ligand-gated ion channels that mediate nociceptive signals in the central nervous system. Previously, we presented a novel analgesic (MJPY1) that alleviates thermal hyperalgesia and mechanical allodynia in rodent models of neuropathic and inflammatory pain. MJPY1 is a selective positive allosteric modulator of α3-containing GlyRs

and binds specifically to a transmembrane domain site for Δ9-tetrahydrocannabinol (THC), one of the major psychoactive components in cannabis.1,2 Here, we show that the glycinergic mechanisms of analgesia are independent of the psychoactive effects and modulation of opioid receptors. A battery of *in vivo* tests was performed with MJPY1 to assess its selectivity for α3-containing GlyRs, potential for psychomotor side effects, and potential for substance use disorder. To determine if MJPY1's analgesic action is mediated by other analgesic receptors, such as opioid receptors, Sprague-Dawley rats with chronic constriction injury induced-neuropathic pain were treated with MJPY1 in the absence and presence of the opioid receptor inhibitor naloxone. The thermal and mechanical hypersensitivities were assessed by Hargreaves and von Frey tests. Psychomotor side effects were examined using the open field and horizontal ladder rung walking tests in rats without pain conditions. Substance use disorder potential, such as drug seeking and reward behaviors, were investigated using the conditioned place preference test in naïve rats with repeated exposures to MJPY1. The results of these tests suggest that MJPY1 produces analgesia independent of opioid receptors, does not cause detrimental locomotor side effects, and does not establish substance use conditioning, making MJPY1 an attractive alternative to current chronic pain treatments. In conclusion, MJPY1 represents a novel class of analgesics that may transform chronic pain managements in the future.

Disclosures: J. Caporoso: None. **L. Jiang:** None. **T.S. Tillman:** None. **P. Tang:** None. **Y. Xu:** None.

Digital Abstract Session

P173. Non-Opioid Treatments

Program #/Poster #: P173.04

Topic: D.03. Somatosensation – Pain

Title: Dt095597 a new NPFFR1 antagonist devoid of addictive properties to fight opioid crisis

Authors: *S. AYACHI[1], C. DOEBELIN[1], M. FER[1], M. GIAMBELLUCO[1], A. DUMONT[1], L. DESHONS[1], E. STEINBERG[1], S. TARLET[1], S. MAYER[1], C. FRANCHET[1], N. LENNE[1], M. GERUM[2], A. MEDINA[2], F. PETIT[3], E. DARCQ[3], B. KIEFFER[3], F. SIMONIN[2], S. SCHANN[1]; [1]DOMAIN THERAPEUTICS, ILLKIRCH GRAFFENSTADEN, France; [2]CNRS, Univ. of Strasbourg, ILLKIRCH GRAFFENSTADEN, France; [3]McGill\Douglas Res. center, Montreal, QC, Canada

Abstract: Opioid analgesics are the cornerstones for treating moderate to severe pain despite numerous side effects including respiratory depression, constipation and itching. Upon chronic administration, they also induce analgesic tolerance, Opioid Induced Hyperalgesia (OIH) and addiction. Because of tolerance, opioids dose escalation is needed to achieve analgesia, unfortunately, this also increases the risk of respiratory depression involved in death by overdoses. In the United States, fatalities involving opioids now account for more deaths than motor-vehicle accident incidents. In the context of this opioid epidemic, there is an urgent need to discover novel strategies to prevent individuals from becoming opioid-dependent and ease

opioid detoxification. NPFF receptors (NPFFR) are GPCRs with an established anti-opioid activity, known to reduce OIH and analgesic tolerance. Based on NPFFR involvement in the pain homeostasis, identification of a selective NPFFR1 antagonist is a promising therapeutic strategy to fight the current opioid crisis. We present here the preclinical results of DT095597, a new orally bioavailable NPFFR1 antagonist candidate. These results include efficacy, selectivity profile and PK data in several species. We also present DT095597 in vivo results on several mice models. Used in combination with opioids, DT095597 reduces Morphine Induced Hyperalgesia which help prevent opioid dose escalation. In addition, DT095597 used in monotherapy is able to prevent and erase Induced Latent pain Sensitization induced by chronic morphine injection or by inflammatory pain (CFA). DT095597 effect on opioid detoxification has also been tested. DT095597 is able to prevent Oxycodone physical withdrawal when given in preventive or curative treatment. Finally, non-addictive properties of DT095597 has also been tested to assess its safety. Using intracranial self-stimulation on rats, we demonstrate that DT095597 induces no change on frequency rate curve indicating that it has no addictive properties and does not induce anhedonia nor dysphoria. In conclusion we have identified a potent and selective NPFFR1 antagonist orally available: DT095597. DT095597 is able to block OIH, and LS induced by chronic morphine or CFA injection, and also to reduce oxycodone physical withdrawal, while having no addictive properties. DT095597 effect on Latent pain Sensitization could prevent the acute to chronic pain transition. Results on opioid withdrawal are also promising concerning DT095597 involvement on opioid detoxification. Overall these data highlight DT095597 potential to reduce and prevent the progression and the severity of opioid epidemic crisis

Disclosures: **S. Ayachi:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **C. Doebelin:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **M. Fer:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **M. Giambelluco:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **A. Dumont:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **L. Deshons:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **E. Steinberg:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **S. Tarlet:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **S. Mayer:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **C. Franchet:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **N. Lenne:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **M. Gerum:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Domain Therapeutics. **A. Medina:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Domain Therapeutics. **F. Petit:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Domain Therapeutics. **E. Darcq:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Domain Therapeutics. **B.**

Kieffer: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Domain Therapeutics. **F. Simonin:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Domain Therapeutics. **S. Schann:** A. Employment/Salary (full or part-time):; Domain Therapeutics.

Digital Abstract Session

P173. Non-Opioid Treatments

Program #/Poster #: P173.05

Topic: D.03. Somatosensation – Pain

Support: Arizona Biomedical Research Commission New Investigator Award #ADHS18-198875
Institutional funds from the University of Arizona

Title: Inhibition of Heath Shock Protein 90 in the spinal cord enhances opioid anti-nociception by disabling an inhibitory GABA circuit

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Abstract: Opioid drugs used in the treatment of pain are in desperate need of replacement due to their deleterious side effects. One strategy on offer is developing dose-reductive compounds that, when co-administered with palliatives, can reduce the dose of opioid required for treatment, retaining analgesic efficacy while reducing unwanted side-effects such as addiction, respiratory depression, and death. We previously showed that intrathecal (IT) administration of the heat shock protein 90 (Hsp90) inhibitor 17-AAG potentiated the efficacy of morphine induced antinociception, while reducing or not changing opioid side effects, enabling a dose-reduction strategy. Proteomic analysis of the translational alterations induced by IT 17-AAG administration revealed an upregulation of GABA transporter 2 (GAT-2). To elucidate the role of GAT-2 in mediating the enhancement of morphine antinociception, 17-AAG treated mice were administered a GAT inhibitor, Nipecotic Acid (NA), in the tail flick assay. NA effectively blocked the 17-AAG enhancement of antinociception compared to controls, while not changing responses in Vehicle-treated mice, suggesting that GAT-2 mediates 17-AAG's antinociceptive enhancement. Since GAT-2 takes up GABA into the cell and reduces GABA activity, we hypothesized that an inhibitory GABA circuit is present in the spinal cord which reduces opioid antinociception and is disabled by Hsp90 inhibition and GAT-2 upregulation. To test this GABA hypothesis, mice were treated with IT GABA-A or GABA-B agonists (muscimol, baclofen) prior to systemic morphine administration. The GABA-A agonist reversed 17-AAG enhanced antinociception much like NA, supporting our hypothesis. Intriguingly, the GABA-B agonist

reversed 17-AAG antinociception in males but not females, suggesting a GABAergic sex-specific difference. To further test the inhibitory GABA circuit hypothesis, naïve mice (no 17-AAG) were injected with IT GABA-A or GABA-B antagonists (bicuculline, CGP54626) prior to morphine and tail flick. Males showed enhanced morphine antinociception with either antagonist, supporting the GABA inhibitory circuit hypothesis; however, neither drug had an impact on female mice, again suggesting mechanistic sex differences. These results taken together suggest that IT 17-AAG potentiates morphine-induced antinociception via upregulation of GAT-2, relieving an inhibitory GABAergic brake on opioid antinociception. This study provides mechanistic insight into how Hsp90 inhibitors can be used to boost opioid antinociception and may have identified a novel inhibitory circuit of opioid antinociception.

Disclosures: C.S. Campbell: None. **P.R. Langlais:** None. **J.M. Streicher:** None.

Digital Abstract Session

P173. Non-Opioid Treatments

Program #/Poster #: P173.06

Topic: C.10. Brain Injury and Trauma

Support: the Canadian Institutes of Health Research
the Canadian Breast Cancer Foundation

Title: Mindfulness-based stress reduction for chronic neuropathic pain relief in breast cancer survivors? The brain says yes!

Authors: *O. BROWN[1], T. HATCHARD[1,2], O. MIODUSZEWSKI[1], Y. SHERGILL[3], Z. FANG[1], P. POULIN[1,3,4], A. SMITH[1];
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Abstract: Rationale and Objective: Chronic neuropathic pain (CNP), often caused by injury to peripheral nerves, affects 25-60% of women following breast cancer treatment. Current pharmacological treatment does not often help with CNP relief. Mindfulness-based stress reduction (MBSR), a psychological intervention, may be able to help. This work summarizes neuroimaging results from a group of women in a randomized control trial that investigated the effects of MBSR on CNP following breast cancer treatment. Methods: Twenty-three women were scanned with a 3T Siemens MRI scanner, including an Emotional Stroop fMRI task, resting state fMRI and diffusion tensor imaging (DTI). All participants were scanned two times, one at baseline after medical optimization and again after an 8 week MBSR course (n=13) or care as usual (n=10). They were also given the Brief Pain Inventory (BPI) at both scanning times. Results: The MBSR group had a significant reduction in pain severity following the intervention, while the control group did not. The Emotional Stroop results found that MBSR was associated with less blood flow in regions involved in pain processing and visual activity. The DTI results

showed that MBSR was related with greater fractional anisotropy (FA) post-intervention in left subcortical regions, which was correlated with lowered pain severity. The final imaging sequence reported greater functional connectivity within the default-mode network (DMN), specifically between the posterior cingulate cortex and medial prefrontal regions, following the MBSR intervention. This increase in functional connectivity within the DMN was associated with decreased pain severity. Conclusions: These studies provide compelling evidence of MBSR as a promising alternative to pharmacological treatment for pain-related problems in women suffering from CNP as a result of breast cancer treatment.

Disclosures: O. Brown: None. **P. Poulin:** None. **O. Mioduszewski:** None. **T. Hatchard:** None. **Z. Fang:** None. **Y. Shergill:** None. **A. Smith:** None.

Digital Abstract Session

P174. Ion Channels

Program #/Poster #: P174.01

Topic: D.02. Somatosensation

Support: CIHR Grant MOP-130282

Title: Activation of TRPM3 induces mitochondrial activity and enhances neurite outgrowth in adult sensory neurons

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Abstract: Peripheral neuropathy affects approximately 50% of the population with diabetes mellitus depending on age and disease severity. It is associated with substantial morbidity and is characterized by induction of pain and loss of sensory function beginning distally in the lower extremities. Recent studies suggest that molecular cascades maintaining mitochondrial function and calcium homeostasis are effective therapeutic targets for diabetic peripheral neuropathy. Interestingly, our lab has recently reported that muscarinic acetylcholine type 1 receptor (M1R) antagonists stimulated neurite outgrowth, in part, by activating Ca^{2+}/calmodulin-dependent protein kinase kinase II (CaMKKII) and mobilization of AMP-activated protein kinase (AMPK). This further augmented mitochondrial function in sensory neurons imparting protection against small and large fiber neuropathy in various rodent models of peripheral neuropathy (including diabetes). Transient receptor potential melastatin receptor 3 (TRPM3) is a TRP type cation channel that triggers Ca^{2+} influx. TRPM3 is quite unique in that it binds calmodulin and is open under high phosphoinositide levels (the latter would be predicted during blockade of the M1R). We hypothesized that opening of TRPM3 could activate CaMKKII and induce neurite outgrowth and may mimic antimuscarinic drug effects. Dorsal root ganglion (DRG) neurons were isolated from adult control and streptozotocin-induced type 1 diabetic male Sprague–Dawley rats. Assessment of neurite outgrowth was performed in response to pregnenolone sulphate (PS) or CIM0216 (selective and specific TRPM3 agonists, respectively). A significant dose-dependent

elevation of neurite outgrowth was observed. These TRPM3 agonists also increased AMPK activation and extracellular signal-regulated kinases (ERK) signaling in control and diabetic DRG neuron cultures. There was also a significant augmentation of mitochondrial activity. Specifically, TRPM3 agonists elevated maximal respiration in diabetic DRG neuronal cultures. Our investigations towards understanding of TRPM3 activation and its downstream molecular cascade will hopefully lead to potential therapeutic targets for impeding detrimental effects of peripheral neuropathy.

Disclosures: S. Chauhan: None. **P. Fernyhough:** None.

Digital Abstract Session

P174. Ion Channels

Program #/Poster #: P174.02

Topic: D.02. Somatosensation

Support: R01 AR051219
DARPA (HR0011-15-2-0054, EK)

Title: Piezo2 channel has critical roles in light-touch mechanosensitive sensory neurons in mouse hairy skin

Authors: *Y. BABA[1], C.-K. TONG[2], *E. A. LUMPKIN[1];
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Abstract: PIEZO2 is a mechanically gated ion channel expressed in somatosensory neurons and mechanosensory epithelial cells. Because global *Piezo2* knockout causes perinatal lethality, Previous studies of PIEZO2 function in neurons have used the tamoxifen-inducible Cre/lox system or caudally restricted Hoxb8-driven Cre, as global knockouts of Piezo2 in the nervous system are perinatal lethal (Ranade et al. 2014; Murthy et al. 2018). These studies have shown that Piezo2 is critical for the function of proprioceptors, low-threshold mechanoreceptors and tactile allodynia. To perform constitutive knockdown of *Piezo2* over a broader anatomical area, we utilized a Cdx2-cre transgenic line. Using a tdTomato reporter line, we confirmed that Cre is expressed broadly in somatosensory neurons from the brachial plexus through thoracic ganglia. $Cdx2^{Cre}$; $Piezo2^{fl/fl}$ (Piezo2CKO) suppressed Piezo2 expression completely in the $Cdx2^{Cre}$ expression domain. Piezo2CKO mice survived until adulthood but were smaller than control mice and displayed abnormal limb movements, consistent with the loss of functional proprioceptors as previously reported. Using this mouse model, genotype-blinded recordings from cutaneous sensory neurons in response to mechanical stimulation were surveyed in ex-vivo skin nerve preparations (n=44 units from 18 mice). Many types of sensory neurons displayed mechanosensory defects in Piezo2CKO mice. Rapidly adapting mechanosensory neurons were not observed in Piezo2CKO mice (n=0/44 units) compared with control (CTRL) genotypes (n=17/36 units). Additionally, low-threshold slowly adapting responses associated elicited by stimulation of guard hair and touch domes were never observed (Piezo2CKO: n=0/20 units,

CTRL: n=28/30). By contrast, A mechanonociceptors (AMs) showed lower firing rates for initial phase of stimulation than control mice (Piezo2CKO: n=11/44 units, CTRL: n=2/19); however, their mechanical sensitivity was not lost completely. The von Frey thresholds of AMs were comparable between genotypes (median & interquartile ranges: Piezo2CKO:5.9, 2.2-19.6; CTRL:3.9, 0.7-19.6 mN). Together, these data confirm that Piezo2 channels are essential for encoding tactile stimuli with high sensitivity, and demonstrate that other mechanosensory transduction mechanisms suffice in A mechanonociceptors.

Ranade et al. (2014) Piezo2 is the major transducer of mechanical forces for touch sensation in mice. PMC4380172.
Murthy et al. (2018) The mechanosensitive ion channel Piezo2 mediates sensitivity to mechanical pain in mice. PMC6709986.

Disclosures: Y. Baba: None. **C. Tong:** None. **E.A. Lumpkin:** None.

Digital Abstract Session

P174. Ion Channels

Program #/Poster #: P174.04

Topic: D.02. Somatosensation

Support: NIH Blueprint award NS115219-01
NSF GRFP Grant No. DGE-114747
NIH Grant R35NS105092

Title: Where does touch take place? Protein micropatterning to study how mechanoelectrical channels find their station

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[3]Departments of Mechanical Engin. and Molecular, Cellular, & Developmental Biol., Univ. of California Santa Barbara, Santa Barbara, Santa Barbara, CA

Abstract: Mechanoelectrical transduction (MeT) ion channels allow for the rapid conversion of physical stimuli into neural impulses. The positioning of these MeT channels along the neurite is hypothesized to play a critical role in setting mechanoreceptor sensitivity. In the roundworm Caenorhabditis elegans, mechanoreceptors called the Touch Receptor Neurons (TRNs) are responsible for detecting gentle touch. TRN function requires the expression of the DEG/ENaC ion channel subunit MEC-4. In wild-type TRNs, MEC-4 clusters into discrete puncta that are distributed along the neurite with inter-punctum spacing that increases distally from the cell body. Previous work established that the positioning of these puncta depends on genes encoding extracellular matrix (ECM) proteins, but did not identify which aspects of MEC-4 trafficking, localization, or anchoring had been disrupted. Aiming to dissect the biological processes underlying MEC-4 positioning, we conducted the first ever exploratory analysis of MEC-4 expression along neurites of isolated TRNs from dissociated C. elegans embryos. To control for

the potentially confounding effects of neurite morphology, and to simplify image acquisition and analysis, we cultured TRNs expressing an mNG::MEC-4 fusion protein on custom micropatterns of peanut agglutinin (PNA). In most cases, as was expected, TRN neurite morphology closely mirrored micropattern geometry. Using custom image analysis code, we quantified the fusion protein distribution along the full length of the neurite and used time lapse imaging to characterize protein mobility. We found that MEC-4 puncta were more sparse and more mobile than their in vivo counterparts. Upon repeating these experiments with TRNs from animals also expressing the mCherry::RAB-3 vesicle marker, we found that most of the MEC-4 puncta were likely sequestered within vesicles and had not reached the plasma membrane. Since laminins are a conserved and ubiquitous element in ECMs, we also asked whether or not the addition of laminin to the micropattern would be sufficient to create denser, more stable MEC-4 puncta. We developed a procedure for patterning PNA stripes alongside randomly distributed laminin microdots, and found this did not appreciably alter MEC-4 puncta. Our results confirm that the punctate distribution of MEC-4 seen in vivo is a primarily non-cell autonomous process, but suggest a potentially cell intrinsic mechanism regulating MEC-4 trafficking. The experimental technique developed here shows promise as a tool for direct, live imaging of protein mobility and may serve as a testbed for further exploring the interplay of neurite shape and channel trafficking.

Disclosures: **J.A. Franco:** None. **A. Das:** None. **B. Pruitt:** None. **M.B. Goodman:** None.

Digital Abstract Session

P175. Barrel Cortex and Sensory Processing

Program #/Poster #: P175.01

Topic: D.04. Somatosensation – Touch

Support: NIH 1 F32 NS114327-01
NIH 1 R37 NS092367

Title: Recent trial history cues attentional selection of whisker stimuli in mice

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Abstract: The ability to selectively direct attention to behaviorally relevant stimuli is critical for animals in complex environments. To examine how stimulus statistics and performance history guide attentional selection for tactile processing, we trained head-fixed mice to perform a Go/NoGo whisker detection task. Mice had nine whiskers inserted in a piezo array. On each trial, one randomly chosen whisker was deflected (Go trials) or no whisker was deflected (NoGo trials). Mice were rewarded for licking on Go trials (hits), but were neither rewarded nor punished for licking on NoGo trials (false alarms). The identity of the deflected whisker on Go trials was randomly selected, with varying probabilities. Thus, sequential trials included random

sequences of whiskers and repetitions of specific whiskers, with NoGo trials interspersed throughout. Behavioral performance was strongly influenced by the history of successful detections on recent trials. Multiple, consecutive hits led to an increase in lick probability on subsequent trials of the same whisker, causing behavioral shifts in both detection sensitivity (d') and decision criterion (c). The increase in d' reflected increased hit rate after consecutive prior hits, which was greater than a more modest elevation in false alarm rate. Improvement in d' was maximal following consecutive hits on the same whisker, and was much weaker following multiple hits on random whiskers or repetitions of a different whisker. Thus, recent hit trials engage a whisker-specific attentional effect. This effect lasted ~10 s before subsiding, and was flexibly shifted among whiskers based on their recent history. It did not occur when whisker repetitions were not successfully detected (misses), indicating that sensory repetition alone did not drive this effect, rather, the conjunction of whisker stimulation and reward was essential. Thus, mice used the recent history of whisker deflections and rewards to modulate sensory processing of spatially specific whisker stimuli on a rapid timescale, consistent with attentional selection. We are now investigating the role of cortical interneurons in gating enhanced sensory detection during this attentional selection process.

Disclosures: **D.L. Ramamurthy:** None. **A. Chen:** None. **G. Krishna:** None. **P. Bharghavan:** None. **K. Casale:** None. **D.E. Feldman:** None.

Digital Abstract Session

P175. Barrel Cortex and Sensory Processing

Program #/Poster #: P175.02

Topic: D.04. Somatosensation – Touch

Support: NIH R37 NS092367

Title: Fine structure and stability of the whisker map in layer 2/3 of somatosensory cortex in awake, behaving mice

Authors: *H. WANG, D. E. FELDMAN;
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Abstract: The whisker map in rodent somatosensory cortex is well characterized under anesthesia, but its organization during awake sensation is unknown. Using a novel behavioral task, we measured whisker receptive fields, map structure, and map stability in awake mice with 2-photon calcium imaging. During a whisker-attentive task, layer 2/3 pyramidal neurons were sharply tuned for one or a few whiskers, and cells tuned to different whiskers were intermixed in each column. This salt-and-pepper organization was superimposed on a sub-columnar tuning gradient and mean barrel-related map. In mice performing a sound-attentive task whisker tuning was more homogeneous in each column, and pairwise firing correlations were increased, indicating that the whisker map becomes decorrelated during whisker-attentive behavior. To assess map stability, we performed the same behavioral task in Drd3-Cre/TIGRE2.0-GCaMP6s

mice, which stably expressed Ca^{2+} sensor in layer 2/3 pyramidal cells, and imaged longitudinally from the same neurons every ~4 days for 2 weeks. Whisker tuning was highly dynamic, with 30% of neurons significantly changing their whisker tuning over a 4-7 day period, and many neurons losing or gaining whisker responsiveness across days. Only 60 % of consistently responsive units maintained constant tuning across sessions. Tuning was more stable for neurons tuned for the columnar whisker than for those tuned to non-columnar whiskers. Tuning dynamics persisted when whisking was abolished with Botox, suggesting that active whisker use does not drive the dynamic instability of the map. The ever-changing nature of the sensory map may provide a substrate for selecting optimal representations to perceive the complex environment.

Disclosures: H. Wang: None. **D.E. Feldman:** None.

Digital Abstract Session

P175. Barrel Cortex and Sensory Processing

Program #/Poster #: P175.03

Topic: D.04. Somatosensation – Touch

Support: NIH R01NS088958
NIH R01NS094450

Title: C-fos reports experience-dependent but not learning-related activity in mouse somatosensory cortex

Authors: *J. LEE[1], M. ZHU[1], D. J. MARGOLIS[2], A. L. BARTH[1];
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Abstract: Immediate-early gene expression induced by neural activity has been used to map discrete neural ensembles involved in sensation, cognition, and behavior. C-fos has commonly been assumed to identify neural engrams associated with learning or experience. Here we investigate how expression of the immediate-early gene c-fos is controlled by sensory experience and learning in mouse barrel cortex, a brain area strongly implicated in learning. Using longitudinal 2-photon imaging in superficial layers of mouse somatosensory cortex in a fosGFP transgenic mouse, we find that sensory association learning using a multiwhisker stimulus paired with a water reward did not enhance or stabilize fosGFP expression compared to baseline conditions. Acute whisker stimulation in awake mice also did not drive elevated fosGFP expression. However, exposure to an enriched tactile environment rapidly increased fosGFP expression across the previously labeled population. Sensory deprivation profoundly shifted fosGFP ensembles, increasing expression in weakly labeled cells while reducing expression in brightly labeled cells. These data indicate that monitoring immediately-early gene expression does not reveal a learning-specific engram in primary sensory cortex. We propose that fosGFP expression in primary sensory cortex is regulated by feedforward sensory drive as well as internal brain dynamics.

Disclosures: **J. Lee:** None. **M. Zhu:** None. **D.J. Margolis:** None. **A.L. Barth:** None.

Digital Abstract Session

P175. Barrel Cortex and Sensory Processing

Program #/Poster #: P175.04

Topic: D.04. Somatosensation – Touch

Support: HHMI Gilliam Fellowship

Title: Context dependent sensorimotor processing during active sensation: elucidating motor cortex's role during whisker movement

Authors: ***G. I. TELIAN**, J. BROWN, H. ADESNIK;
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Abstract: Mice actively adapt their whisking strategy to explore their environment. Vibrissae somatosensory cortex (vS1) is thought to convey sensory information to vibrissae motor cortex (vM1) in order to rapidly update the whisker motor program. However, this hypothesis has not been explicitly tested. Here we compare vM1's role in sensory-motor processing between untrained mice, and trained mice performing a whisker-dependent discrimination task. In both contexts, animals were head-fixed and free to palpate a tactile stimulus while freely running on a circular treadmill. Using high-density extracellular recordings, optogenetic silencing, and high speed videography we probe the sensory representation of tactile stimuli in vM1 and ask whether vM1 activity is necessary for adaptive whisking strategies and selecting appropriate behavioral choices. We find that vM1 represents tactile stimuli independent of vS1, putatively relying on its direct thalamocortical input. In untrained mice, vM1 silencing had no detectable impact on whisking kinematics, but had a dramatic impact when mice were engaged in the tactile discrimination task. During behavior silencing caused a retraction of whisker set-point as well as an early onset of licking. vM1 silencing also dramatically impaired performance on the task which is a potential consequence of both impaired adaptive whisking, the elimination of refined sensory representations in vS1, or an impairment in choosing the appropriate behavioral response. The effects of vM1 silencing on whisking kinematics were only apparent when mice were actively engaged in the discrimination task. Disengaged mice exhibited behavior similar to untrained mice with no changes to their whisker movements. We conclude that vM1 can encode the tactile environment independent of S1, its control over whisker movements is context dependent, being specifically engaged during demanding sensory tasks, and that it may play a role in selecting behavioral choices as changes in whisking did not always coincide with changes in choice.

Disclosures: **G.I. Telian:** None. **J. Brown:** None. **H. Adesnik:** None.

Digital Abstract Session

P175. Barrel Cortex and Sensory Processing

Program #/Poster #: P175.05

Topic: D.04. Somatosensation – Touch

Support: AFOSR FA9550-19-1-13022629
NIH R01 NS088958

Title: Reciprocal inhibition in PV and SST neurons inhibitory networks in mouse barrel cortex during sensory association training

Authors: *E. PARK, D. KULJIS, A. BARTH;
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Abstract: Inhibitory neurons form a highly interconnected network in the neocortex. Althoughreduction of inhibition, or disinhibition, has been proposed as one of the critical mechanismsof learning, it is unknown whether connections between inhibitory neurons are strengthenedto mediate disinhibition. Previous studies have shown that two types of inhibitory neurons,somatostatin (SST)-expressing neurons and parvalbumin (PV) neurons, are the primarysource of inhibition to excitatory pyramidal neurons and that both sources of inhibition maybe reduced during sensory or motor learning. Experimental evidence indicates that SSTneurons and PV neurons are reciprocally connected, but it is unknown whether thesesynaptic connections are altered during learning. We used ChR2 expressed in either PV orSST neurons and targeted postsynaptic inhibitory neurons in layer 2/3 of mousesomatosensory (barrel) cortex for whole cell patch-clamp recording in vitro to compare therelative strength and plasticity of this inhibitory subnetwork. Under baseline conditions, SST-inhibitory postsynaptic currents (IPSCs) in PV neurons were greater than PV-IPSCs in SSTneurons. Next, we investigated whether the strength of reciprocal connectivity between PVneurons and SST neurons change after learning in a whisker-dependent sensoryassociation training paradigm. We hypothesized that increased inhibitory input betweenSST neurons and PV neurons might facilitate disinhibition and network rewiring duringlearning. We found that SST-IPSCs in PV neurons were unaltered after sensory associationtraining. Our results suggest that potentiation of SST synapses onto PV neurons may not beengaged to trigger PV-mediated disinhibition during learning.

Disclosures: E. Park: None. **D. Kuljis:** None. **A. Barth:** None.

Digital Abstract Session

P175. Barrel Cortex and Sensory Processing

Program #/Poster #: P175.06

Topic: D.04. Somatosensation – Touch

Support: NIH R56 NS119599

Title: Long-term sensory association training decreases stimulus-evoked activity inL2/3 pyramidal neurons in mouse barrel cortex

Authors: *M. ZHU[1], A. L. BARTH[2], S. J. KUHLMAN[1];
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Abstract: Sensory learning alters cortical response properties and is linked to site-specific synaptic changes. We used longitudinal in vivo Ca imaging to determine how the stimulus-evoked activity of L2/3 pyramidal neurons in mouse barrel cortex changed throughout the course of training in a sensory association task, where a multi-whisker stimulus is coupled to a delayed water reward in freely-moving animals. Ca imaging was performed each day during a 6-day baseline and 10-day training period in head-fixed mice, where the stimulus alone was used to monitor the evolution of sensory responses in barrel cortex. Stimulus-evoked responses across the entire imaged population for naïve mice (during the pretraining period) were characterized by a brief increase in activity followed by a prolonged inhibition that lasted for several seconds. During the sensory association training process, the stimulus-evoked responses progressively reduced. After a week of sensory association training where all animals displayed behavioral changes indicating that they had learned the association, short-latency stimulus-evoked activity was significantly suppressed in all cells across the population compared to the pretraining period. Notably, the changes in cell activity after training is dependent upon the baseline activity of the cell during the pretraining period. The stimulus-evoked responses of the top 20% of cells that showed strongest baseline activity decreased quickly after training; the stimulus-evoked responses of the middle 60% of cells gradually reduced across training; the bottom 20% of cells showed increased stimulus-evoked activity and reduced post-stimulus inhibition during the early training period, which renormalized back to baseline after a week of training. Our experiments help address the question of how changes in sensory-evoked cell activity are related to both network rewiring and also behavioral improvement during long-term training.

Disclosures: M. Zhu: None. **A.L. Barth:** None. **S.J. Kuhlman:** None.

Digital Abstract Session

P176. Stimulus Features Response Properties, Neural Coding and Plasticity

Program #/Poster #: P176.01

Topic: D.04. Somatosensation – Touch

Support: Thompson Family Foundation Initiative on Chemotherapy Induced Peripheral Neuropathy and Sensory Neuroscience
Berrie Foundation Initiative on the Neurobiology of Obesity
NINDS R01 NS105241

Title: In vivo calcium imaging identifies functionally and molecularly distinct subsets of tongue-innervating mechanosensory neurons

Authors: *Y. MOAYEDI[1], S. XU[2], B. U. HOFFMAN[1], G. J. GERLING[2], E. A. LUMPKIN[3]; [1]Columbia Univ., New York, NY; [2]Univ. of Virginia, Charlottesville, VA; [3]Univ. of California, Berkeley, CA

Abstract: Mechanosensory neurons in the mouth provide essential information for guiding food choice, feeding ability, and speech. Yet, little is known about the functional diversity of mechanosensory neurons that innervate mammalian oral cavity. To address this gap, we previously mapped the distribution of neuronal endings in mouse and human oral cavity. We found that tongue is equipped with putative mechanosensory afferents including Krause end bulbs and perigemmal collars, whose functions in sensation are not known. To interrogate the functional diversity of mouse lingual mechanosensory neurons, we used in vivo calcium imaging of trigeminal ganglia. Experiments were performed in male and female animals with at least 3 animals/experiment. We investigated calcium responses of tongue-innervating trigeminal neurons that respond to thermal and mechanical stimulation (e.g. pressure, fluid flow, temperature changes). Nearly half of all responding neurons responded to mechanical stimulation (pressure or fluid flow). Delivery of somatosensory stimuli revealed stimulus tuning of neurons correlates with cell body size, as in cutaneous sensory neurons. Large-diameter tongue-innervating neurons responded to mechanical stimuli whereas small-diameter neurons responded best to temperature and flow. Based on stimulus-response times, we found distinct subgroups that responded well to brushing, pressure, or pressure & brushing. We next developed an unbiased clustering approach to classify calcium response characteristics to pressure stimulation. This approach revealed that pressure responding neurons displayed three distinct stimulus-response profiles to pressure: on only, on & off, or sustained responses throughout the duration of stimulation. Next, based on known genetic markers for skin mechanoreceptors, we screened for markers that selectively labeled subsets of physiologically distinct tongue-innervating neurons. Based on this screen, we mapped peripheral anatomy of specific types of end organs to response profiles and found that distinct end organs have unique response profiles to stimulation. These studies lay the foundation to define functional differences between somatosensory neurons in the mouth and how they encode tactile features of foods and contribute to oral functions.

Disclosures: Y. Moayedi: None. **S. Xu:** None. **B.U. Hoffman:** None. **G.J. Gerling:** None. **E.A. Lumpkin:** None.

Digital Abstract Session

P176. Stimulus Features Response Properties, Neural Coding and Plasticity

Program #/Poster #: P176.02

Topic: D.04. Somatosensation – Touch

Support: NIH Grant R15DE027844-01

Title: Tactile and multisensory responses to tooth sensation in the neocortex of the naked mole-rat (Heterocephalus glaber)

Authors: *N. J. HITE[1], J. NIE[2], D. K. SARKO[3];
[1]Dept. of Physiol., [3]Dept. of Anat., [2]Southern Illinois University, Sch. of Med., Carbondale, IL

Abstract: Dentition is highly critical to overall health and well-being. Behaviors such as eating and speaking require simultaneous processing of multiple sensory modalities, yet little is understood about the neural substrates related to dentition. In this study, we investigated how individual neurons in the naked mole-rat cortex adjusted their response properties to combinations of auditory, visual, and tactile stimuli, with a focus on neurons responsive to dental and orofacial regions. In addition, we assessed tactile response preferences including response threshold and directionality preferences for the naked mole-rat's highly sensitive and behaviorally critical incisors. Using in vivo electrophysiology in anesthetized naked mole-rats, the integrative properties of neurons were compared between central somatosensory regions and those bordering auditory and visual cortices. We hypothesized that additional sensory stimuli would modulate the dominant tactile responses of the somatosensory cortex, resulting in increased firing rates and reduced response latencies compared to unimodal stimulus presentations. We further hypothesized that cortically expanded somatosensory regions such as the incisors would have greater incidences of multisensory integration compared to other areas. These studies help to elucidate the neural substrates of dental perception and the modulatory role that additional sensory stimuli play regarding dental sensation.

Disclosures: N.J. Hite: None. **J. Nie:** None. **D.K. Sarko:** None.

Digital Abstract Session

P176. Stimulus Features Response Properties, Neural Coding and Plasticity

Program #/Poster #: P176.03

Topic: D.04. Somatosensation – Touch

Support: NSF IIS 2006152

Title: Tactile-thermal interactions influence cutaneous communication systems

Authors: *L. A. JONES, A. SINGHAL;
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Abstract: Much of the fundamental research on tactile-thermal interactions has examined the effect of changing either the temperature of a display in contact with the skin or of the skin itself on threshold-level responses. There have been few studies that have systematically explored the effects of concurrently varying thermal and tactile inputs on the perception of supra-threshold stimuli. It is unclear whether one signal masks the perception of another, facilitates its identification, or even if tactile and thermal cues can be perceived independently when presented concurrently. The objective of the present series of experiments was to determine whether tactile pattern identification is affected by concurrent thermal stimulation and conversely, if the identification of thermal patterns is influenced by concurrent tactile stimulation. A multisensory display was built to provide tactile and thermal cues to the skin. An annular Peltier device with

an outer diameter of 24 mm and contact area of 377 mm2 was used to deliver warm and cool stimuli to the skin on the thenar eminence. A motor was mounted in the center of the Peltier device that could either indent the skin to deliver a low frequency pressure stimulus or vibrate the skin at 100 Hz. The pressure stimuli ranged from 1.7 kPa to 10.9 kPa and the vibrotactile stimuli varied in intensity or duration (0.5-1.5 s). In the first set of experiments, the thermal stimuli were defined relative to baseline skin temperature and either warmed (+5 ºC) or cooled (-7 ºC) the skin. Each tactile stimulus was presented three times to 10 participants who had to identify the tactile pattern using a visual template. The same display was used in the second series of experiments to present six thermal patterns that had to be identified during concurrent tactile stimulation. The thermal patterns varied with respect to waveform (square wave, step and ramp) and the rate of change in temperature (0.7-3 ºC/s). In all experiments performance was compared to that achieved at neutral skin temperature. The results indicate that concurrent thermal stimulation influences the ability to identify tactile stimuli, and that this effect is temperature specific. Warming the skin facilitated identifying the intensity of tactile stimuli (90% correct) but impeded the processing of temporal cues (62% correct). In contrast cooling the skin did not affect perceptual performance. This finding may reflect the enhanced activity of cutaneous thermoreceptors during warming and possibly also the change in the mechanical properties of the skin with warming. Concurrent tactile stimuli influenced thermal pattern identification, with the effect being greater for warm stimuli.

Disclosures: L.A. Jones: None. **A. Singhal:** None.

Digital Abstract Session

P176. Stimulus Features Response Properties, Neural Coding and Plasticity

Program #/Poster #: P176.04

Topic: D.04. Somatosensation – Touch

Support: BBSRC Grant BB/R003971/1
BBSRC Grant BB/R001847/1

Title: Early Sensory Processing of Tactile Texture in the Brain

Authors: *A. R. LOOMES[1], H. A. ALLEN[2], R. D. ROBERTS[1], H. KWOK[1], A. M. WING[3]; [1]Univ. of Birmingham, Birmingham, United Kingdom; [2]Univ. of Nottingham, Nottingham, United Kingdom; [3]Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Psychophysical studies support a duplex theory of tactile texture (Hollins et al 2001) processing with fine textures mediated by vibration signals and coarse textures by signals conveying spatial detail. Here we asked whether auditory and visually selective brain areas contribute to the processing of tactile stimuli. In two fMRI experiments we examined activity in somatosensory, auditory and visual cortices during static (1.5s) and dynamic (20mm/s) application of coarse (spatial period of 1600 μm, ridge width of 400 μm) and fine (spatial period 400 μm, ridge width of 100 μm) textures to the fingertip. A region of interest analysis, using

localisers identified with 10Hz and 200Hz vibrotactile stimuli, and anatomically localised auditory and visual areas revealed contralateral activation in somatosensory and auditory cortex during contact between the textures and skin. Moreover, these areas were significantly more activated with dynamic than static stimulation. In contrast, movement dependent activation was not observed in the visual cortex. These findings were replicated in a control experiment where the static stimulus was applied twice in quick succession. Activation of auditory areas was significantly greater during dynamic compared to static stimulation. Together these findings provide neuroimaging evidence of contrasting cortical processing of dynamic compared to static tactile input consistent with duplex theory. However, in contrast to duplex theory, the effect is seen with both coarse and fine textures.References:Hollins, Sliman J. Bensmaïa, Sean Washburn, M. (2001). Vibrotactile adaptation impairs discrimination of fine, but not coarse, textures. *Somatosensory & motor research*, *18*(4), 253-262.

Disclosures: **A.R. Loomes:** None. **H.A. Allen:** None. **R.D. Roberts:** None. **H. Kwok:** None. **A.M. Wing:** None.

Digital Abstract Session

P176. Stimulus Features Response Properties, Neural Coding and Plasticity

Program #/Poster #: P176.05

Topic: D.04. Somatosensation – Touch

Support: TÜBİTAK grant no: 117F481 under European Union's FLAG-ERA JTC 2107 project GRAFIN

Title: Comparison between psychophysical performance of rats and incremental Bayesian models based on average firing rates of SI cortical neurons

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Abstract: Cortical motor neuroprostheses depend on the simultaneous activity of many neurons for predicting movement parameters. We used a similar approach to predict the detection of a vibrotactile sensory stimulus in a psychophysical yes/no task performed by trained rats. The algorithms presented in this study may be useful for future brain-computer interfaces aimed to provide somatosensory function. 10 awake behaving rats were tested with vibrotactile stimulus applied on the hindpaw glabrous skin (frequency: 40 Hz, duration: 0.5 s, amplitude: 200 μm) while they performed the detection task. Multiunit spike activity was recorded with 16-channel microwire array electrodes implanted in the hindpaw representation of SI cortex. Band-pass filtered (500 Hz - 8 kHz) spike data were sorted into units by using the Wave_Clus spike sorting algorithm. Combinations of average spike rates from all (approximately 35 units in each rat) units (FR, within the stimulus duration) and task-related features (stimulus presence (S) in the current trial; one (P1), two (P2), and three (P3) most recent responses in successive trials) were

input to 12 incremental Bayesian models to predict the vibrotactile stimulus in each rat experiment. The models were implemented in a probabilistic programming framework (PyMC3). Nonparametric sensitivity index (A') and bias index (B'') were used to compare model performances with psychophysical data. Although the primitive models without FR (i.e. with only S, with S+P1, with S+P2) had significantly higher A' values than those obtained from psychophysical data, they had substantially higher bias. Inclusion of FR into the models decreased the bias, but also decreased detectability to values close to experimental data (paired t-tests: p's > 0.1 and p's > 0.2, for A' and B'' respectively). As a matter of fact, a Bayesian model based on average firing rates from a population of cortical neurons seems to be adequate to yield the psychophysical performance in a yes/no detection task. We are currently testing time-dependent neural features to further improve this *in silico* brain based on Bayesian inference.

Disclosures: S. Öztürk: None. **I. Devecioğlu:** None. **B. Güçlü:** None.

Digital Abstract Session

P176. Stimulus Features Response Properties, Neural Coding and Plasticity

Program #/Poster #: P176.06

Topic: D.04. Somatosensation – Touch

Support: Whitehall Foundation Research Grant 2017-05-71
NIH Grant R01NS107599

Title: Cortical Localization of Sensory-Motor Transformation in a Whisker Detection Task in Mice

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Abstract: Responding to a stimulus requires transforming an internal sensory representation into an internal motor representation. Where and how this sensory-motor transformation occurs is a matter of vigorous debate. Here, we trained male and female mice in a whisker detection go/no-go task in which they learned to respond (lick) following a transient whisker deflection. Using single unit recordings, we quantified sensory-, motor- and choice-related activities in whisker primary somatosensory cortex (S1), whisker primary motor cortex (wMC) and anterior lateral motor cortex (ALM). Based on the criteria of having both strong sensory and motor representations and early choice probability, we identify whisker motor cortex as the cortical region most directly related to the sensory-motor transformation. Suppression of both S1 and wMC altered task performance, yet in notably different ways. Our data support a model of sensory encoding originating in S1, sensory amplification and sensory-motor transformation occurring within wMC, and motor signals emerging in ALM after the sensory-motor transformation.

Disclosures: B. Zareian: None. **Z. Zhang:** None. **E. Zagha:** None.

Digital Abstract Session

P176. Stimulus Features Response Properties, Neural Coding and Plasticity

Program #/Poster #: P176.07

Topic: D.04. Somatosensation – Touch

Support: NIH Grant 1R01NS092875

Title: Sensitrak- automated assessment of forelimb sensation in rodents

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Abstract: Somatosensory function is routinely measured in humans by determining the threshold for detection of a vibrotactile stimulus applied to the hand, and this measure correlates with dexterity. Assessment of vibration sensation in animals has been largely missing because of the need to train behaviors that signal detection of the stimulus. Two commonly used somatosensory assessments in rats, the Hargreaves test for heat and the von Frey test for pain, rely on reflexes and do not require training, but they test a separate sensory pathway, and response may be compromised by motor deficits that may hinder withdrawal of the limb. There are currently no turnkey systems for behavioral testing of forelimb touch. We thus created SensiTrak, a behavior system to determine the sensitivity of rodents to vibration applied to the forepaw. Rats are trained to hold to a lever and to release when they feel a vibration. The lever is fitted with a linear resonant actuator for delivering vibration and a force sensor to signal when to start the trial and when the rat releases the lever. Control software is used to deliver stimuli of varying duration and to reward the rats if they release the handle within 0.5 seconds of vibration or do not release during no stimulation (catch) trials. To determine sensitivity, response rate was plotted against duration of the vibration. We hypothesized that this task could be implemented in a semi-automated fashion, and that the resulting psychophysical profiles would allow reproducible sensory thresholds. After associating the handle with a reward (~2 weeks), rats can be trained by the automated reward system to hold and to release when they feel a vibration (~5 weeks). Upon testing, our first three rats produced clear sigmoid-shaped psychometric curves, similar to those observed for somatosensation testing in humans and other sensory functions in rats. We set the threshold as 50% as the difference between the true positive rate and the false positive rate, which we call the corrected positive rate. Rats achieved the 50% rate at a stimulus duration of between 40 and 60 ms (vibration frequency of 166Hz). In addition to this relatively narrow range between rats, each rat showed stability within their respective thresholds as far out as 13 weeks after psychometric testing began. Backed by these results, SensiTrak seems to be a reliable measure of somatosensation in rats. Future studies will test the utility of this system for mice and will measure function after the touch sensory system injury and recovery.

Disclosures: D. Yoo: None. **A. Ramamurthy:** None. **A. Sloan:** None. **J. Carmel:** None.

Digital Abstract Session

P176. Stimulus Features Response Properties, Neural Coding and Plasticity

Program #/Poster #: P176.08

Topic: D.04. Somatosensation – Touch

Support: NIH/NINDS New Innovator Award 1DP2NS105554

Title: Behavioral and Neural Bases of Tactile Shape Discrimination Learning in Head-Fixed Mice

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Abstract: Tactile three-dimensional shape recognition requires perception of object surface angles. We investigated how object surface angles are represented in the brain, how this representation is constructed from sensory inputs, and how it reorganizes across learning. Head-fixed mice learned to discriminate object angles by active exploration with one whisker. Calcium imaging of excitatory neurons in layers 2-4 of barrel cortex revealed maps of object-angle tuning before and after learning. Three-dimensional whisker tracking demonstrated that the sensory input components that best discriminate angle (vertical bending and slide distance) also have the greatest influence on object-angle tuning. Despite high turnover in active ensemble membership across learning, both the population distribution of object-angle tuning preferences and individual preferences remained stable. Motor strategy and sensory inputs were not changed across learning, but population response from touch-response neurons better discriminated object angle after learning. These results show how discrimination training enhances stimulus selectivity in primary somatosensory cortex while maintaining perceptual stability.

Disclosures: J. Kim: None. **A. Erskine:** None. **J.A. Cheung:** None. **S.A. Hires:** None.

Digital Abstract Session

P176. Stimulus Features Response Properties, Neural Coding and Plasticity

Program #/Poster #: P176.09

Topic: D.04. Somatosensation – Touch

Support: NSF IBN-9723178
NIH RR00165
Emory University SIRE Program

Title: Potential correlates of neuronal survival in the dLGN of macaques with longstanding V1 damage

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Abstract: Surviving visual function after damage to primary visual cortex (V1) in primates ("blindsight") was originally believed to depend on alternative pathways through the tecto-pulvinar system. However, more recent studies established a crucial contribution of surviving neurons in the dorsal lateral geniculate nucleus (dLGN) (Schmid et al., *Nature, 466,* 2010). After V1 damage, most retinotopically corresponding dLGN cells degenerate, but a subset of extrastriate-projecting neurons survive, primarily in interlaminar zones, and have roughly normal response properties (Yu et al., *J. Neurosci, 38,* 2018). We investigated whether vascularization patterns and surviving retinal inputs might play a role in the survival of this small population. Subjects were 6 *M. mulatta* (both sexes) with lesions of left V1 made either at 5-6 weeks or in adulthood and survival of 4-7 yr. Cell and blood vessel measurements were made in coronal sections immunoreacted to reveal calbindin-D28K (Cal, a marker of the cell population of interest) or cholera toxin B-subunit label (from retinal injections) and counterstained with Giemsa. Using each animal as its own control, and quantifying blind as to age at lesion, we found that the proportion of the dLGN occupied by blood vessels was significantly greater on the lesioned side, with comparatively greater effects in adult-lesioned cases ($p < 0.05$, Mann-Whitney *U*). Contrary to predictions, we did not find, overall, that surviving dLGN cells within degenerated zones were closer to blood vessels than expected by a random distribution; however, surviving cells in adult-lesion cases were significantly closer to vessels than were comparable neurons in the infant-lesion cases. We also predicted that surviving dLGN cells would be more preferentially clustered in areas of high residual retinal input, relative to the distribution of Cal cells and retinal label on the intact side, but found this to be the case only in one of the infant-lesioned subjects. Finally, with respect to the phenotype of surviving neurons *per se*, we observed a significant proportion of surviving Cal-negative neurons in the adult-lesion cases (approximately 20%), whereas virtually all the surviving cells after lesions in infancy contained Cal. The results are thus not consistent with an important role for access to either vasculature or clumps of retinal label for the precise subset of dLGN neurons that survive V1 damage. However, the analyses revealed several differences in dLGN architecture and surviving cell populations after damage in infancy compared with similar damage in adulthood, suggesting subtle differences in mechanisms of reorganization at the dLGN level.

Disclosures: A.M. Auerbach: None. **H.R. Rodman:** None.

Digital Abstract Session

P176. Stimulus Features Response Properties, Neural Coding and Plasticity

Program #/Poster #: P176.10

Topic: D.04. Somatosensation – Touch

Support: NERSC

Title: Somatosensory and Auditory Loops Support Tactile Frequency Discrimination

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Abstract: Somatosensory and auditory loops support tactile frequency discrimination Auditory scene analysis is essential to many daily activities: understanding speech in noise, localization of sounds, musical perception, etc. Most of the time, several senses are involved in these activities. For example, a violinist plays a note, sounds and vibrations are produced by the instrument. Hearing and tactile systems are activated. Understanding how the brain recruits each of these senses and how they interact with each other remains a matter of study. In order to better understand tactile perception, the study of neuroanatomical correlates is necessary via low-level tasks. This is why the main goal of this study was to investigate neural correlates of tactile perception during a simple frequency discrimination task via the tactile modality only (no auditory perception). We hypothesized that both somatosensory and auditory systems will be recruited during the frequency (pitch) discrimination task even if no sounds were presented to the auditory system. During and EEG recording, participants were presented with a vibrotactile oddball paradigm (left hand vibrotactile stimulation glove) in which deviant stimuli (225Hz, 250 Hz, 500Hz, 800Hz, 1000Hz) differing in frequencies from the standard stimuli (200Hz). To control for auditory perception, participants were asked to listen to a continuous noise during the entire experiment. Source reconstruction of vibro-tactile event related potentials showed right lateralized Mismatch Negativity response in both somatosensory and auditory regions for each deviant stimulus in comparison to the standard stimulus. The somatosensory response was earlier than the auditory response. Also, the MMN amplitude increased with increasing frequency changes except for the 1000Hz condition which is at the limit of the frequency range perceivable by the tactile system. These findings suggest that pitch discrimination without auditory perception is performed by an interplay between tactile and auditory systems. Time-frequency analyses and directed functional connectivity will inform us about the oscillatory patterns supporting this interplay as well as the direction of the connections. These results will allow a better understanding of multisensory interaction during tactile unisensory perception. Also, these new findings can help find ways to improve auditory scene analysis in individuals who have difficulty with activities involving the treatment of several sound sources.

Disclosures: A. Grenier: None. **P. Albouy:** None. **A. Sharp:** None.

Digital Abstract Session

P177. Peripheral Olfactory Mechanisms

Program #/Poster #: P177.01

Topic: D.05. Olfaction and Taste

Support: DFG grant STE531/20-1, SPP1392
DFG grant STE531/20-2, SPP1392

Title: Perforated patch clamp survey of cyclic nucleotide-dependent ionic currents in olfactory receptor neurons of the hawkmoth Manduca sexta

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Abstract: Olfactory receptor neurons (ORNs) of the hawkmoth *Manduca sexta* sensitize via cAMP- and adapt via cGMP-dependent mechanisms on fast time scales, dependent on previous acute odor exposure. Furthermore, circadian rhythms in the concentration of the stress hormone octopamine in hawkmoth antennae generate peaks of cAMP during the moth´s activity phase, modulating olfactory sensitivity and kinetics daytime-dependently on a 24 h time scale. With perforated patch clamp recordings of cultured hawkmoth ORNs employing voltage-step protocols (n=223) we searched for voltage-gated cyclic nucleotide-dependent potassium- and non-specific cation currents that could be targets during fast or slow modulation of odor transduction. First, eleven types of currents were distinguished and named according to reversal potential, current-voltage relation, time course, and pharmacology. Then, the membrane-permeable cyclic nucleotide-derivatives 8bcAMP (n=13) and 8bcGMP (n=13) were applied and currents were evaluated in three different time windows after application in search for statistically significant cyclic nucleotide-dependent changes (Student's t-test vs. no application). Either cAMP- and/or cGMP-derivatives antagonistically affected three of five K^+ currents and two non-specific cation currents. The Ca^{2+}-dependent K^+ current $I_{K(Ca^{2+})}$ and the sensitive pheromone-dependent K^+ current $I_{K(cGMP-)}$ that both express fast kinetics were significantly inhibited by 8brcGMP, while a slow K^+ current $I_{K(cGMP+)}$ was significantly activated by 8bcGMP. Furthermore, application of 8bcAMP significantly blocked the slowly activating, zero mV reversing non-specific cation currents I_{LL} and $I_{cat(PKC?)}$ that remain activated in presence of 8brcGMP. Their activations pull the membrane potential towards their 0 mV reversing potentials, in addition to increasing intracellular Ca^{2+} levels voltage- and I_{LL}-dependently. Twenty minutes after application 8brcGMP significantly blocked a TEA-independent K^+ current $I_{K(noTEA)}$ and the fast cation current $I_{cat(nRP)}$ that both shift the membrane potential to negative values. We conclude that at high levels of cAMP conditions of sensitization are maintained via specific opening/closure of ion channels that allow for fast kinetics, hyperpolarized membrane potentials, and low intracellular Ca^{2+} levels. Adaptation is supported via cGMP that antagonizes cAMP, opening Ca^{2+} permeable channels with slow kinetics that stabilize depolarized resting potentials. [Supported by DFG grants STE531/20-1,2; SPP1392]

Disclosures: **M. Stengl:** None. **K. Schroeder:** None. **J. Dolzer:** None.

Digital Abstract Session

P177. Peripheral Olfactory Mechanisms

Program #/Poster #: P177.02

Topic: D.05. Olfaction and Taste

Support: Spanish Ministerio de Ciencia, Innovación y Universidades Grant FIS PI15/00262

Title: Olfactory neuroepithelium ACE2 downregulation in eosinophilic inflammation: a potential protective factor for COVID-19?

Authors: *C. MARIN, V. TUBITA, C. LANGDON, M. FUENTES, M. LÓPEZ-CHACÓN, A. VALERO, I. ALOBID, J. MULLOL;
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Abstract: Sudden and severe loss of smell is one of the initial symptoms of COVID-19. Via entry proteins (angiotensin-converting enzyme 2 (ACE2)/transmembrane protease serine-2 (TMPRSS2) in sustentacular cells), SARS-CoV-2 may induce inflammation in the olfactory epithelium or enter to the central nervous system damaging olfactory bulbs, causing loss of smell. The impact of COVID-19 in airway chronic diseases, such as chronic rhinosinusitis (CRS), has been found reduced and potentially linked to respiratory tissue eosinophilia and reduced ACE2 expression, suggesting that this downregulation might be a protective factor for COVID-19. However, the ACE2/TMPRSS2 expression in the olfactory epithelium in patients with eosinophilic inflammation has not been investigated yet.Our objective was to investigate whether the olfactory epithelium entry protein levels in eosinophilic CRS are downregulated compared to healthy control cases, potentially suggesting a reduced risk of SARS-CoV-2 infection of the olfactory system.We investigated ACE2 and TMPRSS2 expression in the olfactory epithelium from eosinophilic CRS with nasal polyps (CRSwNP, n=13) patients and healthy controls (n=11). Immunohistological studies were performed to identify olfactory neurons (OMP+), sustentacular cells (Hsp25+), eosinophil infiltration (BMK13+), and ACE2 protein expression. Real-time PCR studies were performed to quantify ACE2 and TMPRSS2 mRNA.Olfactory epithelium from CRSwNP patients showed an increased number of eosinophils (BMK13+) ($p<0.05$) in association with a decrease in olfactory neurons (OMP+) ($p<0.01$) and sustentacular cells (Hsp25+) ($p<0.05$). The expression of ACE2 protein ($p<0.01$) and mRNA ($p<0.05$), and TMPRSS2 mRNA ($p<0.05$) was significantly decreased in the olfactory epithelium.These findings suggest that ACE2/TMPRSS2 downregulation in the olfactory epithelium of CRSwNP patients might be related to tissue eosinophilic inflammation, potentially leading to a decreased risk of olfactory epithelium damage and entry to the olfactory bulbs by SARS-CoV-2.
This study was sponsored by a grant (FIS PI15/00262) from the Spanish *Ministerio de Ciencia, Innovación y* Universidades.

Disclosures: C. Marin: None. **V. Tubita:** None. **C. Langdon:** None. **M. Fuentes:** None. **M. López-Chacón:** None. **A. Valero:** None. **I. Alobid:** None. **J. Mullol:** None.

Digital Abstract Session

P178. Central Olfactory Mechanisms

Program #/Poster #: P178.01

Topic: D.05. Olfaction and Taste

Support: NIH R01-DC017876

BRAIN 1R01NS111673-01

Title: Wiring logic of the rodent olfactory system revealed by sequencing of single cell projections

Authors: ***Y. CHEN**[1], X. CHEN[1], ***B. BASERDEM**[2], J. M. KEBSCHULL[3], H. ZHAN[1], Y. LI[1], M. DAVIS[1], A. M. ZADOR[1], A. KOULAKOV[1], D. F. ALBEANU[1];
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Abstract: The olfactory system relies on hundreds of odorant receptors to recognize numerous volatile compounds. However, the neuronal substrates of odor perception remain elusive, partially due to lack of insight into how the brain sorts the inputs from the sensory periphery. In contrast to the structured long-range connectivity and spatial representations in other sensory modalities, previous axonal tracing studies found that olfactory bulb (OB) outputs, the mitral and tufted cells, project in a highly distributed and seemingly random fashion to their largest cortical target, the piriform cortex (PC). However, previous studies were limited by the small number of neurons whose projections were traced, which may obscure any potential spatial structure in OB output. To investigate the wiring logic of the olfactory system, here we analyze the projections of 5,309 OB output neurons and 30,433 PC output neurons at single-cell resolution. We employ highly multiplexed barcode sequencing-based mapping techniques (MAPseq and BARseq), which enable discriminating different types of OB output neurons by their soma locations and brain-wide projections. We identify structured OB (mitral cell) projection modules, reproducible across mice, which tile the anterior-posterior (A-P) axis of the piriform cortex and co-innervate distinct brain regions. In addition, we find characteristic sets of PC output neurons which project to different brain targets, and are enriched at specific A-P locations in the piriform cortex. Drawing from these results, an organizing principle emerges: matched input-output, parallel circuit motifs span the A-P axis of the piriform cortex and co-innervate specific sets of OB target areas. These results challenge the canonical framework of piriform cortex as a random network, at the core of current understanding of olfactory processing. They encourage further investigation into the function of these neural circuits, and the formulation of novel computational models to account for the logic of information flow in the olfactory system.

Disclosures: **Y. Chen:** None. **X. Chen:** None. **B. Baserdem:** None. **J.M. Kebschull:** None. **H. Zhan:** None. **Y. Li:** None. **M. Davis:** None. **A.M. Zador:** None. **A. Koulakov:** None. **D.F. Albeanu:** None.

Digital Abstract Session

P178. Central Olfactory Mechanisms

Program #/Poster #: P178.02

Topic: D.05. Olfaction and Taste

Support: Autonomous Government of Castilla-La Mancha SBPLY19 180501 000216

Title: Comparative Anatomy of the olfactory peduncle in nonhuman and human primates

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Abstract: The human olfactory system has been historically considered as a system mainly involved in odor recognition. Nevertheless, during the last decade, olfactory dysfunction has been studied as a preclinical symptom related to Parkinson's and Alzheimer's diseases. The olfactory system appears to be a direct pathway to other cortical olfactory regions of the brain for some pathogens or prion-like diseases, and the anatomy of sensory neurons, directly exposed to the environment in the nasal cavity allows it. In parallel, most mammals generate new neurons during adulthood which incorporate into a preexisting olfactory circuitry, presumably to preserve its functionality intact. However, in adult humans this process has not been demonstrated, and the information concerning cellular composition of different olfactory structures as the olfactory peduncle is still scarce. In this work, we studied 28 humam olfactory nerves ranging from 19 to 93 years old and 5 cases of young adult primates (*Macaca fascicularis*). The olfactory nerves of both species were divided into main olfactory bulb, and anterior, medial and posterior regions of the olfactory peduncle. One sample of each region was embedded in Durcupan resin and transversely sectioned in semithin sections (1.5µm). Other samples were transversely sectioned at 30µm in a freezing microtome. We then studied morphology and the cytoarchitecture of all different portions of the olfactory peduncle along adulthood. Furthermore, by using stereological and statistical analysis, we assessed the presence and distribution of blood vessels and corpora amylacea (spherical bodies that accumulate in some areas). Immunohistochemical techniques and electronic and confocal microscopy were performed with the aim of identifying the distribution of different cell populations such as glia, mature and putative young neurons and undifferentiated cells in the olfactory peduncle. Regarding Macaca, we observed a very homogeneous peduncle, with few corpora amylacea and blood vessels, mainly present in the core. In humans, we observed different morphologies following patterns. All the cases showed a vertex in the dorsal portion, corresponding to the region below the olfactory sulcus and higher density of cells and fibers in the medial region. In addition to the anterior olfactory nucleus (sometimes present), we also distinguished different layers. Our findings advance our knowledge of the structure of the olfactory peduncle and may be of use in understanding adult neurogenesis and the processes underlying normal and pathological aging.

Disclosures: J. Raspeño-Garcia: None. **J. Aguilar-Gómez:** None. **P. Torrente-Garcia:** None. **F. Sancho-Bielsa:** None. **A. Cózar-Cuesta:** None. **S. González-Granero:** None. **E. Artacho-Pérula:** None. **R. Insausti:** None. **J. Garcia-Verdugo:** None. **C. De la Rosa-Prieto:** None.

Digital Abstract Session

P178. Central Olfactory Mechanisms

Program #/Poster #: P178.03

Topic: D.05. Olfaction and Taste

Support: KVPY Fellowship SB1712051

Title: Odor coding by attractor switching in the locust antennal lobe

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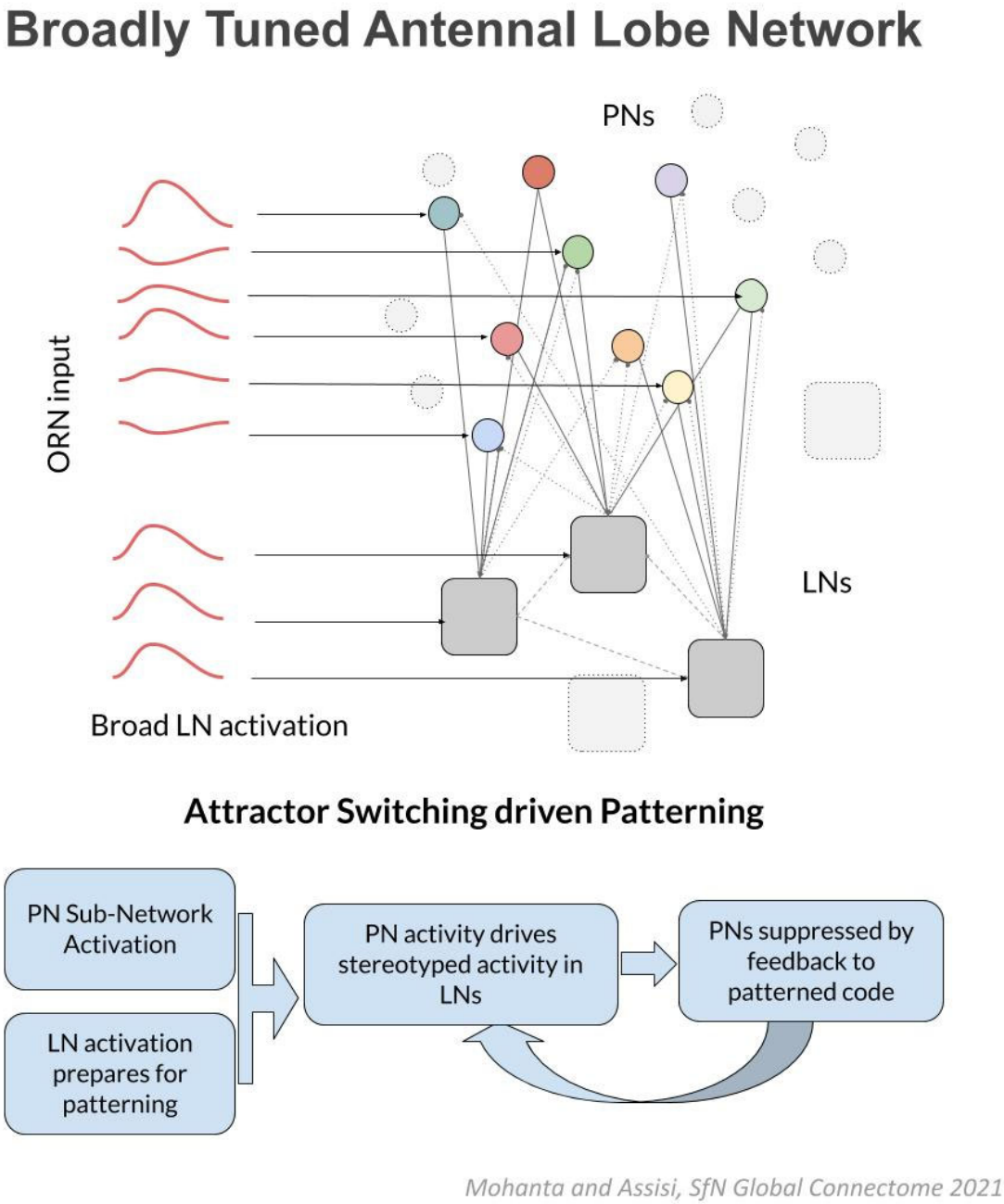
Abstract: The antennal lobe, the insect equivalent of the olfactory bulb in mammals, is a dense network of excitatory projection neurons (PNs) and inhibitory local interneurons (LNs). In the locust, this network is known to generate elaborate spatiotemporal patterns of activity in response to an odor. Computational models of the antennal lobe have typically assumed that the input to the inhibitory interneurons is narrowly tuned. That is, each interneuron receives input from a small fraction of olfactory receptor neurons (ORNs) and responds reliably to only a small subset of odor inputs. However, we know from a number of experiments, this is not the case. Interneurons arborise extensively across the antennal lobe and receive input from nearly 70% of all ORNs in the antennae. Therefore, odor input to different LNs is not as distinguishable as one would expect if the neurons were narrowly tuned. Using a realistic computational model of the locust antennal lobe, we sought to understand the effect that broad odor tuning has on the repertoire of spatiotemporal patterns the network can reliably generate. We modeled PNs and LNs as conductance-based neurons with properties matching in vivo observations. Spiking ORN input drove PNs and LNs in a manner that replicated experimentally observed statistics of PN responses. Earlier studies have shown that competitive interactions between interneurons can result in distinct patterns of activity; spatiotemporal attractors that are defined by the topology of the inhibitory sub-network. The inhibitory network can, in turn, entrain projection neurons. Here, we explore the hypothesis that excitatory input from projection neurons act as perturbations that drive the inhibitory network through a sequence of attractors. We examined the dynamics of switching as a function of the balance between excitatory (PN and ORN drive to the interneurons) and inhibitory (reciprocal interactions between interneurons) drive in the LN network.



Disclosures: **R. Mohanta:** None. **C. Assisi:** None.

Digital Abstract Session

P178. Central Olfactory Mechanisms

Program #/Poster #: P178.04

Topic: D.05. Olfaction and Taste

Support: ERC consolidators grant #616063
German Israeli Foundation grant I-1479-418.13/2018
Israeli Science Foundation grant #224/17

Title: Flexible Categorization in the Mouse Olfactory Bulb

Authors: *E. KUDRYAVITSKAYA, E. MAROM, H. SHANI-NARKISS, D. PASH, A. MIZRAHI;
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Abstract: The ability to group sensory stimuli into categories is crucial for efficient interaction with a rich and ever-changing environment. In olfaction, basic features of categorical representation of odours were observed as early as in the olfactory bulb (OB). Categorical representation was described in mitral cells (MCs) as sudden transitions in responses to odours that were morphed along a continuum. However, it remains unclear to what extent such response dynamics actually reflect perceptual categories and decisions therein. Here, we tested the role of learning on category formation in the mouse OB, using *in vivo* two-photon calcium imaging and behaviour. We imaged MC responses in naïve mice and in awake behaving mice as they learned two tasks with different classification logic. In one task, a 1-decision boundary task, animals learned to classify odour mixtures based on the dominant compound in the mixtures. As expected, categorical representation of odours, which was evident already in naïve animals, further increased following learning. In a second task, a multi-decision boundary task, animals learned to classify odours independent of their chemical similarity. Here, odour discrimination was based on the meaning ascribed to them (either rewarding or not). Following the multi-decision boundary task, odour representations by MCs reorganized according to the odour value in the new category. This functional reorganization was also reflected as a shift from predominantly excitatory odour responses to predominantly inhibitory odour responses. Our data shows that odour representations by MCs is flexible, shaped by task demands, and carry category-related information.

Disclosures: E. Kudryavitskaya: None. **E. Marom:** None. **H. Shani-Narkiss:** None. **D. Pash:** None. **A. Mizrahi:** None.

Digital Abstract Session

P178. Central Olfactory Mechanisms

Program #/Poster #: P178.05

Topic: D.05. Olfaction and Taste

Support: NIH Grant R-25-GMO5826416
NSF MRI Grant 1626326

Title: Chemoanemotaxic responses to selected odorants by house crickets

Authors: C. E. WEAVER, *V. D. SHIELDS;
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Abstract: The olfactory system of insects is a prominent model in neuroscience that allows us to address how insects detect, encode, and process olfactory stimuli. Stimulation of olfactory receptors may allow insects to detect and identify food, mating partners, and avoidance of predators. These receptors are located in cuticular sensory organs (sensilla) found on their long, paired, multi-segmented antennae. The cuticle of these sensilla is pierced by numerous small pores with underlying olfactory receptor cells, responsible for detecting odorants. Here, we studied the olfactory capability of the house cricket, *Acheta domesticus*, an omnivorous scavenger from the Gryllidae family. These insects use their antennae to detect chemosensory (smell and taste), mechanosensory (touch), and possibly thermo-hygrosensory (temperature and humidity) information. The overall aim of this study was to determine if food samples, deemed biologically-relevant from the habitat of the crickets (e.g., fruits, vegetables, proteins), elicited positive anemotaxis (oriented movement in response to odor from the food source) and acted as food baits. We tested fruit samples including, blueberries, strawberries, oranges, apples, red grapes, and bananas. Vegetable samples included, tomatoes, carrots, cauliflower, broccoli, onion, green beans, and green peppers. Sources of protein included, various flavors of cat food (e.g., poultry and fish). Female and male juveniles and adults were tested. We hypothesized that some of the food samples tested would elicit significant positive anemotaxis, while others would not. The fruits and vegetables tested proved to be ineffective food baits, while initial testing of protein samples showed promising results. If attractive, these samples could serve as possible baits, as house crickets are pests and can contaminate foods with their feces. This raises concerns about foodborne illnesses associated with microbial pathogens and creates health concerns, especially in developing countries.

Disclosures: C.E. Weaver: None. **V.D. Shields:** None.

Digital Abstract Session

P179. Taste: From Periphery To Central Circuits

Program #/Poster #: P179.01

Topic: D.05. Olfaction and Taste

Support: CIHR grant FDN-148424

Title: Mechanisms of lactic acid taste in Drosophila melanogaster

Authors: *M. STANLEY, B. GHOSH, Z. F. WEISS, J. CHRISTIAANSE, M. D. GORDON;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Sour has been studied almost exclusively as an aversive taste modality. Yet, recent work in the fruit fly, *Drosophila melanogaster,* shows that carboxylic acids differ in their behavioural valence and molecular mechanisms of detection. Here, we investigate the less explored pathway of gustatory acid attraction focusing on lactic acid. Lactic acid is produced by

fermentative bacteria and the smell is attractive to mammals and insects, but attractive taste qualities have not been described. We found that lactic acid is appetitive to *Drosophila*, even without olfaction, and sought to uncover the cellular and molecular mechanisms of this gustatory attraction by using multiple behavioural assays and *in vivo* calcium imaging of fly gustatory receptor neurons (GRNs). Silencing of sweet GRNs completely abolishes these attractive behaviours, and flips the valence to aversion, suggesting that lactic acid activates both attractive and aversive sensory neurons. Calcium imaging of GRNs with labellar lactic acid stimulation revealed strong activation of sweet GRNs and weaker responses in bitter GRNs. The calcium responses in sweet GRNs have unique kinetics with distinct peaks accompanying both stimulus onset and removal. Mutants for the broadly expressed ionotropic receptor 25a (IR25a) or the GR64 cluster of sweet gustatory receptors each display partially reduced attraction to lactic acid, while combined mutants avoid it. Strikingly, each receptor class is required for distinct aspects of the sweet GRN calcium response: IR25a mutants show a dramatic reduction in the onset response to lactic acid stimulation, while the GR64 cluster mutants show a significant reduction in the response due to lactic acid removal. Thus, lactic acid drives robust behavioural attraction by acting through multiple classes of receptors to activate a single sensory neuron class in physiologically distinct ways.

Disclosures: **M. Stanley:** None. **B. Ghosh:** None. **Z.F. Weiss:** None. **J. Christiaanse:** None. **M.D. Gordon:** None.

Digital Abstract Session

P179. Taste: From Periphery To Central Circuits

Program #/Poster #: P179.02

Topic: D.05. Olfaction and Taste

Support: UTSA Brain Health Consortium Graduate and Postdoctoral Seed Grant
NIH-SC2-GM130411

Title: In vivo calcium imaging of taste responses in the vagal ganglion

Authors: *B. E. FOWLER, S. HUMAYUN, L. J. MACPHERSON;
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Abstract: The majority of taste research has focused on the pathway from the frontal papillae (FP) through the chorda tympani nerve to the geniculate ganglion. This has left the pathway from the circumvallate (CV) through the glossopharyngeal nerve to the petrosal of the vagal ganglion largely unexplored. Relative to the FP, the CV boasts an enriched population of bitter responding TRCs, and whole nerve studies have shown that taste signals carried through the glossopharyngeal nerve favor bitter and sour tastants.Calcium imaging of the geniculate ganglion has profiled the responses of individual gustatory neurons to taste stimuli. These studies have shown highly specific responses by individual neurons to behaviorally relevant concentrations of tastants representing all five tastants. What are the response patterns of individual petrosal

neurons to taste stimuli? To image the activity of vagal neurons to taste stimuli applied to the posterior tongue, we surgically exposed vagal ganglia in transgenic animals of both sexes expressing GCaMP under the Snap25 promoter. We then imaged the exposed ganglia while perfusing the CV with a panel of taste stimuli. The imaging protocol was repeated three times per ganglion. We then analyzed the videos by taking the dF/F of the video and measuring changes in brightness for individual cells. Once responses had been verified, response profiles were compared to determine the proportion of cells responding to each taste. These results were compared to results obtained using this protocol to image geniculate neurons while stimulating the FP with the same tastant panel.

Disclosures: B.E. Fowler: None. **S. Humayun:** None. **L.J. Macpherson:** None.

Digital Abstract Session

P179. Taste: From Periphery To Central Circuits

Program #/Poster #: P179.03

Topic: D.05. Olfaction and Taste

Support: FSU Department of Biological Science

Title: Sex differences in sodium butyrate enhancement of conditioned taste aversion

Authors: *D. MUKHERJEE, T. HOUPT;
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Abstract: Animals can easily develop new food preferences and aversions through associative learning processes. If they consume a novel flavor and experience malaise, they develop an aversion towards that particular flavor, food or fluid. They also learn to prefer a particular flavor that provides positive consequences. Conditioned taste aversion (CTA) requires gene expression and histone acetylation, which can be enhanced by HDAC inhibitors such as sodium butyrate (NaB). Previously, it was shown that CTA is enhanced in male rats by NaB (Kwon and Houpt 2010). Here, we sought to determine whether enhancement of CTA using NaB is subject to sex-specific difference through changes in histone modification. Adult male (n=8) and female (n=8) Sprague-Dawley rats were placed on water restriction for 7 days with ad lib food to ensure intake on conditioning day. On the conditioning day they were given 30 min access to saccharin (0.125%) and then injected with lithium chloride (LiCl) (0.15M, 3ml/kg). Ten minutes later, half the rats were injected with NaB (0.3M, 12ml/kg) or NaCl (0.3M, 12ml/kg). Beginning the next day, 2-bottle 24-h preference tests of water vs. saccharin was carried out for 10 days. In preliminary results, the saline-injected rats showed moderate CTA which extinguished over the 10 days of preference testing. Male rats injected with NaB appeared to have a greater CTA then saline-injected rats. However, NaB did not appear to enhance CTA in the NaB-injected females. Thus, female rats appear to be less sensitive to the CTA-potentiated effects of NaB. This could be due to (a) differences in pharmacokinetics or (b) differences in gene-regulation during CTA learning and its modulation by histone acetylation. Future studies could explore the role of

estrogen on NaB effects, e.g., by testing the enhancement of CTA by NaB in ovariectomized female rats.

Disclosures: **D. Mukherjee:** None. **T. Houpt:** None.

Digital Abstract Session

P179. Taste: From Periphery To Central Circuits

Program #/Poster #: P179.04

Topic: D.05. Olfaction and Taste

Title: Gustatory cortex responses evolve through incidental taste exposures

Authors: ***V. L. FLORES**[1], J.-Y. LIN[2], D. B. KATZ[3];
[1]Furman Univ., Greenville, SC; [3]Dept Psychol, [2]Brandeis Univ., Waltham, MA

Abstract: Experience has been widely shown to impact learning and sensory perception. In the field of taste, familiarity with taste stimuli that later become the conditioned stimulus (CS) has long been known to reduce the strength of aversion learning when subsequently pairing with gastrointestinal illness (unconditioned stimulus [US]; Lubow 1973; De La Casa and Lubow 1995). Recently, we have demonstrated that even experience with "incidental" (i.e., non-CS and non-US) stimuli can also influence later learning: specifically, female Long Evans rats pre-exposed to salty and sour tastes developed a stronger learned aversion to novel sucrose. This work suggests that incidental taste experience changes the neural dynamics underlying the processing of familiar and new tastes in gustatory cortex (GC). Here, we begin to explore this possibility by evaluating how GC cortical responses to tastes themselves change over three days of exposure. Our results demonstrate that familiarity with tastes increases the discriminability of water and sodium chloride-evoked GC responses. Furthermore, as animals are familiarized to tastes, the overall percentage of cortical neurons showing identity- and/or palatability-related activity increases. In the held-unit analysis, we found that single-neuron responses to tastes show a greater absolute change in firing rate following taste exposure when compared to water exposure. These findings begin to characterize the impact of familiarity on neural dynamics of taste processing and give insight into how incidental taste experience impacts future learning.

Disclosures: **V.L. Flores:** None. **J. Lin:** None. **D.B. Katz:** None.

Digital Abstract Session

P179. Taste: From Periphery To Central Circuits

Program #/Poster #: P179.05

Topic: D.05. Olfaction and Taste

Support: F31 DC017660 LO
NIDCD 5R01DC007176 RFK

Title: Taste neuronsvary in degree of converging input from taste receptor cells.

Authors: *L. OHMAN, T. HUANG, Z. WHIDDON, R. F. KRIMM;
ASNB, Univ. of Louisville Sch. of Med., Louisville, KY

Abstract: Individual taste neurons show tremendous variation in peripheral morphology. The next step is to determine whether these morphological variations dictate variation peripheral connectivity to taste receptor cells (TRCs). Using a whole mount immunohistochemistry to stain taste buds combined with sparse cell genetic labeling of individual chorda tympani neurons, we analyzed the proximity of individual chorda tympani nerve fibers to TRCs using the Imaris software. We examined 77 separate fiber branches entering the taste bud, of these 52% contact (come within a distance below the resolution of the light microscope) one taste receptor cell (mean = 1.4, range= 0-4) whereas 36% contact more than one and 12% form no contacts. Of the fibers contacting TRCs, 87% of fibers contact TRCs of the same type and 13% contact TRCs of multiple types. Some fibers follow along a taste receptor cell for a considerable distance (13µm, long contacts), while others do not (short contacts, 3µm). Contacts are formed by all branch order types (1st-7th order) but most frequently with 3rd order branches. Next, we traced whole neurons from the base of the tongue. Some neurons (37.5%) innervate only one taste bud, branch sparsely (3-5 branch ends), and making 1-5 contacts with 1-2 taste receptor cells of the same type. Other neurons (62.5%) branch more robustly to innervate 3-5 taste buds, with 13-27 branch ends, making 7-14 contacts with 4-13 taste receptor cells of both types. The total length of the neuron in the taste bud predicts the range of contacts made with taste receptor cells innervated (r = 0.92). We suggest that sparsely branched neurons contacting only a few TRCs of the same type may be narrowly tuned, whereas neurons that branch robustly to innervate many taste receptor cells of different kinds may be broadly-tuned.

Disclosures: L. Ohman: None. **T. Huang:** None. **Z. Whiddon:** None. **R.F. Krimm:** None.

Digital Abstract Session

P179. Taste: From Periphery To Central Circuits

Program #/Poster #: P179.06

Topic: D.05. Olfaction and Taste

Support: The University of Nebraska at Omaha Office of Research and Creative Activity
Nu Rho Psi G. Andrew Mickley Undergraduate Research Grant

Title: Lingual nerve transection induces diffuse microglia response in nucleus of the solitary tract in Sprague-Dawley rats

Authors: *B. D. ANDERSEN, A. J. RIQUIER, S. I. SOLLARS;
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Abstract: The anterior tongue contains fungiform papillae which are innervated by the somatosensory lingual nerve (LN). In rats, each papilla contains a single taste bud that is

innervated by the gustatory chorda tympani nerve (CT). Despite the CT and LN relaying different types of sensation, damage to either nerve can result in loss in both sensory systems, with both the taste bud and papillae degenerating during developmental injury (Sollars, 2005; Omelian et al., 2016). This sensory interaction, while common in the brain, is highly unusual in the peripheral nervous system. Both the CT and LN project to adjacent, but distinct, subnuclei within the nucleus of the solitary tract (NTS) of the brainstem. Since the two nerves are interrelated in the periphery, and project to closely related subnuclei within the brain, we examined whether the CT and LN may share an immune response following damage to a single nerve. To that end, microglia, the primary indicators of a central immune response, were examined in both the lateral LN subnuclei and the medial CT subnuclei. Twelve male and 12 female Sprague-Dawley rats received unilateral LN transection at 65 days of age, with the CT kept intact. Brains were extracted 1, 2, 3, 5, 8, or 16 days post-surgery and the NTS sectioned (40 μm). Every other section was stained with Luxol Fast Blue and cresyl violet to visualize nerve-specific subnuclei. Alternating sections were used to analyze microglia in respective NTS subnuclei using an Iba1 antibody. Medial (CT) and lateral (LN) microglia response densities were quantified using Neurolucida (MBF Bioscience). The ventral subnuclei, innervated by neither nerve served as a control for the specificity of the response. Preliminary data indicate that although microglia increased in all transected-side NTS subnuclei by 5 days post-surgery, this increase in microglia density was significantly weaker in the medial subnuclei of the intact CT relative to the lateral subnuclei of the transected LN. Additionally, an increase in microglia density observed in the control ventral subnuclei indicates there may be a diffuse microglia response occurring more broadly in the NTS following transection of the LN.

Disclosures: B.D. Andersen: None. **A.J. Riquier:** None. **S.I. Sollars:** None.

Digital Abstract Session

P179. Taste: From Periphery To Central Circuits

Program #/Poster #: P179.07

Topic: D.05. Olfaction and Taste

Support: UTSA
Brain Research Foundation

Title: Using GRASP to Map the Connectivity of Peripheral Taste Circuits

Authors: *S. M. LANDON, L. MACPHERSON;
Biol., Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Precise re-wiring of gustatory neurons to distinct taste receptor cells (TRCs) within the taste bud happens continuously as TRCs undergo renewal. This rewiring of peripheral taste circuits is crucial for the preservation of taste perception over time; however, we do not currently have a way to monitor the formation and turnover of taste synapses during this dynamic process. Therefore, I have redesigned and employed GRASP (GFP Reconstitution Across Synaptic

Partners) in the peripheral taste system to visualize the synaptic connections between gustatory neurons and TRCs, and to probe the connectivity of candidate synaptic partners. GRASP is a novel technique that utilizes the reconstitution of genetically encoded split GFP proteins, called pre- and post-GRASP, in potential synaptic partners to visualize sites of synapse. First, I have created transgenic mice that express the cyan variant of the pre-GRASP (CRASP) protein under the PKD2L1 promoter, a marker for sour TRCs. Using immunohistochemistry, I have confirmed the expression of pre-CRASP proteins in sour TRCs. To complete CRASP reconstitution at taste synapses, I used a post-GRASP AAV which traffics the post-GRASP protein to the synaptic cleft and marks neurons with td-Tomato. After injecting the post-GRASP AAV into the NST of a PKD2L1 pre-CRASP mouse, I observed td-Tomato positive neurons contacting sour TRCs which elicited CFP puncta, marking the synaptic connections between sour TRCs and gustatory neurons. Recently, a population of geniculate ganglion neurons, marked by the expression of Penk, have been shown to be involved in relaying sour taste signals. To determine if Penk neurons make synaptic contacts with sour TRCs, I injected a PKD2L1 pre-CRASP; Penk-cre double transgenic mouse with cre-dependent post-GRASP AAV and found that these Penk neurons indeed make synaptic contacts with sour TRCs. Because I have validated that GRASP works in the peripheral taste system, I am expanding these studies by engineering more pre-GRASP transgenic mice for other taste modalities (bitter-GFP variant and sweet/umami-YFP variant) to map peripheral taste circuits and observe the dynamics of taste synapse turnover.

Disclosures: S.M. Landon: None. **L. Macpherson:** None.

Digital Abstract Session

P180. Hair Cells & Auditory System

Program #/Poster #: P180.01

Topic: D.06. Auditory & Vestibular Systems

Support: NIH DC012347
HHMI Gilliam Fellowship for Advanced Study

Title: The impact of persistent and resurgent voltage-gated sodium currents on excitability in vestibular ganglion neurons

Authors: *S. BAEZA LOYA, R. EATOCK;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: The vestibular inner ear transmits head-motion information to the brain via two populations of primary vestibular ganglion neurons (VGNs) which differ in the regularity of action potential (AP) timing (regular and irregular) and represent rate and temporal encoding, respectively (Jamali et al., Nat Comm 7:13229, 2016). Understanding the impact of diverse ionic currents on spike timing regularity is crucial to understanding how different sensory coding strategies arise. Although voltage-gated sodium (Nav) currents drive the rising phase of APs, their contributions to AP regularity differences are not fully understood. Nav currents through a

given α subunit can be transient (inactivating current), persistent (non-inactivating current), and/or resurgent (current flow after relief from inactivation block). We are interested in how these Nav current modes influence AP firing in VGNs. Whole-cell recordings were taken from mouse VGNs (postnatal days, P, 3-25) that were isolated and cultured overnight. However, it is difficult to experimentally distinguish the effects of these currents on spiking because we lack methods to isolate components. We therefore modified a computational conductance-based VGN model (Hight and Kalluri, J Neurophysiol 116:503, 2016; Ventura and Kalluri, J Neurosci 39:2860, 2019), which included generic transient Nav current, by adjusting and adding expressions for transient, persistent, and resurgent Nav components with properties based on our data. In a sample of 68 VGNs, all had large transient Nav currents that were blocked by 1 μM TTX; 37 had persistent Nav current (P3-25), 5 had resurgent Nav current (P17-20), and 3 (P17-20) had both persistent and resurgent currents. Application Nav1.6 blocker 4,9-anhydro-tetrodotoxin (4,9-ah-TTX) partially blocked both transient and persistent currents, indicating substantial portion of each is carried through Nav1.6 channels. In current clamp, 4,9-ah-TTX decreased neuronal excitability: increasing current threshold for spiking in all VGNs, and decreasing AP rate in sustained (regular) VGNs. In the computational model, adding persistent and resurgent current components had a negligible effect on firing by the model transient (irregular) VGN. For the model sustained (regular) VGN, adding persistent and resurgent currents decreased spike latency (delay relative to current step onset) and increased spike rate. Increasing Nav channel availability in the after-spike interval with persistent and resurgent currents may enhance the excitability of regular VGNs and so help to shape sensory encoding by the vestibular inner ear.

Disclosures: S. Baeza Loya: None. **R. Eatock:** None.

Digital Abstract Session

P180. Hair Cells & Auditory System

Program #/Poster #: P180.03

Topic: D.06. Auditory & Vestibular Systems

Support: NIH-NIDCD R01 DC012347

Title: Computational Model of Non-Quantal Synaptic Transmission at the Vestibular Hair Cell-Calyx

Authors: *A. GOVINDARAJU[1], A. LYSAKOWSKI[2], R. EATOCK[3], R. RAPHAEL[1]; [1]Rice Univ., Houston, TX; [2]Dept. of Anat. & Cell Biol., Univ. of Illinois at Chicago, Chicago, IL; [3]Neurobio., Univ. of Chicago, Chicago, IL

Abstract: Vestibular Type I hair cells detect and relay information on head motion to afferent neurons, which in turn guide motor reflexes that maintain gaze, balance, and our sense of orientation. Type-I hair cells transmit to a unique enveloping structure (Calyx) of the afferent neuron by both **quantal** release of glutamate from vesicles and **non-quantal (NQ)** flow of ions

through the pre-synaptic membrane of the hair cell into the synaptic cleft and through the post-synaptic calyx membrane. Recording electrodes cannot access the synaptic cleft between the vestibular hair cell-calyx (VHCC) synapse without disrupting its structure and function. As a result, ion concentrations [Ion] and electric potential (ϕ) within the synaptic cleft (SC) cannot be measured and the voltage of the post-synaptic membrane cannot be directly obtained. This has posed a barrier to understanding NQ transmission. We have developed a computational biophysical model of the synapse to overcome this limitation. The input to the model is a step or sinusoidal deflection of the hair bundle, and the outputs include the spatio-temporal profile of $[K^+]_{SC}$, $[Na^+]_{SC}$, ϕ_{SC} and ϕ_{Calyx}. To simulate the behavior of the system, the VHCC model uses expressions for K^+ and Na^+ electrodiffusion in the cleft, simplified Hodgkin-Huxley-style ion currents based on whole-cell recordings, and the cable equation. Electrical potentials within the hair cell and the afferent neuron are calculated as a function of $[K^+]_{SC}$ (K^+ modulation), and ϕ_{SC} (Ephaptic coupling) which alter driving forces of currents across the presynaptic and postsynaptic membranes. By allowing $[K^+]_{SC}$ or ϕ_{SC} to be held constant as required, the model allows the separation of these two processes which cannot be achieved experimentally. Currents through the low-voltage-activated K^+ conductance ($g_{K,L}$) on the hair cell and Kv7.4 and HCN channels on the post-synaptic membrane were compared. At rest and during stimulation of the hair bundle, the currents through $g_{K,L}$ and Kv7.4 were an order of magnitude greater than current through HCN. $g_{K,L}$ and Kv7.4 appear to be the foremost mediators of transmission during physiological operation. Model simulations suggest that both fast, frequency independent ephaptic coupling and low-pass K+ modulation can account for, and make non-quantal transmission between the hair cell and afferent neuron faster than chemical transmission.

Disclosures: **A. Govindaraju:** None. **A. Lysakowski:** None. **R. Eatock:** None. **R. Raphael:** None.

Digital Abstract Session

P180. Hair Cells & Auditory System

Program #/Poster #: P180.04

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01 DC016413
McKnight Scholar Award
Pew Scholar Award

Title: Topographic map for a developing vestibular peripheral circuit

Authors: *Z. LIU[1], D. G. HILDEBRAND[2], M. W. BAGNALL[1];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Harvard Med. Sch., Boston, MA

Abstract: In most sensory systems, a topographic map is formed by orderly projections from the periphery to the central nervous system. Although hair cells in the vestibular end organ are oriented topographically, the organization of their postsynaptic afferents is as yet unknown. Here

we report that the topographic map of vestibular afferents, at the level of the ganglion, is organized by both sensory tuning and developmental age. Using serial-section electron microscopy, we reconstructed all synaptic connections between 91 hair cells and 105 afferents of one utricle in the larval zebrafish. The relative position of the kinocilium and stereocilia of each hair cell reflected its orientation tuning. Near the line of the polarity reversal, a band of hair cells had the longest kinocilium and stereocilium with their (k/s) length ratio close to 1. These hair cells were presumed most mature and identified as striolar on the lateral edge of the macula. Outside this band, we categorized hair cells as extrastriolar (k/s ratio larger than 1.7) or developing / newborn (small kinocilium absolute length). We found that 16/105 utricular afferents were fully myelinated, presumably the earliest born, while other afferents were only partially myelinated or not myelinated at all. Myelinated utricular afferents preferentially innervated early-born striolar hair cells, with more synaptic ribbons, than unmyelinated afferents. The somata of these older myelinated afferents were also positioned more laterally in the utricular ganglion, with younger unmyelinated afferents located more medially. In adult animals, afferents derived from striolar and extrastriolar zones are known to encode phasic and tonic vestibular signals, respectively. Thus, our data suggest that the phasic pathway matures earlier to compensate for fast self-movements, and the tonic pathway develops later for slow self-movements. Moreover, we find the vestibular ganglion also organized by sensory tuning: most afferents in the rostral region of the ganglion innervated hair cells tuned to rostral tilt, whereas those in the caudal region innervated hair cells tuned to ipsilateral tilt. In all, we show that vestibular afferents contact hair cells with selective orientation and striolar identity, and they are topographically organized by both sensory tuning and age.

Disclosures: Z. Liu: None. **D.G. Hildebrand:** None. **M.W. Bagnall:** None.

Digital Abstract Session

P180. Hair Cells & Auditory System

Program #/Poster #: P180.05

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD R01 DC012347

Title: Bouton afferent terminal activity in the mouse utricle

Authors: *A. GONZALEZ GARRIDO, O. LOPEZ RAMIREZ, R. EATOCK;
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Abstract: Vestibular afferent neurons drive reflexes that stabilize gaze and head position. In mammals, vestibular afferent neurons terminate on hair cells with either bouton endings on type II hair cells or calyceal endings on type I hair cells. These terminals distribute differently in the functional zones of the sensory epithelium. The utricular epithelium, has striolar (central) and extrastriolar (peripheral) zones with anatomical and functional differences, including a difference in spike timing regularity. While whole-cell recordings from vestibular afferent calyces are

available in some rodent species, there have been no reported recordings from vestibular bouton terminals. Here, we report preliminary observations on the membrane conductances and action potential properties of mammalian vestibular bouton terminals. We used whole-cell patch clamp to record from boutons in the in vitro semi-intact preparation of CD1 mouse utricle (P10-21). This preparation conserves the hair cells, primary afferent innervation and mechanosensory pathway, allowing the study of primary afferent synapse mechanisms. Bouton morphology was revealed by fluorescent dye from the recording pipette. Bouton endings were tested for voltage-dependent conductances, voltage responses to current injection, and synaptic transmission evoked by deflecting the bundles of type I and type II hair cells. Boutons had HCN and voltage-gated K (KV) channels (IKDR, KLV and A-type). Striolar boutons were larger (3.4 ± 0.7 pF) than extrastriolar (2.1 ± 0.1 pF; $p = 0.03$) and, similar to calyces, had more negative resting potentials (-74.3 ± 1.4 mV vs. -67.7 ± 1.7 mV; $p = 0.04$), input resistances did not differ significantly. As with calyces, positive current steps evoked sustained and transient firing patterns. In dimorphic afferents, we demonstrated electrotonic propagation of non-quantal calyceal responses to bouton terminals in the same afferent. We recorded both regular and irregular spontaneous activity of bouton endings, without clear correlation with zone. In one case, we recorded a bouton on a bouton-only extrastriolar afferent; it had relatively small capacitance (1.8 pF), high input resistance (691.2 MΩ vs 255.7 ± 51.7 MΩ for 8 dimorphic ES boutons), small voltage-gated currents, and a transient spiking response to injected currents with a low current threshold (40 pA). These preliminary bouton data show a range of voltage-gated conductances and firing properties that have also been described in calyceal recordings. These data will help to build a model of how information from boutons and calyces is electrically integrated in vestibular afferent firing. Supported by NIDCD (R01 DC012347).

Disclosures: A. Gonzalez Garrido: None. **O. Lopez Ramirez:** None. **R. Eatock:** None.

Digital Abstract Session

P180. Hair Cells & Auditory System

Program #/Poster #: P180.06

Topic: D.06. Auditory & Vestibular Systems

Support: NIH grant R21-DC013181
NIH grant P41-RR004050

Title: Characterizing inner ear mitochondria

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Abstract: The main hypothesis of this study is that mitochondria in hair cells and inner ear sensory epithelia are non-homogeneous and that the structural and molecular differences they exhibit are related to their differential responses to ototoxic insults, such as aminoglycoside antibiotic toxicity, chemotherapeutics and noise, which can lead to deafness and dizziness. We utilized EM tomography and computer reconstruction methods in our structural analysis. Our results indicate that there are three populations of mitochondria of different sizes within the vestibular sensory epithelium (identified using surface area and volume measurements): large (found in the subcuticular plate region of central zone type I vestibular hair cells); medium (in all hair cells and afferents); and small (in efferent boutons). By constructing examples of each type, we can determine average volume and surface area for each type, as well as the number and type of cristae within each (lamellar, tubular or tubulo-lamellar). In addition, we are also studying the number, size, and locations of crista junctions (small intersections of cristae with the inner mitochondrial membrane) with respect to other important structures within the cell. For example, our structural findings have shown that in many hair cells, the cristae of mitochondria adjacent to the cuticular plate or to a striated organelle (SO) are perpendicular to, and polarized toward, the organelle. This indicates that these cristae, which are lamellar, align end-on with the cuticular plate and that the crista junctions (CJs) open towards the SO on that side of the mitochondrion. Crista junctions, which function as barriers to the diffusion of molecules inside mitochondria, thus concentrating the oxidative phosphorylation complexes that generate ATP, are thought to be key regulators for the release of apoptotic effectors. Our structural studies are also investigating the role of cytoskeletal filaments, known as tethers, that connect mitochondria to key structures in hair cells, such as striated organelles, cuticular plates or each other. We are exploring the many potential roles these tethers may serve, including positioning mitochondria near structures with high demands for energy or Ca^{2+} and facilitating crosstalk between organelles. We conclude that there are significant differences in morphology among these three mitochondrial sub-populations.

Disclosures: V. Babu: None. **N. Laban:** None. **R. Bahari:** None. **Z.S. Abdul:** None. **D. Ruyyashi:** None. **D.N. Marbina:** None. **S. Irukulla:** None. **A.S. Al-Rifai:** None. **P.B. Satani:** None. **I. Bhuptani:** None. **B.O. Zakkar:** None. **A.W. Al-Najjar:** None. **A.A. Patel:** None. **M. Lopez:** None. **S.D. Price:** None. **G. Perkins:** None. **A. Lysakowski:** None.

Digital Abstract Session

P180. Hair Cells & Auditory System

Program #/Poster #: P180.07

Topic: D.06. Auditory & Vestibular Systems

Support: ERC Grant project RATLAND
ISF F.I.R.S.T. Grant

Title: Spatial learning in a complex environment

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Abstract: We explore rat behavior and electrophysiology in a complex setting (the Rat Interactive Fantasy Facility, RIFF). The RIFF consists of a large circular arena (160 cm diameter) with 6 interaction areas (IAs) that each have a water port, a food port, and two loudspeakers. Rat behavior is monitored online using video tracking and nose-poke identification. Neural responses are recorded using telemetry (a 64-channel TBSI transmitter integrated in an Alpha-Omega SNR data acquisition system) or a logger on the head of the animal (RatLog-64, Deuteron Technologies). We trained rats to perform a sound localization task in the RIFF. Auditory cues consisted of 6 different modified human words that were played from each IA separately. When a rat reached the center of the arena, one of the sounds started playing (once every 2 seconds) and the rat had to identify the correct location and collect a reward (food or water) within 20 seconds. Trials were otherwise terminated by access to a wrong port or by time out. The rats were able to learn the task rapidly without guidance. Different rats showed different strategies of exploration and reward collection. Recording neural activity in primary auditory cortex during behavior, we found significant correlations between neuronal activity and a range of non-auditory behaviorally-related variables. Interestingly, behavioral outcomes were correlated with the neural responses before sound presentation, but not with the neural responses to sounds.

Disclosures: A. Polterovich: None. **M.M. Jankowski:** None. **A. Kazakov:** None. **J. Niediek:** None. **I. Nelken:** None.

Digital Abstract Session

P181. Auditory Processing: Temporal, Frequency, and Spectral Processing

Program #/Poster #: P181.01

Topic: D.06. Auditory & Vestibular Systems

Title: The effect of MAP2 gene deficiency on hearing

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Abstract: Microtubule-Associated Protein 2 (MAP2) is thought to critically contribute to the polymerization and stabilization of microtubules in neurons. MAP2 gene knockout C57BL/6 (MKO) mice, however, were fertile, developed normally and had no apparent abnormality. Thus, detailed physiological functions of MAP2 in living organisms have not been fully clarified. We recently found that the strain shows poor auditory responsiveness including weak auditory startle reflex. Here focused on the functions of the MAP2 gene in hearing by examining the auditory brainstem response (ABR) in MKO and wild-type (WT) mice. A measurement electrode was placed in the inferior colliculus, and sound stimuli were presented using 10 frequencies of 2, 4, 8,

16, 32, 50, 60, 64, 68, and 72 kHz. The sound pressure level was varied from 0 to 90 dB SPL in 5 dB steps. The recording was performed under anesthesia. The minimum audible sound pressure levels (auditory thresholds) were estimated from the presence of wave V of ABR, the inferior colliculus response. The auditory thresholds of WT mice were comparable to those of previous reports over all frequencies. The auditory thresholds of MKO mice were significantly higher than those of WT in the 2-32 kHz range with 16 kHz stimulus showing the maximum increase in the auditory threshold by 40 dB (2&4 kHz : $p<0.05$, 8&32 kHz : $p<0.01$, 16 kHz : $p<0.001$). In the frequency above 50 kHz, the ABR was not confirmed in MKO mice. In addition, decrees of ABR amplitude from wave I to V were relatively equivalent, suggesting that the increment of the threshold was primarily caused by unresponsiveness related to wave I (i.e., cranial auditory nerve), or earlier auditory process. We further assessed the morphology of MKO mice's auditory periphery and found no physical defects in the tympanic membrane, ossicles, or the organ of Corti. Together with a previous study showing the MAP2 expression in cochlea hair cells, our data suggest that MAP2 gene deficiency might cause an increase in the auditory threshold by disrupting the function of hair cells.

Disclosures: K. Tanaka: None. **T. Miyasaka:** None. **A. Harada:** None. **K.I. Kobayasi:** None.

Digital Abstract Session

P181. Auditory Processing: Temporal, Frequency, and Spectral Processing

Program #/Poster #: P181.02

Topic: D.06. Auditory & Vestibular Systems

Title: Effects of NMDA Receptor Antagonism on Mismatch Negativity in Rodents

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Abstract: Various neuropsychiatric conditions are associated with impairments in cortical information processing that can be measured via electroencephalography (EEG) using sensory evoked responses. Furthermore, these deficits can be modeled in rodents and are widely considered a robust translatable biomarker between humans and rodents. Mismatch negativity (MMN) is a type of sensory-evoked response that probes the ability of the cortex to store a mnemonic memory of repeating tones (standard tones) in order to respond differentially to a qualitatively different tone (e.g. pitch deviant tone). Deficits in MMN generation are one of the most widely replicated neurophysiological findings in schizophrenia and can be re-produced by N-methyl d-aspartate (NMDA) receptor antagonism, suggesting a role for NMDA signaling in both the generation of MMN responses and schizophrenia pathology. The current study sought to determine whether and to what extent NMDA modulation can impact rodent MMN. Two approaches were explored: 1) acute NMDA antagonism with (+)-MK-801 hydrogen maleate (MK-801) and 2) a sub-chronic phencyclidine hydrochloride (PCP) paradigm to explore possible sustained effects. Male Sprague Dawley rats were implanted with subcutaneous telemeters that wirelessly transmit EEG signal recorded over the frontal cortex. After recovery, separate cohorts

were administered either an acute dose of MK-801 (0.1 or 0.3mg/kg, i.p.) or were enrolled in a sub-chronic PCP (scPCP) paradigm (7 days of 5mg/kg PCP, BID). Auditory stimuli were delivered in a sound-attenuating chamber at 6kHz and 8kHz in a flip-flop sequence. Average evoked responses to 8kHz standard tones were subtracted from 8kHz deviant evoked responses to calculate a difference wave (i.e. the mismatch response). Rats displayed a similar neuro-oscillatory signature to human MMN with deviant tones producing significantly larger evoked responses in the negative (N1: 35-50ms post-tone) and positive (P2: 50-120ms) components of the MMN-complex compared to standard tones. NMDA antagonism with MK-801 and scPCP differentially impacted these components. Overall, MK-801 was able to produce acute and reversible dose-dependent MMN deficits in both the peak amplitude and area under the curve of the N1 (0.3mg/kg) and P2 (0.1 & 0.3mg/kg) components. Conversely, scPCP administration only impacted the P2 component but this effect was sustained for 7 weeks post-washout. The current results demonstrate that NMDA antagonism impacts deviance detection in rodents and highlights two putative models of pharmacologically-induced MMN deficits. The differential impact of these models on the MMN-complex should be considered.

Disclosures: S. Kabu: None. **D.J. Graziano:** None. **M.M. Sidor:** None.

Digital Abstract Session

P181. Auditory Processing: Temporal, Frequency, and Spectral Processing

Program #/Poster #: P181.03

Topic: D.06. Auditory & Vestibular Systems

Title: Clozapine dose-dependently improves 40 Hz auditory steady-state response but partially protects against MK-801-induced disruption.

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Abstract: Auditory steady-state response (ASSR) refers to the entrained activity of cortical neurons to a repetitive auditory stimulus, as measured by electroencephalography. Entrainment is quantified by time-frequency measures such as evoked power, intertrial coherence (ITC), and induced power. ASSR at 40 Hz is especially reduced in schizophrenia patients, likely due to reduced NMDA-mediated sensory transmission, a proposed pathophysiological mechanism. In the current study, we confirmed this hypothesis using a high affinity, non-competitive NMDA receptor inhibitor, MK-801, in chronically implanted female rats (n=12). Auditory stimuli were presented (20 clicks for 0.5 seconds (40 Hz), 65 dB, 2 sec inter-stimulus interval) at 30, 60, 90- and 120-minutes post-MK-801 administration (IP). Recordings were made from the vertex electrode using a cerebellar reference. Evoked power and PLF were reduced in a dose-dependent manner by MK-801 (0.05-0.15 mg/kg), while induced power was increased. These effects mimic changes seen in schizophrenia patients. Next, we tested the effect of clozapine (an atypical antipsychotic; 2.5, 5 & 10 mg/kg, sc), which has been shown, among other effects, to augment NMDA transmission. Clozapine doses were chosen to reflect clinically relevant D2 occupancy.

Clozapine by itself improved the 40 Hz ASSR (evoked power, ITC, and induced power) in a dose and time-dependent manner. Next, we tested if clozapine can protect against MK-801-induced 40 Hz ASSR deficits. For this, clozapine (5 or 10 mg/kg, sc) or vehicle were given 20 minutes before MK-801 (0.1 mg/kg, IP) administration. A vehicle/vehicle group was a negative control. ASSR was recorded at 30, 60, 90- and 120-minutes post-MK-801 injection. Compared to veh+veh, veh+MK801 produced robust deficits in 40 Hz ASSR measures at all time points. Clozapine (10 mg/kg only) provided significant but partial and temporally short-lived (~ 60 min) protection. These studies highlight the following: 1. Clozapine improves sensory transmission by itself; 2. The partial efficacy of clozapine in protecting against NMDA disruption points to its therapeutic limitation in normalizing NMDA neurotransmission; 3. Our work illustrates the utility of the 40 Hz ASSR as a circuit-based pharmacodynamic biomarker for drug development.

Disclosures: M.U. Raza: None. **S.V. Digavalli:** None.

Digital Abstract Session

P181. Auditory Processing: Temporal, Frequency, and Spectral Processing

Program #/Poster #: P181.04

Topic: D.06. Auditory & Vestibular Systems

Support: NINDS F31-NS111849
NINDS T32-NS007431
NIDCD R01DC017516
Pew Biomedical Scholarship
Whitehall Foundation
Klingenstein-Simons Fellowship
Foundation of Hope

Title: Discrete Functional Subnetworks within Secondary Auditory Cortex Integrate Multi-Frequency Sounds with Synchronous Onsets

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Abstract: Integration of multi-frequency sounds into a unified perceptual object is critical for recognizing syllables in speech. This “feature binding” relies on the precise synchrony of each component’s onset timing, but little is known regarding its neural correlates. We find that multi-frequency sounds prevalent in vocalizations, specifically harmonics, preferentially activate the mouse secondary auditory cortex (A2), whose response deteriorates with shifts in component onset timings. The temporal window for harmonics integration in A2 was broadened by inactivation of somatostatin-expressing interneurons (SOM cells), but not parvalbumin-expressing interneurons (PV cells). Importantly, A2 has functionally connected subnetworks of neurons encoding harmonic, but not inharmonic sounds. These subnetworks are stable across days and exist prior to experimental harmonics exposure, suggesting their formation during

development. Furthermore, A2 inactivation impairs performance in a discrimination task for coincident harmonics. Together, we propose A2 as a locus for harmonic integration, which may form the circuit basis for vocal processing.

Disclosures: A.M. Kline: None. **D.A. Aponte:** None. **H. Tsukano:** None. **A. Giovannucci:** None. **H.K. Kato:** None.

Digital Abstract Session

P181. Auditory Processing: Temporal, Frequency, and Spectral Processing

Program #/Poster #: P181.05

Topic: D.06. Auditory & Vestibular Systems

Support: Tsvi Yanai
ERC
ISF

Title: Gap responses in awake rats: on-off response adaptation and omission responses reveal a representation of prediction errors

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Abstract: The detection of sensory deviance is an important computational task that may provide an evolutionary advantage to organisms. Deviance can be detected by using a memory trace of past stimuli to predict what may occur next. Large prediction errors mark sensory deviance. In human studies, mismatch negativity (MMN) has been suggested to indicate automatic deviance detection. In animal models, stimulus-specific adaptation (SSA) shows some corresponding features. However, while MMN has been shown in response to omitted stimuli, omission responses are rare in animal studies. Using temporal gaps, we show that offset responses, which were absent in anesthetized rats, were widely present in awake rats. These offset responses showed SSA, similar to the onset responses. Most importantly, continuous noise bursts were embedded as deviants in a sequence of gap stimuli, robust omission responses to the expected gaps that did not occur were evoked, reflecting prediction error under adaptation. These omission responses, together with the on-off response SSA, reveal a representation of prediction error in auditory cortex neurons.

Disclosures: B. Awwad: None. **M. Jankowski:** None. **A. Poltorovich:** None. **S. Bashari:** None. **I. Nelken:** None.

Digital Abstract Session

P181. Auditory Processing: Temporal, Frequency, and Spectral Processing

Program #/Poster #: P181.06

Topic: D.06. Auditory & Vestibular Systems

Support: Human Frontier Fellowship LT000220/2018
NIH Grant 5T32DC000023-35
BRAIN Initiative NCS-FO 1734744
AFOSR FA9550-14-1-0398NIFTI
ONR N00014-17-1-2736

Title: Effect of clutter on the neural representation of acoustic objects in the bat midbrain

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Abstract: Auditory processing in complex environments is a challenge for humans and animals alike. Auditory objects are the individual sound sources that comprise auditory scenes. Deciphering the neural encoding of auditory objects is a first step to understanding how rich soundscapes are perceived. Echolocating bats are a powerful animal model to explore this problem. Aerial hawking bats hunt on the wing, and actively probe auditory objects in their surroundings via echolocation. Bats produce brief ultrasound signals, interrupted by periods of silence, rendering echo snapshots of auditory objects which change with viewing angle, distance, and the presence of clutter and acoustic distractors. Relying on dynamic and discontinuous echo information, bats simultaneously track prey and avoid collisions with obstacles in highly cluttered environments. This natural task requires that bats process spatially and temporally overlapping echoes to make split-second decisions, exemplifying the need for rapid object discrimination and classification. The effects of both physical clutter and masking noise on bat sonar discrimination have been explored. However, it is unknown if there are conditions in which acoustic clutter may enhance the representation of auditory targets. In this work we studied the responses of neurons in the auditory midbrain of the big brown bat, *Eptesicus fuscus*, to echoes from 3-dimensional objects in the vicinity of physical clutter. Our analysis reveals population coding of echo features that can be used to discriminate between auditory objects. We further investigated the effects of environmental clutter on the discrimination of auditory objects. We discovered that effect of clutter on object discrimination is highly variable, depending on object properties and object distance from clutter. In many conditions clutter predictably impaired discrimination of auditory objects. However, in some instances clutter enhanced object features, leading to higher levels of discrimination. These findings suggest that in natural settings, environmental clutter may actually provide beneficial information to bats engaged in target tracking and provides further evidence in a growing body of literature that noise is not universally detrimental to sensory encoding.

Disclosures: **K.M. Allen:** None. **A. Salles:** None. **S. Park:** None. **M. Elhilali:** None. **C.F. Moss:** None.

Digital Abstract Session

P181. Auditory Processing: Temporal, Frequency, and Spectral Processing

Program #/Poster #: P181.07

Topic: D.06. Auditory & Vestibular Systems

Support: RO1 AG050548, F31 AG055236, McKnight Brain Research Foundation

Title: Auditory and visual system function and white matter condition is differentially impacted by normative aging in macaques

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Abstract: Normative brain aging results in decreased function across multiple sensory systems that compromises an older individual's ability to detect and process behaviorally relevant information and can substantially reduce quality of life. Deficits in auditory and visual processing, in particular, are commonly encountered by older individuals due to age-associated pathologies at the level of the cochlea and eye, and from multiple changes that occur along the ascending auditory and visual pathways that further reduce sensory function in each domain. One fundamental question that remains to be directly addressed is whether the structure and function of the central auditory and visual systems follow similar trajectories across the lifespan, or sustain the impacts of brain aging independently. Advances in the quality and precision of sensory prosthetics have made it clear that distinct sensory deficits require unique approaches, and precisely understanding how particular age-associated neurobiological changes impact different facets of sensory processing is critical for optimizing intervention strategies that maintain sensory function in older individuals. The present study used diffusion magnetic resonance imaging and electrophysiological assessments of auditory and visual system function in adult and aged macaques to better understand how age-related changes in white matter connectivity at multiple levels of each sensory system might impact auditory and visual function. Sensory processing and sensory system fractional anisotropy (FA) were both reduced in older animals compared to younger adults. Corticocortical FA was significantly reduced only in white matter of the auditory system of aged monkeys, while thalamocortical FA was lower only in visual system white matter of the same animals. Importantly, these structural alterations were significantly associated with sensory function within each domain. Together these results indicate that age-associated deficits in auditory and visual processing emerge in part from microstructural alterations to specific sensory white matter tracts, and not from general differences in white matter condition across the aging brain. Accounting for this sort of

specificity will aid in designing individualized intervention strategies that optimally correct and maintain brain function across the lifespan.

Disclosures: **D.T. Gray:** None. **N.M. De La Peña:** None. **L. Umapathy:** None. **S.N. Burke:** None. **J.R. Engle:** None. **T.P. Trouard:** None. **C.A. Barnes:** None.

Digital Abstract Session

P182. Auditory Processing: Vocalizations and Natural Sounds

Program #/Poster #: P182.01

Topic: D.06. Auditory & Vestibular Systems

Support: Autism Speaks Predoctoral Fellowship 11100

Title: Methyl CpG Binding Protein 2 in Parvalbumin Cortical Interneurons is Critical for Processing Ultrasonic Vocalizations

Authors: *D. RUPERT, A. PAGLIARO, S. SHEA;
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Abstract: Title: Methyl CpG Binding Protein 2 in Parvalbumin Cortical Interneurons is Critical for Processing Ultrasonic Vocalizations
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Background: Rett Syndrome (RTT) is a pervasive, neurodevelopmental disorder previously classified as an Autism Spectrum Disorder and caused by loss of function mutations in the X-linked gene methyl CpG binding protein 2 (*MeCP2*). *MeCP2* codes for the protein by the same name, a ubiquitously expressed transcription factor that maintains mature neuronal synapses.
Objectives: Our goal is to determine the contribution of cell-type specific *Mecp2* mutation to disruptions of auditory cortical circuitry and plasticity that degrade social vocal perception in a mouse model of Rett syndrome.
Methods: To achieve this goal, we use a cell-type restricted *Mecp2* mutant mouse model in combination with a behavioral paradigm that relies on vocal communication. We employ pup retrieval a natural, auditory-dependent, learned maternal behavior that we report to be impaired by *Mecp2* mutation. Maternal retrieval is cued by ultrasonic vocalizations (USVs)- high-pitched distress calls emitted by isolated infant pups. Our previous work suggests lack of plasticity in auditory cortex (ACtx), a brain region required to detect and interpret vocalizations, underlies impaired retrieval performance. Here we build on these findings by parsing out the contributions of specific subclasses of inhibitory interneurons (i.e. parvalbumin-, somatostatin-, and vasoactive intestinal peptide- positive populations) to auditory processing. We examine the effects of restricted *Mecp2* mutation in these inhibitory interneuron subclasses on pup retrieval behavior, *in vivo* neural circuit activity, and calcium imaging in the ACtx in response USVs.
Results: We find *Mecp2* mutation restricted to the parvalbumin positive (PV+) cell type is

impairs pup retrieval behavior, and degrades ACtx neuronal activity in response to pup vocalizations.
Conclusions: This work focuses on functional consequences of *Mecp2* mutation to auditory processing circuitry that expands our understanding of the underlying etiology of RTT and informs pathophysiological mechanisms underlying neurodevelopmental disorders more broadly.

Disclosures: D. Rupert: None. **S. Shea:** None. **A. Pagliaro:** None.

Digital Abstract Session

P182. Auditory Processing: Vocalizations and Natural Sounds

Program #/Poster #: P182.02

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01 MH106656

Title: Vocal perceptual category boundaries are modified by maternal experience in female mice

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Abstract: Only a fraction of sounds an organism encounters are behaviorally significant. One example is vocalizations, which occur against a backdrop of sounds with overlapping features, and also exhibit substantial variability. This presents two competing challenges: detection must be selective enough to minimize false alarms while also being tolerant to natural variation. The equilibrium of these demands can result in 'categorical perception', in which steep perceptual boundaries exist along a continuous stimulus dimension. Here we use a vocally-motivated pup retrieval behavior to reveal how the brain adjusts and enforces perceptual boundaries between vocal signals and other sounds. When pups are separated from their nest, they emit an ultrasonic vocalization (USV) that maternally-experienced mice can use to locate and retrieve the pups back to the nest. We have developed a high-throughput, quantitative behavioral paradigm where nulliparous female mice are water-deprived and trained in a probabilistic go/no-go task. They are conditioned to lick a water spout in response to playback of a USV, and to withhold licking in response to a synthetic tone. Once stable criterion performance (>80%) is achieved, the mice are separated into two groups: mice that are housed with pups and gain maternal experience ('surrogates'), and mice that have never been exposed to pups ('naïves')., We next present a set of novel sounds to the mouse as infrequent and unrewarded catch trials (including bandwidth-limited noise and pitch-shifted USVs), and we use licks to the novel stimuli as a measure of perceived similarity to natural USVs. These infrequent stimuli are never rewarded, and USVs are only rewarded on 80% of trials, thus the mouse receives no instruction with regard to catch trials. Nevertheless, we find that responses to the synthetic sounds show evidence of learning between initial and late trials that differs in naives and surrogates. Initial responses of surrogates towards broadband sounds in the range of USVs are similar to those of the Go-Cue, while naïve mice tend to initially have lower response rates to those same sounds. Late responses of surrogate

mice toward those same sounds were generally lower than those of the naïve mice. These results align with electrophysiological data in the primary auditory cortex done before and after behavioral training, suggesting that auditory cortex neurons adjust their firing rate following both pup exposure and task learning. Taken together, our results shed light on how vocal perceptual filters are optimized during maternal experience, and will provide insight into how categorical perception contributes to speech processing and learning.

Disclosures: L. Shen: None. **S.D. Shea:** None.

Digital Abstract Session

P183. Auditory Processing: Cellular and Circuits

Program #/Poster #: P183.01

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Brain Initiative R01NS104911
NIH P41GM103412

Title: Partial reconstruction of the canonical microcircuit of owl ICX

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Abstract: We report new findings towards the long-term effort to reconstruct the canonical microcircuit of the owl auditory space map. Previous work applied the volume electron microscopy (EM) method of serial block face EM (SBEM) to analyze a 0.25 x 10^6 μm^3 volume of tissue containing a peroxidase-labeled space-specific neuron (SSN) from the owl inferior colliculus. We discovered that SSNs are studded with a novel type of dendritic spine, toric spines, named for their topological holes. Here we report SBEM imaging and preliminary analysis of an unlabeled SSN in a larger volume (2.4 x 10^6 μm^3), revealing the cytoplasmic features of the toric spines with significantly more detail throughout. Axons projecting horizontally and onto SSNs within the external nucleus of the inferior colliculus (ICX) were labeled *in vivo*, prospectively imaged by 2-photon microscopy, and the axon-laden region was isolated for SBEM. This volume contains >50 SSNs and an estimated >5,000,000 synapses. In comparison to the previous volume: 1. Asymmetric synapses with spherical vesicles are clearly distinguishable from symmetric synapses with pleiomorphic vesicles. 2. The internal structure of putative toric spines is apparent. They contain mitochondria, not found in typical mushroom spines, and smooth endoplasmic reticulum (SER). 3. Most postsynaptic structures are rife with SER, likely involved in Ca sequestration and lipid metabolism. 4. Astrocytic processes identified by the presence of glycogen particles and intermediate filaments (presumably GFAP) are intimately associated with SSNs. 5. The spatial density of myelinated axons is higher than in the previous volume, likely reflecting a more lateral position within ICX. 6. Preliminary, incidental observations also indicate that Nodes of Ranvier on different myelinated axons appear spatially

clustered, speculatively suggesting a possible potential role in coordinating action potential firing properties among nearby axons. 7. Nuclear pores, sub-surface cisternae, heminodal organization and many other classical features of neuronal ultrastructure are clearly apparent. To extract the microscale connectome, we are currently using a team of annotators to produce dense reconstructions of small sub-volumes. These will be used to train newly developed machine learning algorithms for automated segmentation. Finally, segmented axons will be registered to the 2-photon volume to identify functional connections within ICX. In summary, the results to be shared are expanding our knowledge of the ultrastructure of toric spines and SSNs, and establish a clear path towards mapping the local synaptic network in which they are embedded.

Disclosures: **P. Harrast:** None. **E. Bushong:** None. **D. Sanculi:** None. **M. Madany:** None. **W. Wong:** None. **P. Jain:** None. **V. Pierce:** None. **C. Resngit:** None. **C. Sterner:** None. **M. Ellisman:** None. **W.M. DeBello:** None.

Digital Abstract Session

P183. Auditory Processing: Cellular and Circuits

Program #/Poster #: P183.02

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant SC1 GM118242-03
UMass startup funds

Title: The role of PnC glycinergic interneurons in prepulse inhibition in the context of aging

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Abstract: BACKGROUND: Prepulse inhibition (PPI) of the auditory startle reflex is the suppression of an acoustic startle response to an intense stimulus when a weak stimulus (prepulse) precedes the startle stimulus (pulse). PPI is the classic operational measure of sensorimotor gating, and is used both in humans and animal models. As a hallmark of schizophrenia, PPI deficits are also found in other neuropsychiatric disorders and are associated with deficits in attention. In addition, both human and rodent studies show age-dependent alterations of PPI, but the underlying mechanisms remain unclear. The brainstem caudal pontine reticular nucleus (PnC) is at the core of the PPI pathway, relaying sensory inputs from several brain regions to motor neurons. The PnC contains neurons expressing the glycine transporter type 2 (GlyT2), which are widely distributed and closely intermingle with startle-mediating giant neurons. $GlyT2^+$ PnC inhibitory neurons are found in both humans and mice and were shown to play a major role in behavioral arrest (Giber et al, 2015). Although glycine inhibits PnC giant neurons and reduces the startle response in rats (Koch and Friauf, 1995; Geis and Schmid, 2011), the contribution of $GlyT2^+$ PnC inhibitory neurons to PPI remains unknown. Therefore, here, we investigated the role of $GlyT2^+$ PnC inhibitory neurons in PPI and in the context of aging. METHODS: To drive Archaerhodopsin expression (Arch3.0; optogenetic inhibitory tool

sensitive to green light) in GlyT2$^+$ PnC neurons, adult GlyT2-Cre mice (3-6 months old) were injected with AAV-Dj-EF1a-DIO-Arch3.0-eYFP in the PnC. The acoustic startle response (ASR) and PPI were performed 4 weeks post injection, both in the presence and in the absence of the photo-inhibition in GlyT2-Cre mice, which were compared to control adult C57BL/6J mice. In addition, the effect of age on PPI was assessed in another cohort of older GlyT2-Cre mice, aged between 9-12 months. Using these mice, GlyT2$^+$ PnC neurons were also photo-inhibited to determine their contribution to the age-related reduction in PPI.
RESULTS: Photo-inhibiting GlyT2$^+$ PnC neurons had no effect on ASR in adult GlyT2-Cre mice, their control C57BL/6J littermates and older GlyT2-Cre mice. Interestingly, photo-inhibition significantly decreased PPI ($p=.028$) but only in adult GlyT2-Cre mice, particularly at the 30 ms ($p=0.048$) and 50 ms ($p=0.023$) inter-stimulus intervals between the prepulse and the pulse.
DISCUSSION: The findings suggest that: 1) GlyT2$^+$ PnC inhibitory neurons contribute to PPI in adult mice and 2) the contribution of GlyT2$^+$ PnC inhibitory neurons to PPI declines with age, providing a possible explanation for age-related PPI impairments.

Disclosures: W. Huang: None. **K. Fenelon:** None.

Digital Abstract Session

P183. Auditory Processing: Cellular and Circuits

Program #/Poster #: P183.03

Topic: D.06. Auditory & Vestibular Systems

Support: European Research Council ERC-StG-678120 (RobSpear)

Title: A convolutional neural-network model of the inner-hair-cell and auditory-nerve-fiber complex

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Abstract: Analytical descriptions of inner-hair-cell (IHC) and auditory-nerve-fiber (ANF) processing have evolved over the years, with IHC transduction models shifting from simplified low-pass filters to detailed conductance models which include basolateral outward K^+ currents. State-of-the-art models of the IHC-ANF synapse complex describe the vibrations of the IHC stereocilia based on the mechanical drive to the IHC and model ANF spikes or instantaneous firing rates as resulting from the depletion and replenishment of different neurotransmitter stores. While such sensory models have progressed to accurately capture the nonlinear and dynamic properties of the neuronal processes associated with hearing, they typically comprise mechanistic descriptions and coupled sets of ordinary differential equations, rendering these models slow to compute.Here, we present a hybrid computational neuroscience - deep neural network (DNN) framework which simulates auditory IHC-ANF processing, to offer a fast and differentiable model which can be used in large-scale neuronal network models. Based on a state-of-the-art

biophysical model of the auditory periphery (including human cochlear mechanics, IHC and ANF processing), we trained several convolutional neural network (CNN) architectures to learn the computations performed by the IHC-ANF complex (CoNNear$_{IHC-ANF}$). We determined the hyperparameters in these architectures (different layers, filter lengths, input and context windows, activation functions) on the basis of well-known single-unit IHC and ANF properties derived from experimental neuroscience studies. The model was trained using an acoustic speech corpus, and its performance evaluated using basic acoustic stimuli which were not included in the training set. The final CoNNear$_{IHC-ANF}$ model offers a 70-fold speed-up factor on a CPU and a 280-fold factor on a GPU when compared to the analytical IHC-ANF model processing computations. When connected to a DNN-based cochlear model, preferably CoNNear$_{cochlea}$, population responses (e.g. CAP, ABR wave-I) can be simulated across a large number of cochlear tonotopic locations, and be used for backpropagation purposes. Our auditory periphery framework can be applied for the development of large-scale neural-network circuits aimed to advance our knowledge of unknown neuronal systems such as the brainstem and subcortical pathways, or the human auditory cortex. Work supported by European Research Council ERC-StG-678120 (RobSpear)

Disclosures: **F. Drakopoulos:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ghent University. **D. Baby:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ghent University. **S. Verhulst:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ghent University.

Digital Abstract Session

P183. Auditory Processing: Cellular and Circuits

Program #/Poster #: P183.04

Topic: D.06. Auditory & Vestibular Systems

Support: BSF-NSF Grant 2016688
ANR grant 17-NEUC-0005-01a

Title: The geometry of sound representation along the auditory pathway

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Abstract: Ongoing activity in the absence of stimulation is known to reflect aspects of the evoked activity but much remains to be understood about how sounds are represented. Here, we study features of the spontaneous activity of the sound-responsive (R) and sound-unresponsive

(U) neurons at three stages of the ascending auditory pathway. Units were recorded extracellularly in the Inferior colliculus (IC, n=4), Medial Geniculate Body (MGB, n=4) and primary Auditory Cortex (A1, n=5) of anesthetized rats using Neuropixel probes. Sound stimulation included pure tones - 37 frequencies (6 tones/octave) at 8 possible attenuations (30-100 dB SPL). The ongoing activity was recorded before each session for about 10 seconds. Units were extracted using Kilosort followed by manual curation. For each stimulus, the total number of spikes, k, was counted and the spontaneous rate, λ, estimated. These were used to compute the probability, ps, of emitting k spikes or more in response to stimulus s assuming a homogeneous Poisson process with rate λ. A cluster was classified R if ps<0.05/nstim for one stimulus at least. We observed that i) the spontaneous firing rates of R and U were distinct and their difference increased from IC to A1; ii) the average pairwise spike correlations between nearby R clusters in MGB and A1 were higher than the correlations between R and U clusters or between U clusters; and iii) the spatial mixing between R and U increased on average from IC to A1. Overall, R and U populations exhibited distinct activities despite their anatomical mixing. We also analyzed the topological structure of the activity in the three stations. This activity does not allow for low-dimensional Euclidean representations, and instead requires mappings with hyperbolic geometry. This was true for all three stages of auditory processing. However, responses only within the IC, and not in the MGB and A1, were consistent with a uniform distribution of points in a three dimensional hyperbolic space. Because hyperbolic geometry is congruent with hierarchical tree-like organization, the hyperbolic coordinates make it possible to arrange neurons in a hierarchical way. We find that hyperbolic coordinates in IC aligned with the latency of neural responses in a way that allows for gradual refinement of auditory responses following their representation. This code is well suited for fast and organized communication of dynamic acoustic features. Together, these studies unravel the effects of the recurrent connectivity, both within and across stations, on sound representation in the ascending auditory system. We develop theoretical models to reproduce these results.

Disclosures: **A. Berrou:** None. **H. Zhang:** None. **M. Jankowski:** None. **M. Harpaz:** None. **T. Sharpee:** None. **D. Hansel:** None. **I. Nelken:** None.

Digital Abstract Session

P183. Auditory Processing: Cellular and Circuits

Program #/Poster #: P183.05

Topic: D.06. Auditory & Vestibular Systems

Support: ERC grant no. 340063 - RATLAND

Title: Integration of auditory information in the insular cortex and secondary somatosensory cortex

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Abstract: We used Neuropixels probes to study processing of sounds in the auditory field located in the Insular cortex (InsC) in anesthetized rats. InsC is a multi-modal cortical area composed of functionally distinct subregions that differ in their connectivity with other brain areas. Detailed histological analysis of electrode tracks revealed auditory responsive neurons in Insular cortex (InsC, n=508), as well as more dorsally, in the adjacent secondary somatosensory cortex (S2, n=372). The band of auditory responsive neurons extended continuously from ventral S2 and into granular insular cortex (GI). Units recorded throughout this band had short minimal onset latencies that were sometimes shorter than for A1 units. These short-latency responses were dominant in S2 while InsC contained in addition a subpopulation of units with longer latencies. Auditory units in S2 and InsC showed pronounced adaptation to repeated pure tones and moderate stimulus-specific adaptation (SSA) to pure tones, smaller than in A1. They had minor (InsC) or no (S2) SSA to complex spectrally balanced tone clouds. Units in InsC and S2 auditory field had different profiles of sensitivity to the structure of tone sequences compared to A1. In conclusion, under anaesthesia, despite the similarity in their responses to simple sounds, neurons in rat InsC and S2 perform computations that differ significantly from those performed by neurons in A1.

Disclosures: **M. Karayanni:** None. **M.M. Jankowski:** None. **M. Harpaz:** None. **A. Polterovich:** None. **I. Nelken:** None.

Digital Abstract Session

P183. Auditory Processing: Cellular and Circuits

Program #/Poster #: P183.06

Topic: D.06. Auditory & Vestibular Systems

Support: NIH/NIBIB (P41-EB018783)
NIH/NIBIB (R01-EB026439)
NIH/NINDS (U24-NS109103)
NIH/NINDS (U01-NS108916)
NIH/NIMH (P50-MH109429)
Fondazione Neurone
NIH/NINDS (R37 NS21135)

Title: Phase Reset in Ongoing Oscillations -Response to, or Prediction of External Sensory Stimuli?

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Abstract: Ongoing cortical oscillations are known to exhibit phase resets related to external sensory stimuli. It is thought that phase reset lowers the membrane potential and increases the excitability of neuronal ensembles. However, whether phase reset is a response to or prediction of an external sensory stimulus remains unclear. To answer this question, we investigated phase resets in the ongoing cortical oscillations of five human subjects that attended to repetitions of the 'La-La-Ba La-La-Ga' syllable pattern. This pattern created a temporal expectation of the 'Ba' or 'Ga' syllables. To ensure the subjects' attention, we asked them to respond with a button press to a 'Ta' syllable that occurred randomly in place of 12% of the 'Ga' syllables. Similarly, to determine whether phase reset occurs in prediction of a stimulus, we randomly omitted 30% of the 'Ga' syllables. The syllables were 410ms in duration and separated by each other by a 200ms ISI. Over the 40 minutes of this experiment, we recorded 1280 sequences that contained 160 omitted and 64 'Ta' syllables. In our analysis, we were interested in detecting phase resets in the ongoing cortical oscillations. For this purpose, we first identified those cortical locations that exhibited a statistically significant high frequency activity (HFA;70-170 Hz) response to the syllables. For each location and each syllable, we then calculated the likelihood of phase reset over time using phase-amplitude coupling (PAC) between the phase of ongoing low-frequency oscillations (<40Hz) and the amplitudes of high gamma activity. Finally, we determined whether phase reset occurred before or after the syllable onset by grouping the PAC results into 'Ba,' 'Ga' and omitted syllables, and calculating the cumulative likelihood of phase reset. In this analysis, we first determined the Gaussian distribution of PAC onsets. Since PAC is known to immediately follow phase reset, we defined the phase reset onset as the Gaussian distribution onset. This analysis shows that, on average, PAC occurs within the superior temporal gyrus approximately 200ms after syllable onset and that it is preceded by phase reset by 100ms. Further, we found a peak in the high gamma activity during the trough period that immediately followed the phase reset. In summary, these results show that the onset of PAC follows the onset of the 'Ba', 'Ga' and omitted syllables. Thus, our study supports the notion that phase reset is a response to an external sensory stimulus and not a prediction.

Disclosures: H. Cho: None. **R.T. Knight:** None. **P. Brunner:** None.

Digital Abstract Session

P184. Auditory Processing: Adaptation, Learning, and Memory

Program #/Poster #: P184.02

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC015543
NIH Grant DC009635
NIH Grant DC012557

Title: Distinct synaptic plasticity mechanisms determine the diversity of cortical responses during behavior

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Abstract: Spike trains recorded from the cortex of behaving animals can be complex, highly variable from trial to trial, and therefore challenging to interpret. A fraction of cells exhibit trial-averaged responses with obvious task-related features such as pure tone frequency tuning in auditory cortex. However, a substantial number of cells (including cells in primary sensory cortex) do not appear to fire in a task-related manner (Jaramillo et al, 2010; Rodgers & DeWeese 2014) and are often neglected from analysis. Even classically responsive cells lose their stimulus representation during task-engagement without impairing behavior (Otazu et al, 2009; Bagur et al, 2018). These results suggest that non-classically responsive cells may play an underappreciated role in sensory processing and cognition. We recently used a novel single-trial, spike-timing-based analysis to show that both classically responsive and non-classically responsive cortical neurons recorded from auditory and frontal cortex contain significant information about sensory stimuli and behavioral decisions (Insanally et al, 2019). In addition, we showed that non-classically responsive neurons can predict behavioral errors and encode abstract cognitive variables better than classically responsive cells. Here we expand this investigation to explore the synaptic origins and potential contribution of these cells to network function. To do so, we trained a novel spiking recurrent neural network (RNN) model that incorporates spike-timing-dependent plasticity (STDP) mechanisms to perform the same task as behaving animals. By leveraging excitatory and inhibitory plasticity rules this model reproduces neurons with response profiles that are consistent with our previously published experimental data, including classically responsive and non-classically responsive neurons. We found that both classically responsive and non-classically responsive neurons encode behavioral variables in their spike times as seen in vivo. Interestingly, heterosynaptic plasticity in excitatory-to-excitatory synapses increased the proportion of non-classically responsive neurons and may play a significant role in determining response profiles. Finally, our model also makes predictions about the synaptic origins of classically and non-classically responsive neurons which we compare to *in vivo* whole-cell recordings taken from the auditory cortex of behaving animals. This approach successfully recapitulates heterogeneous response profiles measured from behaving animals and provides a powerful lens for exploring large-scale neuronal dynamics and the plasticity rules that shape them.

Disclosures: M. Insanally: None. **B. Albanna:** None. **B. Depasquale:** None. **S. Fadaei:** None. **K. Rajan:** None. **R. Froemke:** None.

Digital Abstract Session

P184. Auditory Processing: Adaptation, Learning, and Memory

Program #/Poster #: P184.03

Topic: D.06. Auditory & Vestibular Systems

Support: NIMH DIRP ZIA MH002928-01 to BA
NIMH DIRP ZIA MH001101-25 to MM

Title: Correlates of auditory decision making in prefrontal, auditory and basal lateral amygdala cortical areas

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Abstract: Spatial selective listening and auditory choice underlie important processes including attending to a speaker at a cocktail party and knowing how (or if) to respond. To examine task encoding and relative timing of potential neural substrates underlying these behaviors, we developed a spatial selective detection paradigm for monkeys, and recorded activity in primary auditory cortex (AC), dorsolateral prefrontal cortex (dlPFC) and the basolateral amygdala (BLA). A comparison of neural responses among these three areas showed that, as expected, AC encoded the side of the cue and target characteristics before dlPFC and BLA. Interestingly, AC also encoded the monkey's choice before dlPFC and around the time of BLA. Generally, BLA showed weak responses to all task features except the choice. Decoding analyses suggested that errors followed from a failure to encode the target stimulus in both AC and dlPFC, but again, these differences arose earlier in AC. The similarities between AC and dlPFC responses were abolished during passive sensory stimulation with identical trial conditions, suggesting that the robust sensory encoding in dlPFC is contextually gated. Thus, counter to a strictly PFC-driven decision process, in this spatial selective listening task, AC neural activity represents the sensory and decision information before dlPFC. Unlike in the visual domain, in this auditory task, the BLA does not appear to be robustly involved in selective spatial processing.

Disclosures: J.L. Napoli: None. **C.R. Camalier:** None. **A.L. Brown:** None. **J. Jacobs:** None. **M.M. Mishkin:** None. **B.B. Averbeck:** None.

Digital Abstract Session

P184. Auditory Processing: Adaptation, Learning, and Memory

Program #/Poster #: P184.04

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01DC018621

Title: Activity-dependent Kv1.1 membrane expression drives intrinsic plasticity in the zebra finch auditory cortex

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Abstract: In zebra finches and other songbirds, the auditory system undergoes a sensitive period where the general acoustic environment shapes auditory processing that support auditory perception of vocalizations. In the caudal mesopallium (CM), a cortical-level auditory area implicated in discriminating and learning species-specific vocalizations, there is subset of neurons that only fire transiently at the onset of current injections (i.e., phasic firing), and phasic firing can enhance the reliability and selectivity of neural responses to complex acoustic stimuli. Previous work shows phasic excitability peaks during the sensitive period for song memorization. Changing side by side with phasic excitability is the proportion of CM neurons that express Kv1.1, a low-threshold potassium channel that facilitates phasic firing. These correlated changes in intrinsic plasticity and Kv1.1 expression only occur in a noisy acoustic environment, suggesting that an experience-dependent mechanism regulates expression of Kv1.1 and its localization to the plasma membrane. Here, we investigated the cellular mechanisms underlying this intrinsic plasticity. We stimulated CM neurons in brain slices under current clamp to determine the intrinsic dynamics, followed by post-hoc Kv1.1 expression analysis. We found that Kv1.1-positive neurons were significantly more phasic than Kv1.1-negative neurons. Furthermore, stimulating CM neurons with a complex, broadband current caused neurons to become more phasic over the course of several minutes. These data suggest Kv1.1 may be rapidly relocalized to the cell membrane in an activity-dependent manner, thus driving a change towards phasic excitability.

Disclosures: Y. Lu: None. **C.D. Meliza:** None.

Digital Abstract Session

P184. Auditory Processing: Adaptation, Learning, and Memory

Program #/Poster #: P184.05

Topic: D.06. Auditory & Vestibular Systems

Support: DGE1342536
NIDCD DC012557

Title: Categorical representation by suppression or enhancement of stimuli during auditory perceptual learning

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Abstract: Auditory perceptual learning reliably enhances cortical representations of task-relevant stimuli in trainedanimals relative to naive ones. Despite being associated with perceptual improvement, such changesin neural tuning are typically not measured throughout learning and do not investigate differences inactivity between neuronal subtypes. To address these limitations, we developed an experimental andcomputational framework for describing how sensory representations in both excitatory and inhibitoryneurons change during auditory

perceptual learning.We performed longitudinal two-photon imaging in auditory cortex throughout two-alternative forced-choice auditory conditioning in head-fixed mice. We recorded from excitatory and inhibitory neurons inlayer 2/3 and inhibitory neurons in layer 1. To obtain water reward, animals learned to classifyfrequencies as a single, center frequency (11-16 kHz) or non-center by licking left and right,respectively. Discrimination between center and non-center frequencies improved over 9-21 days. Intrained animals, despite similar behavioral performance, we observed two distinct tuning profiles ofexcitatory neurons in across different animals. Specifically, animals exhibited either a relativeenhancement or suppression of the center frequencies reported as center. This was seen at both thesingle cell and population level as well. We examined when these response profiles emerged duringlearning. We could detect whether an animal would likely exhibit enhancement or suppression as earlyas day 6 in behavioral learning.To make sense of these findings, we developed a network model of excitatory and inhibitory neuronsto explore what may account for these individual differences and how they arise over learning. Wetrained a network model of excitatory and inhibitory neurons by reward-modulated Hebbian synapticlearning (Williams, 1992) to solve the same task. We found that the simulated network learns at similarrates as real animals and captures the across-animal variability in tuning. Overall, both data and modelreveal nontrivial learning dynamics associated with perceptual learning in auditory cortex, with initialtuning driving across-animal variability in the emerging representations.

Disclosures: K.A. Martin: None. **A.M. LeMessurier:** None. **R.C. Froemke:** None. **C. Bredenberg:** None. **C. Savin:** None. **E.P. Simoncelli:** None.

Digital Abstract Session

P184. Auditory Processing: Adaptation, Learning, and Memory

Program #/Poster #: P184.06

Topic: D.06. Auditory & Vestibular Systems

Support: NIH-NIDCD F30-DC017351
NIH-NIDCD R01-DC012557
NIH-NIDCD R01-DC003937

Title: Neuromodulation enhances plasticity in a rat model of cochlear implant use

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Abstract: Rates of auditory perceptual learning with cochlear implants are highly variable across patients. Adaptation to cochlear implants is believed to require neuroplasticity within the central auditory system. However, mechanisms by which behavioral training enables plasticity and improves outcomes are poorly understood. Here we hypothesize that neural mechanisms promoting plasticity in the rat auditory system are key to optimizing cochlear implant usage. Specifically, we examined the effect of pairing locus coeruleus stimulation with an auditory

stimulus on auditory learning when the animal has to relearn a tone identification task (self-initiated, go/no-go) using a cochlear implant. Initial training was done using acoustic stimuli in normal hearing animals. Animals were then bilaterally deafened, unilaterally cochlear-implanted, and retrained with the new target delivered by intracochlear electrical stimulation. Prior to daily behavioral training sessions, rats underwent 5-10 min pairing sessions either with optogenetic locus coeruleus or sham stimulation. Pairing accelerated learning with cochlear implants. We then conducted multi-unit recordings in the auditory cortex to assess responses to the cochlear implant. Animals that had been trained with the cochlear implant had increased activation of the cortex, and those that underwent pairing had a sharper representation of the target cochlear implant channel. We used fiber photometry to monitor activity of noradrenergic locus coeruleus neurons. During auditory learning, cochlear implanted animals display dynamic locus coeruleus activity, specifically during the acquisition of the meaning of reward relevant tones. These studies indicate that neuromodulation can play a powerful role in shaping outcomes with cochlear implant use and training.

Disclosures: E.G. Glennon: None. **R.C. Froemke:** None. **M. Svirsky:** None. **A. Zhu:** None.

Digital Abstract Session

P184. Auditory Processing: Adaptation, Learning, and Memory

Program #/Poster #: P184.07

Topic: D.06. Auditory & Vestibular Systems

Support: Institut de recherche Robert-Sauvé en santé et en sécurité du travail (IRSST)
Natural Sciences and Engineering Council of Canada (NSERC)
Fonds de recherche du Québec (FRQS)
Centre de recherche de l'Institut universitaire de gériatrie de Montréal (CRIUGM)
Bourse d'études sur la recherche en gérontologie et gériatrie UdeM

Title: Impact of presbycusis on static postural control

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Abstract: Falls represent the second leading cause of injuries and deaths among older adults. One common factor associated with increased risks of falls is age-related decline in postural control. Recent neuro-otology studies suggest that hearing plays a role in postural control. However, the effect of age-related hearing loss on postural control is still unknown. Age-related hearing loss, also known as presbycusis, affects nearly two-thirds of the US population over 70 years and represents an important risk factor for increasing falls among older adults. The aim of this study was to to determine the influence of presbycusis on static postural control in normal-hearing and hearing-impaired older adults. Thirty participants underwent a comprehensive audiological evaluation by a certified audiologist with the aim to determine their hearing

thresholds . The modified Clinical Test of Sensory Integration and Balance was used to measure body sway control using a force platform. Participants were required to stand still on a force platform or on a foam surface with eyes open or with eyes closed. A significant group difference was found for both sway velocity and path length measures. Postural sway was increased among hearing-impaired older adults, suggesting poorer postural control as compared to normal-hearing older adults. Our findings suggest that a reduction in auditory inputs due to hearing loss (i.e. presbycusis) significantly affects postural control. Thus, presbycusis could contribute to the decline in postural control among older adults.

Disclosures: L. Behtani: None. **M. Maheu:** None. **F. Champoux:** None.

Digital Abstract Session

P184. Auditory Processing: Adaptation, Learning, and Memory

Program #/Poster #: P184.08

Topic: D.06. Auditory & Vestibular Systems

Support: UC Davis MIND Institute
Robert Shoes Fund
Scott & Jennifer Fearon
UC Davis Deans' Distinguished Graduate Fellowship
NIH Grant 1R01 MH089626-01
NIMH Grant U24MH081810
NICHD Autism Center of Excellence Grant P50 HD093079

Title: Opposing Intensity Adaptation Effects in Auditory Event-Related Potentials from Young Children

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Abstract: Introduction Although the intensity of a stimulus preceding a sound is known to affect the subjective loudness of that sound, a prior study has found no effect of prior stimulus intensity on the neural response to a second stimulus (Ninomiya et al., 2000). However, that study had a relatively small sample. The present study examined intensity adaptation in ERPs from a larger sample of young children.
Methods Participants with usable ERPs were 81 typically-developing children (52 male, M_{age} = 37.09 mos, range 25-57 mos, M_{DQ} = 107.37, range 79-129). While watching a quiet video, participants heard, via headphones, brief complex tones pseudo-randomly varying in intensity between 50, 60, 70 and 80 dB SPL (~200-300 trials/intensity) with the restriction that tones of the same intensity were never presented in succession. ISI randomly varied between 1-2 s. 61-channel EEG was sampled at 1000 Hz. P1 amplitudes were quantified as mean amplitudes over fronto-central channels ±50 ms around the grand-average peak for each intensity: 73-173 ms (50 dB), 60-160 ms (60 dB), 45-145 ms (70 dB), and 42-142 ms (80 dB). We computed differences

between averaged responses to the 2nd stimulus presented in each of the 12 intensity pairs and an overall average of responses to the 2nd stimulus across all prior intensities. More positive difference values indicate a more positive (i.e., larger) P1 response for a given prior intensity. ANOVA was used to examine effects of prior stimulus and hemisphere on difference values from each 2nd stimulus intensity condition.
Results There was a main effect of prior intensity on responses to 50 dB tones, $F(2,160) = 4.23$, $p = .02$. P1 responses to 50 dB tones were significantly larger after 60 dB than after 70 dB or 80 dB sounds, both corrected $p < .05$.
For 60 dB tones, there was an interaction between prior stimulus intensity and hemisphere, $F(2,160) = 4.17$, $p = .02$. Responses were modulated by preceding stimulus intensity over the left hemisphere, corrected $p < .05$. Left hemisphere responses to 60 dB sounds trended towards being smaller after 50 dB tones than after either 70 dB, corrected $p = .08$, or 80 dB, corrected $p = .10$. There were no effects of prior intensity on responses to 70 dB or 80 dB sounds.
Discussion Refractoriness or adaptation may suppress responses to 50 dB sounds after 70 and 80 dB sounds, but responses to 60 dB sounds after 50 dB sounds may be attenuated due to diminished attention capture by the latter. Responses to louder 70 dB and 80 dB sounds were not modulated by prior intensities, suggesting these stand on their own more than soft sounds. Overall, these data suggest effects of prior stimulus intensity on neural responses are shaped by immediate context.

Disclosures: P. Dwyer: None. **C.D. Saron:** None. **S.M. Rivera:** None.

Digital Abstract Session

P184. Auditory Processing: Adaptation, Learning, and Memory

Program #/Poster #: P184.09

Topic: D.06. Auditory & Vestibular Systems

Support: HK Research Grants Council GRF 14606417
HK Research Grants Council GRF 14602418

Title: Illuminating the abnormalities of brain dynamics during pre-attentive change detection in Autism Spectrum Disorder (ASD)

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Abstract: Autism Spectrum Disorder (ASD) is known for deficits in processing emotional information. However it is still largely unknown regarding the brain dynamics involved in cognitive processing of ASD. We examined the difference in frontotemporal brain dynamics involved in the pre-attentive detection of emotional (angry and happy speech) versus non-emotional changes (neutral speech and pure tone) between children with ASD and normal control. Pre-attentive change detection is the ability of human brain to automatically extract

regularities from environment for predicting future event. When a prediction violation occurs, mismatch responses in the frontotemporal network are elicited. Different activation patterns of the frontotemporal network have been revealed for detecting salient (a temporal-followed-by-frontal pattern) versus ambigious change (an early frontal-temporal-late frontal pattern) using Event-related optical signal (EROS) technique. EROS measures changes in optical properties associated with neural responses with superior spatial and temporal resolution, thus was adopted in the current study to capture the dynamics of the frontotemporal network during pre-attentive change detection. Compared to the normal control, children with ASD was hypothesized to be more sensitive to non-emotional change and less sensitive to emotional change indicated by the different activation patterns in the frontotemporal networks. Children diagnosed with ASD (6-12 years old; N=19) and their age and gender matched normal controls (N=19) watched a self-selected silent movie with subtitles and were told to ignore the auditory events presented at the background. Four types of changes, including angry, happy, and neutral speeches as well as pure tone, were presented, while EROS mismatch responses were recorded from the frontal and temporal cortices. The control group showed the (salient) temporal-frontal mismatch response pattern to the angry speech and pure tone changes, while the (ambigious) frontal-temporal-frontal pattern to the happy and neutral speech changes in both hemispheres. Children with ASD only demonstrated the (ambigious) frontal-temporal-frontal pattern for happy speech, neutral speech, and pure tone changes in the right hemisphere, while no mismatch response was observed for the angry speech change. Children with ASD were able to detect non-emotional and emotional (happy) change by adopting a frontotemporal brain network for ambigious change and showed deficits in detecting angry speech change.

Disclosures: **Y. Wang:** None. **X. Xiao:** None. **Y. Shum:** None. **C. Tse:** None.

Digital Abstract Session

P185. Cross-Modal Processing: Spatial and Temporal Factors

Program #/Poster #: P185.01

Topic: D.09. Multisensory Integration

Support: JSPS KAKENHI Grant 18H01414
JSPS KAKENHI Grant 19K20093

Title: Evaluation of operational feeling of rotating seat by the vestibulo-ocular reflex

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Abstract: The vestibulo-ocular reflex (VOR) is a reflex eye movement generated in the direction opposite to the head movement to suppress blurring of the image reflected on the retina. Some studies have indicated that a gain in VOR (ratio of eye to head rotation velocity) is greater during active than during passive head movement (Derner, 1993), although some studies revealed no significant difference (Hanson, 1998). Furthermore, a parameter in a mathematical model for

estimating VOR is known to be different during active and passive operations of a rotating seat (Uefune, 2018). However, it is unclear whether or not VOR can be used to evaluate the operational feeling of a rotating seat. Therefore, in this study, we investigated the relationship between the operational feeling of the rotating seat and VOR. The subjects (n = 12) sat on a seat that rotated in the yaw direction by operating a joystick. While gazing at a fixation point, the subjects performed a task of rotating the seat to the target angle (± 30°) for 60 s following the metronome sound with a frequency of 0.5 Hz. The experimental parameters were set as the operating parameters of the rotating seat, particularly, the gain and time constant in the first-order lag transfer function from the joystick angle to the motor torque attached to the rotating seat, which determines the time response of the rotation by the joystick operation. There were two types: operating parameters A and B. Because operating parameter B has a larger gain and time constant than operating parameter A, the rotation torque is large, and the time response for the joystick operation is slow. Dependent variables were set as the operational feeling of the rotating seat and VOR. The operational feeling was evaluated using a questionnaire (visual analog scale). The VOR was evaluated by a parameter in a mathematical model for estimating the VOR. Under the condition of operation parameter A, the subjects practiced the operation of the rotating seat for 5 min and then performed the task once. Subsequently, the task was performed four times under the condition of operation parameter B. The subjects did not know which operational parameters were applied to each task. As a result, when the operation parameter changed from A to B, the operational feeling deteriorated, and the VOR model parameter decreased. Furthermore, there was a significant positive correlation between the operational feeling and the VOR model parameter ($r = 0.41$, $p < 0.01$, Spearman's). These results suggest that the operational feeling of the rotating seat can be objectively evaluated by VOR.

Disclosures: **Y. Sato:** None. **T. Wada:** None. **Y. Kashiwagi:** None. **Y. Takebayashi:** None.

Digital Abstract Session

P185. Cross-Modal Processing: Spatial and Temporal Factors

Program #/Poster #: P185.02

Topic: D.09. Multisensory Integration

Support: JSPS 19J22981

Title: Detection performance improves based on the three principles of audiovisual integration in head-fixed Mongolian gerbil

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Abstract: We can gain behavioral benefits such as increased accuracy and rapid response by integrating auditory and visual information. The sensory integration is governed by three principles: temporal rule, spatial rule and principle of inverse effectiveness. Few researches, however, examine these principles at the same species. we focused on Mongolian gerbil

(*Meriones unguiculatus*). They have relatively sensitive low-frequency hearing comparable to humans and show more diurnal behavioral pattern than mice or rats. we consider gerbils as appropriate animal model for audiovisual integration research. the purpose of this study was to investigate whether gerbil's behavior improved by audiovisual integration.Total of 7 Mongolian gerbils (4 males and 3 females) were used. the age of the gerbils was about 10 weeks at the first day of behavioral training. We anesthetized gerbils with isoflurane and attached custom-made head plate to the skull before behavioral training. The head-fixed gerbils were trained to lick in response to the auditory or visual stimulus. The duration of stimulus was 500 ms at beginning of training and was then shortened to 10 ms. We presented 11 different intensity of auditory stimuli and 9 different intensity of visual stimuli to measure auditory and visual detection threshold, respectively. First, we examined whether gerbil's behavior follows principles of inverse effectiveness. Subjective stimulus intensity was classified into 4 levels based on the detection rate: Sub threshold (under 35 % detection rate), Around threshold (35 ~ 65 %), Supra threshold (65 ~ 85 %) and max intensity (above 85 %). Auditory, visual and audiovisual stimuli of 4 levels intensity was presented. Gerbil showed higher detection rate to audiovisual stimuli compared to unisensory stimuli. Particularly, behavioral enhancement was prominent when their intensities were around perceptual threshold. Next, we examined the temporal rule in the same experimental setup. Audiovisual stimuli that had different temporal lag between the sound and light (± 200, ± 160, ± 120, ± 80, ± 40, 0 ms) were presented. As a result, Reaction time to audiovisual stimuli was faster than that to unisensory stimuli, and the reaction time to simultaneous audiovisual stimuli was faster than that to asynchronous audiovisual stimuli. These data showed that gerbil's behavior was improved when combination stimuli of auditory and visual stimulus was presented, and suggest that their behavior follows the principle of inverse effectiveness and temporal rule, similar to other animals. In the future study, we will investigate how three principles of integration are neuronally implemented.

Disclosures: **Y. Ito:** None. **T. Osuka:** None. **K.I. Kobayasi:** None.

Digital Abstract Session

P185. Cross-Modal Processing: Spatial and Temporal Factors

Program #/Poster #: P185.03

Topic: D.09. Multisensory Integration

Support: Sir Henry Wellcome Postdoctoral Fellowship (210924/Z/18/Z)

Title: Auditory detection is modulated by theta phase of silent lip movements

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Abstract: Audiovisual speech perception relies, among other things, on our expertise to map a speaker’s lip movements with speech sounds. This multimodal matching is facilitated by salient

syllable features that align lip movements and acoustic envelope signals in the 4 - 8 Hz theta band. Although non-exclusive, the predominance of theta rhythms in speech processing has been firmly established by studies showing that neural oscillations track the acoustic envelope in the primary auditory cortex. Equivalently, theta oscillations in the visual cortex entrain to lip movements, and the auditory cortex is recruited during silent speech perception. These findings suggest that neuronal theta oscillations may play a functional role in organising information flow across visual and auditory sensory areas. We presented silent speech movies while participants performed a pure tone detection task to test whether entrainment to lip movements directs the auditory system and drives behavioural outcomes. We show that auditory detection varies depending on the ongoing theta phase conveyed by lip movements in the movies. In a complementary experiment, presenting the same movies while recording participants' electro-encephalogram (EEG), we find that silent lip movements entrain neural oscillations in the visual and auditory cortices with the visual phase leading the auditory phase. Together, these results suggest that the brain's natural reaction to visual speech stimuli might be to align the excitability of the auditory cortex with sharp mouth-openings because that is when one expects to hear corresponding acoustic syllable edges. Such a neural process could be a very effective filtering method to increase the sensitivity of the auditory cortex in these relevant time windows for speech comprehension.

Disclosures: E. Biau: None. **D. Wang:** None. **H. Park:** None. **O. Jensen:** None. **S. Hanslmayr:** None.

Digital Abstract Session

P185. Cross-Modal Processing: Spatial and Temporal Factors

Program #/Poster #: P185.04

Topic: D.09. Multisensory Integration

Support: NIH R00 EY026994

Title: Effects of eccentric viewing in orientation discrimination

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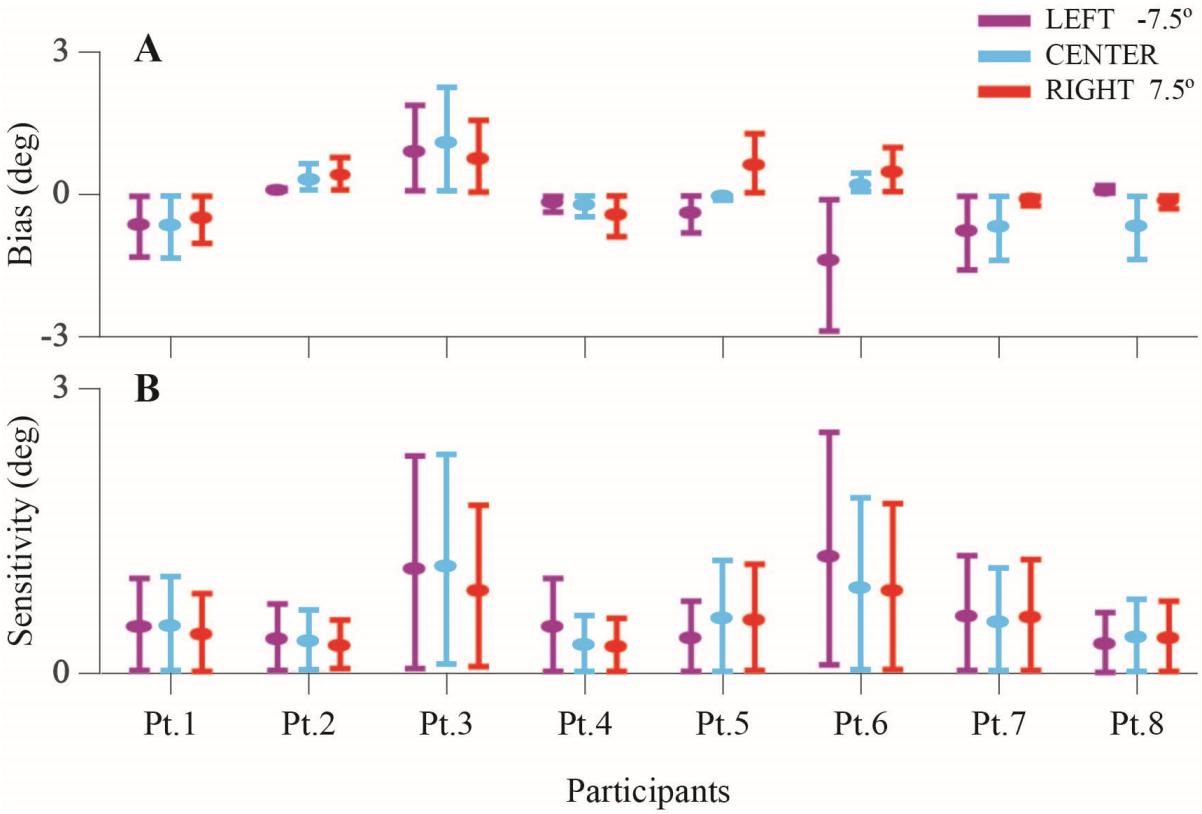
Abstract: Vestibular deficits are particularly evident in individuals with central visual field loss (CFL): two thirds of those with CFL complain of dizziness and instability, and have an increased rate of falls, injury, oscillopsia, and a fear of falling. Correct assessment of spatial orientation and movement is essential for fall-free walking and standing. Orientation information is normally provided through a weighted combination of visual, vestibular, proprioceptive and somatosensory signals. In CFL the problem is compounded by a largely deteriorated visual signal and a potentially mis-calibrated vestibular system. To assess these changes, we first set out to study the effects of eccentric viewing alone on orientation judgements in 8 healthy controls (6F) across the lifespan (22-74yo). Participants were seated in complete darkness with the head

upright. A bar (length=10.5º vis. ang.) was flashed on a monitor either in the center or 7.5º to the right or left of fixation for 41 ms. The width of the bar was scaled with eccentricity (M-scale= 0.333), but the length of the bar was kept the same. Observers indicated if it was rotated clockwise or counterclockwise of vertical in a 2AFC design. Participants performed 504 trials (7 orientations, 2 directions, 3 positions, 12 repetitions), after a short set of practice trials. The bias (Fig1A) and the discrimination sensitivity (Fig1B) were estimated (probit fit) at each eccentricity, for each participant. There was no significant difference in bias or sensitivity for bars presented at the three eccentricities across our observers ($p > 0.2$, 1-way rm ANOVA, Tukey correction). Our pilot data suggest orientation discrimination is preserved when viewing peripherally, up to 7.5º visual angle. For large, high visibility stimuli any changes in subjective visual vertical observed in individuals with CFL are unlikely to be due to differences in viewing eccentricity due to the loss of the fovea. Changes in judgement bias are likely to be representative of a less reliable reference frame re gravity due to degradation of available vestibular information.



Disclosures: **A. Velisar:** None. **N.M. Shanidze:** None.

Digital Abstract Session

P185. Cross-Modal Processing: Spatial and Temporal Factors

Program #/Poster #: P185.05

Topic: D.09. Multisensory Integration

Title: Can the short interval of several minutes reduce motion sickness in repetitive experience of the motorcycle simulator?

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Abstract: Using simulators can safely evaluate the behavior of motorcycle drivers. However, the simulators often induce motion sickness (simulator sickness). Previous studies have reported that the degree of the motion sickness is reduced by repetitive experience of the simulators with intervals of one day or more. If utilizing such an adaptation effect, we can develop a method of reducing motion sickness. However, there are many unclear points in the mechanisms or properties for the adaptation to stimuli inducing motion sickness. In this study, we examined whether the adaptation to a motorcycling simulator occurs even by a short interval of several minutes. Moreover, we measured the degree of motion sickness not only during the simulator experience but also during the non-simulator period to clarify the recovery processes of the sickness. Twenty male volunteers participated in the experiment. The participants wore a head-mounted display (HMD) and sat on a motorcycle chassis. The HMD presented a motorcycling scene along a winding road from a first-person viewpoint. Sounds or vibrations were not presented. The participants continuously experienced two sets of a 6-minute motorcycling scene (simulator period) and 6-minute non-scene period (non-simulator period) [i.e., simulator→non→simulator→non]. They verbally reported the degree of motion sickness by number from 0 (no sickness at all) to 20 (frank sickness) [fast motion sickness (FMS) scale] every 30 seconds. As a result, the FMS scores during the simulator and non-simulator periods were significantly greater for the second set than for the first set, although there was no difference in the peak FMS scores at the ends of the simulator periods between the first and second set. In addition, the decrease of the FMS score immediately after the end of the simulator period (i.e., the beginning of the non-simulator period) was significantly smaller in the second set than in the first set. These results suggest that the 6 minutes of the short interval does not induce adaptation to the stimulator. Rather, motion sickness increased in the second set. Notably, the results also showed that the FMS scores did not reduce to zero at the end of the non-simulator period in the first set. The remained motion sickness might cause the accumulation of the sickness in the subsequent second set.

Disclosures: **M. Hayashi:** None. **Y. Itaguchi:** None. **K. Aigou:** None. **M. Miki:** None. **T. Kimura:** None. **M. Miyazaki:** None.

Digital Abstract Session

P186. Vestibular Perception and Posture

Program #/Poster #: P186.01

Topic: D.06. Auditory & Vestibular Systems

Support: Natural Sciences and Engineering Research Council of Canada-RGPIN-2016-05211

Title: Vestibular function modulates the impact of nGVS on postural control in older adults

Authors: *M. NOORISTANI[1], C. BIGRAS[1], L. LAFONTAINE[1], B.-A. BACON[2], M. MAHEU[1], F. CHAMPOUX[1];
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Abstract: Previous studies have reported an important relationship between increasing age, vestibular impairment and increased risk of falls. Recently, noisy galvanic vestibular stimulation (nGVS) has been shown to improve postural control in older adults during and potentially following stimulation. However, this effect of nGVS in older adults has not been examined in interaction with the integrity of the vestibular function. We aimed at determining the effect of nGVS on postural control in older adults with and without vestibular impairment and ii) at examining the sustained effect of nGVS as compared to a sham stimulation. 36 older adults were randomly assigned to the nGVS group [n=24] or the sham group [n=12]. In the nGVS group, 12 participants had normal vestibular function and 12 had vestibular impairment. Static postural control was assessed prior to stimulation, during stimulation and immediately following 30 minutes of nGVS. Results showed that nGVS induced a significant improvement in sway velocity ($p<0.001$) and path length ($p<0.001$) compared to sham stimulation. Furthermore, nGVS induced a significantly greater improvement of sway velocity ($p<0.05$) and path length ($p<0.05$) in older adults with vestibular impairment, compared to older adults with normal vestibular function. Improvements in sway velocity ($p<0.001$) and path length ($p<0.001$) induced by nGVS were sustained immediately following stimulation. These findings suggest that nGVS improves postural control in older adults, and that the effect of nGVS varies depending on the integrity of the vestibular function. Results also show that nGVS effect on postural control, compared to a sham stimulation, can be sustained after the end of stimulation.

Disclosures: M. Nooristani: None. **C. Bigras:** None. **L. Lafontaine:** None. **B. Bacon:** None. **M. Maheu:** None. **F. Champoux:** None.

Digital Abstract Session

P186. Vestibular Perception and Posture

Program #/Poster #: P186.02

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant 90079498

Title: Nonhuman primate responses to platform tilts resemble those of humans in health and vestibular loss

Authors: *O. M. E. LEAVITT, K. E. CULLEN;
Johns Hopkins Univ., Baltimore, MD

Abstract: An important development for restoration of profound vestibular loss is a vestibular implant which stimulates vestibular afferents in response to head rotations. Vestibular implants are in clinical trials but further research can improve their performance. Experimentation in animal models is essential to further prosthesis development by probing how vestibular circuits involved in balance respond to prosthetic stimulation. In response to sinusoidal tilt perturbations, human subjects transition from a strategy maintaining body position relative to the surface (platform-fixed) to maintaining the head position in space (head-fixed) at 0.5 Hz. Prediction is involved; during unpredictable pseudorandom tilts subjects are less stable with either strategy. Further, regardless of predictability, subjects with bilateral vestibular loss (BVL) demonstrate greater postural instability at low frequencies during platform tilts.
Here, to determine whether postural strategies persist in a monkey model, we used sinusoidal and pseudorandom stimuli. Two rhesus macaques, one normal and one with BVL, stood in an enclosure on a motion platform. The animal was tracked using markerless pose estimation (DeepLabCut), a head-mounted IMU, and a force plate. Sinusoid stimuli included 6 frequencies of sine wave roll tilts of constant peak velocity, and pseudorandom stimuli were constructed from a pseudorandom ternary sequence. Like humans, during sinusoidal roll tilts the normal monkey demonstrated a transition from a platform-fixed to a head-fixed strategy. At low frequencies the body moved little relative to the platform, and at high frequencies the head remained stationary in space. The monkey's transition frequency was 1 Hz, higher than humans' due to biomechanics. The transition frequency persisted during pseudorandom stimuli, but with greater instability than sinusoidal, as with normal humans. The BVL animal's postural responses mirrored those of human subjects with BVL: greater instability during low-frequency sinusoidal stimuli, and less stable response to pseudorandom than sinusoidal stimuli. These results indicate that rhesus provide a useful model of posture in normal and BVL conditions for probing how vestibular circuits involved in balance respond to prosthetic stimulation.

Disclosures: O.M.E. Leavitt: None. **K.E. Cullen:** None.

Digital Abstract Session

P186. Vestibular Perception and Posture

Program #/Poster #: P186.03

Topic: D.06. Auditory & Vestibular Systems

Support: VA Grant 1IK2RX003271
NIH Grant R00 EY026994
NIH Grant R56 DC018003-01
NIH Grant F32 DC017063
NIH Grant R21 DC015097
NIH Grant T32 DC000011

Title: Functional Correlates of Noise-Induced Damage to the Vestibular Periphery

Authors: *C. E. STEWART[1,2], D. S. BAUER[1], A. VELISAR[3], N. M. SHANIDZE[3];
[1]Kresge Hearing Res. Inst., Univ. of Michigan, Ann Arbor, MI; [2]Res. Service, VA Ann Arbor Healthcare Syst., Ann Arbor, MI; [3]Smith-Kettlewell Eye Res. Inst., San Francisco, CA

Abstract: Despite the accumulating evidence that noise exposure damages the vestibular periphery (for review, Stewart et al., 2020), it is often overlooked as a potential source of vestibular damage. Reduced vestibular function plays a significant role in elevated fall risk (Agrawal et al., 2012); however, diagnosis of vestibular disorders can be difficult. Symptoms are often nonspecific and can be attributed to disturbances of other senses, such as vision, hearing or proprioception (Agrawal et al. 2009). Simple measures of otolith organ dysfunction are of considerable value in detection and tracking of noise-induced vestibular loss in human subjects, while rodent studies provide direct measures of physiological and cellular damage with controlled noise exposure dosing. To understand the impact of degree of noise exposure on otolith organ dysfunction, rat and human vestibular data were correlated with degree of noise exposure. In rats, vestibular short-latency evoked potentials (VsEPs) were used to evaluate central and peripheral vestibular activity arising from the otolith organs. In humans, ability to judge static orientation using the subjective visual vertical task was used as a measure of otolith organ function (Alberts et al., 2019).
Rat VsEP loss was related to degree of noise exposure (110-120 dB, 2-6 hrs). While lesser noise exposures produced transient attenuation of VsEP responses, more intense noise exposures produced loss of responses to small head-jerk stimuli (0.32-1.1 g/ms) and significant, persistent attenuation of responses to larger head-jerk stimuli (2.2-5.5 g/ms).
In a preliminary human study, we recruited 4 individuals with a history of noise exposure (3F, age: 35-49) and 4 age-matched controls (1F, age: 36-71). We assessed degree of noise exposure using the Noise Exposure Structured Interview (NESI, Guest et al. 2018). All participants viewed a monitor with the head upright while seated in complete darkness. A bar (length=12º, width=0.22º visual angle) was flashed for 17 ms and observers indicated if it was rotated clockwise or counterclockwise of vertical in a 2AFC design. Participants performed 120 trials (6 orientations, 2 directions, 10 repetitions), after a set of suprathreshold practice trials. In our pilot data we observed decreased sensitivities and greater bias in orientation judgements of individuals with a history of noise exposure across age groups.
Taken together, our data suggest that noise-induced otolith organ dysfunction is dependent on degree of noise exposure, providing a potential link between physiological effects of noise exposure on the vestibular periphery and functional outcomes to be probed further.

Disclosures: C.E. Stewart: None. **D.S. Bauer:** None. **A. Velisar:** None. **N.M. Shanidze:** None.

Digital Abstract Session

P186. Vestibular Perception and Posture

Program #/Poster #: P186.04

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD R01-DC018287

Title: The reduction in the velocity storage time constant due to unilateral hypofunction is a response to changing peripheral signal-to-noise characteristics

Authors: *F. KARMALI, A. MADHANI, R. F. LEWIS;
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Abstract: The velocity storage mechanism in a central mechanism that processes peripheral vestibular cues. This includes the elongation of the semicircular time constant of approximately 5 s to the central velocity storage time constant, which is roughly 15-25 s in individuals with no known vestibular pathology. The velocity storage time constant is an important clinical parameter which varies with age, pathology and stimulus amplitude. In fact, the velocity storage time constant is a key diagnostic parameter for peripheral loss, with a time constant of 6-12 s suggesting unilateral hypofunction. Despite its clinical utility, there is little mechanistic understanding of why unilateral hypofunction should result in a lower velocity storage time constant. It has been hypothesized that Bayesian optimal processing determines velocity storage dynamics based on the statistics of vestibular noise and experienced motion. Specifically, while a longer time constant would be advantageous because this would make the vestibulo-ocular reflex accurate over a longer period of time, it has been argued that this would amplify neural noise and thus, make the VOR less precise. In particular, it has been hypothesized that the brain determines the optimal velocity storage time constant based on vestibular noise to determine the optimal tradeoff between being accurate and being precise. In this study we applied a Bayesian optimal Kalman filter model to determine the ideal velocity storage time constant for unilateral hypofunction. Since the exact effect of unilateral hypofunction on vestibular noise is unknown, we developed four scenarios based on findings in the literature. In all scenarios, the models predicted a velocity storage time constant that was substantially lower than for normal subjects. In particular, one plausible model predicted velocity storage time constants between 7 and 10 s, which is consistent with clinical findings. This suggests that this clinically-relevant change results from the brain optimizing velocity storage in response to a change in the peripheral signal-to-noise ratio. These results complement our existing work showing that age-related time constant variations are explained by an optimal adjustment in response to hair cell death.

Disclosures: F. Karmali: None. **A. Madhani:** None. **R.F. Lewis:** None.

Digital Abstract Session

P186. Vestibular Perception and Posture

Program #/Poster #: P186.05

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01 DC2390

Title: Restoring vestibular afferent dynamics improves accuracy of prosthesis-evoked vestibulo-ocular reflex (VOR) responses

Authors: *K. P. WIBOONSAKSAKUL, D. C. ROBERTS, C. C. DELLA SANTINA, K. E. CULLEN;
Johns Hopkins Univ., Baltimore, MD

Abstract: An exciting and emerging approach to treat patients with impaired vestibular function is a prosthesis that senses head rotation and transforms this movement into vestibular afferent stimulation, substituting for the damaged periphery. Early results from clinical trials, while encouraging, have shown only partial functional improvement. Bridging a gap between basic science knowledge and clinical applications, we implemented biomimetic dynamics in vestibular prostheses for the first time. We asked whether representing the natural dynamics of vestibular afferents in the mapping between head motion and afferent stimulation would result in better performance. To test this proposal, we compared vestibulo-ocular reflex (VOR) responses evoked by the static mapping used by all current devices (no dynamics) to those evoked by 4 newly implemented mappings representing and exceeding the characteristic high-pass dynamics of vestibular afferent processing. Testing was done in two monkeys with profound bilateral vestibular loss that had been implanted with a prosthesis. VOR eye movements were first quantified in response to sinusoidal stimulation that spanned the natural frequency range (0.2 - 20 Hz). We found that afferent-like high-pass mappings evoked more robust VORs with more precise timing. In contrast, the standard static mapping showed a gain decline and increasingly sluggish timing with increasing frequency. Furthermore, mappings with high-pass dynamics exceeding natural range, produced an undesirable phase advance. VOR eye movements were also quantified in response to transient stimulation and similar trends were observed. Overall, using a mapping that mimicked the afferent subclass known to provide primary contribution to the VOR yielded optimal performance. This suggests that endogenous afferent dynamics are well matched to produce accurate VOR response and advocates for a more biomimetic prosthesis design. Together, these results confirm that the implementation of biomimetic mappings in vestibular prostheses can optimize functional outcomes for patients.

Disclosures: K.P. Wiboonsaksakul: None. **D.C. Roberts:** None. **C.C. Della Santina:** None. **K.E. Cullen:** None.

Digital Abstract Session

P186. Vestibular Perception and Posture

Program #/Poster #: P186.06

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant 90084097

Title: Predictive processing by Purkinje cells in the anterior vermis during active versus passive self-motion

Authors: *O. ZOBEIRI[1], K. E. CULLEN[2];
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Abstract: The ability to distinguish between self-generated (reafference) vs. externally-applied (exafference) sensory signals is fundamental for ensuring accurate motor control as well as perceptual stability. This is particularly evident in the context of the vestibular system, in which the same central neurons that receive direct afferent input also project to motor neurons that control vestibulo-spinal reflexes (VSR). Notably, while VSRs are essential for providing a postural response to unexpected perturbation, they are impeding during self-generated head motion. Previous studies by our group have shown that central VSR neurons selectively code passive head movements. Accordingly, here we recorded from Purkinje cells in the vestibular cerebellum (anterior vermis) in rhesus monkeys during comparable active & passive rotational and translational head movements. We first recorded neuronal responses to vestibular-only and neck proprioceptive-only passive stimulation. We found that the Simple spike activity encoded both stimuli in a direction-dependent manner. Accordingly, for each Purkinje cell, we first developed a model of the dynamics of simple spike response based on passive head and body movements kinematics in each direction. We then passively applied both vestibular and proprioceptive stimuli simultaneously (i.e., passive head-on-body rotations) and found that Purkinje cells linearly integrated these two inputs. Then to compare each neuron's responses to active versus passive movements, we fit comparable models to neuronal responses during preferred and non-preferred active head movements. We found that neuronal sensitivities were markedly attenuated in the active condition (~60%, $p<0.01$). Finally, we tested whether the attenuated responses during the active movement is a result of neck motor inputs to the Purkinje cells. We found that while in the majority of the Purkinje cells, the neck motor signals affect the simple spike firing, a simple linear model that integrates motor signal with the sensory feedback cannot explain the suppressed simple spike response during active movements. Taken together, these results provide new insights into the computations performed by Purkinje cells in anterior vermis that underlie the suppression of vestibular reafference, suggesting that the cerebellar Purkinje cells implement nonlinear sensorimotor integration to differentially encode externally-applied vs. self-generated head movements and suppress vestibular reafference signal.

Disclosures: O. Zobeiri: None. **K.E. Cullen:** None.

Digital Abstract Session

P187. Retina- Photoreceptors

Program #/Poster #: P187.01

Topic: D.07. Vision

Support: NEI
FFB

Title: Retinal degeneration in KCNV2 retinopathy

Authors: *S. M. INAMDAR[1], J. G. LAIRD[2], E. A. MELTON[2], M. PUFALL[2], S. A. BAKER[2]; [1]Univ. of Iowa, IOWA CITY, IA; [2]Univ. of Iowa, Iowa City, IA

Abstract: Retinal degeneration in KCNV2 retinopathyShivangi M. Inamdar, Joseph G. Laird, Elizabeth Melton, Miles A. Pufall, Sheila A. BakerKCNV2 retinopathy also known as cone dystrophy with supernormal rod response (CDSRR) is a progressive retinal disorder that affects visual acuity beginning in childhood. It is caused by mutations in *KCNV2* that encodes for Kv8.2, a regulatory subunit of a voltage-gated potassium channel that controls photoreceptor signaling. The molecular pathogenesis of this disease is unknown. To model this disease, we generated a Kv8.2 KO mouse. The abnormal electrical signaling diagnostic of CDSRR patients that is measured using electroretinogram (ERG) recordings was phenocopied in Kv8.2 KO. Both supernormal rod activity and reduced cone activity were observed. Optical coherence tomography (OCT) imaging revealed a slow progressive degeneration resulting in 32% photoreceptor loss at 10 months of age. However, histological analysis of cone density showed no significant change. Transmission electron microscopy (TEM) indicated normal rod and cone ultrastructure at 3 months of age with the exception of the presence of autophagic-like structures in the Kv8.2 KO. RNA seq was performed to gain insight into any differential signaling occurring in the retinas of these mice. One cluster of upregulated genes included GFAP and vimentin indicating Muller cell gliosis. Upregulation of GFAP protein in KV8.2 KO was validated by immunohistochemistry and western blotting. In conclusion these data lead to a model where loss of the critical ion channel subunit, Kv8.2, prevents normal photoreceptor signaling that triggers autophagy and Muller cell stress.

Disclosures: S.M. Inamdar: None. **J.G. Laird:** None. **E.A. Melton:** None. **M. Pufall:** None. **S.A. Baker:** None.

Digital Abstract Session

P187. Retina- Photoreceptors

Program #/Poster #: P187.02

Topic: D.07. Vision

Support: EY007031
EY006641

Title: In Vivo 13C-Tracing of Metabolism Reveals Physiological Reversal of Succinate Dehydrogenase in the Retina

Authors: *D. T. HASS[1], C. BISBACH[2], B. ROBBINGS[3], *J. B. HURLEY[2];
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Abstract: Intro: Investigations of energy metabolism are frequently performed in cell culture or in ex vivo tissue preparations. While such studies have yielded valuable information, these systems are removed from the physiological context of the body. Succinate dehydrogenase (SDH) is a key mitochondrial enzyme that fuels energy metabolism by converting succinate to fumarate. Our investigations in the *ex vivo* retina show a substantial ‘reverse’ SDH activity where succinate is instead formed by fumarate. We sought to determine whether this reversal

occurred *in vivo*, and whether it was unique to the retina.
Methods: We infused a bolus of 100 mg/kg $^{13}C_4$-malate into the bloodstream of freely moving C57BL6J mice through external jugular catheters (n=3 mice/endpoint). 0 (un-infused), 1, 2, 3 and 5 minutes following the infusion, we dissected retina and eyecup (containing retinal pigment epithelium and choroid) tissue. Five minutes following the infusion, we dissected white (WAT) and brown adipose tissue (BAT), liver, lung, and cerebellum tissue. To compare metabolism of malate, tissues were quickly dissected and snap-frozen before metabolite extraction, derivitization, and analysis using gas-chromatography-mass spectrometry.
Results: Malate flux into tissues was approximated using % abundance of $^{13}C_4$-malate. Tissue malate labeling from highest (%) to lowest (%) was: WAT (64) > Lung (50) > BAT (21.7) > Eyecup (20.7) > Liver (15.5) > Cerebellum (5.1) > Retina (2.5). $^{13}C_4$-fumarate labeling followed the same pattern. To adjust for tissue malate import and conversion to fumarate, we determined the relative % abundance of $^{13}C_4$ -succinate assuming 100% $^{13}C_4$-fumarate labeling. This metric of SDH reversal was highest in the retina, and followed the order; retina (20.9) > Liver (12.3) > Eyecup (9.5) > BAT (4.9) > Cerebellum (4.8) > Lung (4.6) > WAT (2.9). Each tissue has succinate pools of different sizes, and after adjusting to pool size and tissue protein, retinas still appear to produce $^{13}C_4$-succinate from $^{13}C_4$-fumarate to a greater extent than other tissues (~71 pmol/mg protein/min, R^2=0.73).
Discussion: This study revealed a surprisingly low degree of penetration of labeled malate into any neural tissue in this panel, suggesting that malate transport across the blood-brain barrier is heavily regulated. The malate that was transported into tissues equilibrated with fumarate and become $^{13}C_4$-succinate in the less conventional SDH reverse reaction. Our data supports that this reverse reaction occurs in the retina, to roughly twice the extent that it occurs in any other tissue. This suggests that other aspects of retina metabolism are likely quite unique from other tissues as well.

Disclosures: **D.T. Hass:** None. **C. Bisbach:** None. **B. Robbings:** None. **J.B. Hurley:** None.

Digital Abstract Session

P187. Retina- Photoreceptors

Program #/Poster #: P187.03

Topic: D.07. Vision

Support: R01 EY020542

Title: Hcn1 is required for proper rod but not cone function

Authors: ***C. LANKFORD**[1], Y. UMINO[2], E. SOLESSIO[2], S. BAKER[1];
[1]Biochem., Univ. of Iowa, Iowa City, IA; [2]Ophthalmology, SUNY Upstate Med. Univ., Syracuse, NY

Abstract: Rod and cone recovery from light-triggered hyperpolarization is controlled at two levels. A fast reset is driven by the transient depolarizing current carried by HCN1 channels,

while a slower reset is driven by inactivation and resetting of the biochemical phototransduction cascade. Inactivation of the phototransduction cascade is rate-limited by a GTPase activating complex and mutations in components of this complex, RGS9 and R9AP, result in the visual disorder Bradyopsia. Bradyopsia is characterized by impaired visual perception for moving stimuli which is evident by reduced temporal contrast sensitivity, as well as photophobia which in mice presents as light aversion. Because HCN1 and RGS9/R9AP both function to accelerate the photoreceptor response to light, we predict that HCN1 loss will result in Bradyopsia-like phenotypes. Retinal signaling measured by electroretinography (ERG) is altered in both RGS9 and R9AP knockout models of Bradyopsia and in HCN1 knockout mice, with prolonged rod activation resulting in extended rod driven retinal signaling leading to dampened responses to subsequent stimulation, particular to higher frequency flickering light. We predict that loss of HCN1 would also result in impaired visual perception similar to that observed in Bradyopsia, namely reduced temporal contrast sensitivity and light aversion. Because HCN1 is expressed throughout the brain including in other neurons of the retina, we tested this prediction using conditional HCN1 KO bred to rod or cone specific Cre-drivers. Immunohistochemistry was used to verify loss of HCN1 from rods or cones, respectively in HCN1-Rod KO or HCN1-Cone KO. Next ERG was used to characterize retinal signaling in these lines. HCN1-Rod KO mice phenocopy the ERG phenotype of global HCN1 KO, exhibiting prolonged rod driven responses and dampened cone driven responses, including response to flickering light. Conversely, HCN1-Cone KO mice exhibit no detectable phenotype suggesting that cones do not require HCN1 to respond normally to single flashes of light or a high frequency flicker. In line with that observation, the HCN1-Cone KO mice do not have altered temporal contrast sensitivity or light aversion. Future studies will continue to test for Bradyopsia-like phenotypes in HCN1-Rod KO animals using behavioral assays and explore the functional role of HCN1 in cones which may be required for dynamic vision under high intensity light or may be dispensable due to compensatory activity of other channels such as the heteromeric $K_v2.1/K_v8.2$ channels that carry the complementary current I_{kx}.

Disclosures: **C. Lankford:** None. **Y. Umino:** None. **E. Solessio:** None. **S. Baker:** None.

Digital Abstract Session

P187. Retina- Photoreceptors

Program #/Poster #: P187.04

Topic: D.07. Vision

Support: NIH R01EY026817

Title: Analysis of Cav1.4 Dependent Cone Synaptic Development

Authors: ***J. G. LAIRD**, A. KOPEL, C. LANKFORD, E. THORNBURG, S. A. BAKER; Univ. of Iowa Carver Col. of Med., Iowa City, IA

Abstract: The voltage-gated calcium channel, Cav1.4, mediates synaptic transmission and maintenance at the ribbon synapses of rods and cones. Mutations in the pore forming subunit, a1F, or the trans-synaptic accessory subunit, a2d4, result in congenital synaptic disorders such as, congenital stationary night blindness 2 (*CSNB2)*, and cone-rod dystrophy. Previous studies of mouse models of these diseases demonstrated that rods and cones have differing requirements for the Cav1.4 subunits. Since cones only make up a small percentage of photoreceptors, we decided to study synaptic development in a double-mutant mouse strain where all photoreceptors are cones. We refer to this strain as 'Conefull'. We crossed conefull and Cav1.4 mutants to generate 'cone:a1F KO' and 'cone:a2d4 KO' to further investigate the role of Cav1.4 in the development and function specifically of cone synapses. As a measure of vision, a visually guided water maze was used. Cone:a1F KO could not pass the visually guided water maze whereas the cone:a2d4 KO could navigate the water maze as efficiently as the control group. Retinal function was measured by electroretinogram (ERG) to show that each animal had an intact a-wave indicative of phototransduction. However, the b-wave, indicative of synaptic transmission was absent in cone:a1F KO and reduced in cone:a2d4 KO. Optical coherence tomography (OCT) imaging of the retinas in living animals revealed progressive retinal degeneration. Retinal thinning over 2-6 months of age progressed from 32-49% loss for cone:a1F KO but only 12-19% loss for cone:a2d4 KO. Histological analysis of cone:a1F KO demonstrated that photoreceptor loss begins as early as post-natal day 11, before eye opening. In summary we have created a useful model for detailed analysis of Cav1.4 dependent cone synaptic development.

Disclosures: J.G. Laird: None. **A. Kopel:** None. **C. Lankford:** None. **E. Thornburg:** None. **S.A. Baker:** None.

Digital Abstract Session

P187. Retina- Photoreceptors

Program #/Poster #: P187.05

Topic: D.07. Vision

Support: NIH Grant R01 EY026216

Title: Frequency-dependent light adaptation in rod-driven trans-retinal ERGs

Authors: *Y. GUO, *Y. UMINO, S. LAMAGNA, E. SOLESSIO;
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Abstract: Human vision adapts to ambient light levels spanning > 8 orders of magnitude, maintaining an exquisite sensitivity to small differences in light (*i.e*. contrast). One consequence of adaptation is that visual responses speed up as light levels increase, enhancing flicker sensitivity at high temporal frequencies. Previously, we have shown that flicker sensitivity is mainly set by rod photoreceptors, whose temporal response kinetics to dim flashes also adaptively speed up. However, it is not known whether rod flash responses alone can fully

account for adaptive rod temporal frequency responses. Thus, the purpose of this work is to determine the mechanisms that shape the rod-driven frequency response in light adaptation. *Methods*: Trans-retinal flicker ERGs were recorded from WT mice. Retinas were perfused with Ames medium supplemented with DL-AP4 (50 μM), CNQX (20 μM) and BaCl2 (100 μM) to isolate the photoreceptor response. Rod-driven photoresponses to sinusoidal flicker of 75% contrast at 0.75 - 28 Hz were recorded at backgrounds ranging from 10 - 3200 R*/rod/s. *Results*: 1. The rod-driven frequency response have a characteristic low-pass shape at low backgrounds (10-200 R*/rod/s) that switches to band-pass shape as background levels rise above 200 R*/rod/s. Superfusion with cesium, a blocker of HCN1 channels, selectively increases the magnitude of the responses to low frequencies at high backgrounds.2. Light adaptation extends the bandwidth of the frequency response functions from a minimum of 1.75±0.03 Hz (Mean±SEM) at 10 R*/rod/s to an asymptote of 5.68±1.47 Hz at 3200 R*/rod/s. R9AP95 mice, with accelerated Gtα*-E* inactivation (the slowest step in the phototransduction cascade), display wider bandwidths than WT mice (2.35±0.07 Hz at 10 R*/rod/s to 8.13±0.29 Hz at 3200 R*/rod/s). 3. For low frequencies (0.75-3 Hz), gain decreases monotonically and phase speeds up as background levels rise (>10 R*/rod/s). For high frequencies (6-28Hz), gain remains constant up to 800 R*/rod/s and decreases for backgrounds >800 R*/rod/s. In this regime, phase remains constant <800 R*/rod/s and speeds up >800 R*/rod/s. *Conclusion*: This study has uncovered that light adaptation in rod-driven frequency responses has two regimes. Rod-driven signals to low frequencies adapts to light similarly as flash responses, while rod-driven signals to high frequencies have constant gain and phase. These results are in line with studies on horizontal cells (Tranchina, D., Gordon, J., & Shapley, R. M., 1984) and psychophysical temporal contrast sensitivity functions of mice (our lab) and humans (Kelly's data).

Disclosures: **Y. Guo:** None. **Y. Umino:** None. **S. LaMagna:** None. **E. Solessio:** None.

Digital Abstract Session

P188. Retinal Circuitry

Program #/Poster #: P188.01

Topic: D.07. Vision

Support: JSPS KAKENHI Grant Number JP16K08504

Title: The functional role of TRPM1 channels in the synaptic transmission between retinal rod bipolar and AII amacrine cells

Authors: ***F. TAMALU**[1], K. SHIBASAKI[2], M. SHIIBASHI[1], S.-I. WATANABE[1], N. MIWA[1]; [1]Saitama Med. Univ., Moroyama, Japan; [2]Univ. of Nagasaki, Nagayo-cho, Japan

Abstract: Retinal rod bipolar cells (RBCs) express the TRPM1 channel, which is a non-selective cation channel that opens in response to light resulting in depolarization. We show that RBCs depolarized approximately 20 mV as the temperature was raised from 22 ºC to 34 ºC, which showed significant differences (86.4 mV at 22 ºC and 66.8 mV at 34 ºC). When using

extracellular ruthenium red, which is a TRP channel inhibitor (RR), there was no significant difference in the membrane potentials of RBCs between 22 ºC and 34 ºC. Similarly, insignificant depolarization by heat was observed in RBCs of TRPM1 KO mice. Moreover, we found that both the frequency and amplitude of EPSCs observed in the AII amacrine cells, that are post-synaptic to RBCs, dramatically increased at a higher temperature. The electric charge of the EPSCs at 34 ºC was 4.3-times greater than that at 22 ºC and 3.1-times greater than that at 34 ºC with RR, respectively. The EPSC frequency increased significantly as the temperature increased from 22 ºC to 34 ºC, but decreased by adding RR at 34 ºC. Similarly, the EPSC amplitude increased at a higher temperature, but is partially inhibited by ruthenium red. In TRPM1 KO mice, there was no significant difference between 22 ºC and 34 ºC observed in terms of frequency, but the amplitude increased significantly at a higher temperature. Our findings indicate that the TRPM1 plays a role in the glutamate release frequency from RBCs at physiological temperature. It is likely that the body temperature may expand the dynamic range of glutamate release from RBCs by increasing the open probability of TRPM1 channel contributing to scotopic vision. Thus, TRPM1 deficiency might relate to retinal diseases such as the nyctalopia, in particular.

Disclosures: **F. Tamalu:** None. **K. Shibasaki:** None. **M. Shiibashi:** None. **S. Watanabe:** None. **N. Miwa:** None.

Digital Abstract Session

P188. Retinal Circuitry

Program #/Poster #: P188.02

Topic: D.07. Vision

Support: NIH Grant EY015290-15A1

Title: Depth perception perturbation in mice with an altered retinofugal pathway

Authors: ***N. SLAVI**[1], C. MASON[2];
[1]Zuckerman Inst., [2]Pathology & Cell Biology, Neuroscience, and Opthalmology, Columbia Univ., New York, NY

Abstract: In animals with frontally positioned eyes, partial decussation of retinal ganglion cell (RGC) axons at the optic chiasm in specific proportions allows binocular integration of visual information in the brain, forming the basis of disparity-based stereopsis. The albino visual system is characterized by a reduction of ipsilaterally projecting RGCs, and a concomitant increase of the contralateral projection. Here, we examined whether aberrations in the eye-to-brain circuitry in albinism lead to impaired visually guided behavior. We compared the performance of pigmented and albino mice in the binocularly driven visual cliff behavioral task to assess depth perception. Mice were positioned in the center of an open-top platform with a shallow and a deep side, both displaying the same sized checkerboard pattern. Pigmented mice almost always chose to step on the shallow side, whereas albino mice failed to distinguish

between the shallow and deep side of the platform. To confirm that this behavior resulted from ipsi-/contralateral RGC axon misrouting in albino mice, we performed an additional visual task that does not depend on binocular disparity, but instead relies on a monocular cue, relative size. For this task, both sides of the behavioral apparatus were at the same height, but its two sides displayed a low- versus a high-spatial frequency checkerboard pattern. Pigmented and albino mice behaved similarly in this monocularly driven task. Our results thus show that perturbations in the ratio of ipsi-/contralateral RGC projection lead to reduced depth perception in albino mice, indicated by their compromised performance in the visual cliff task. This study provides a framework for assaying the functional integrity of the visual circuit in experimental models interrogating neurogenesis and proper output of ipsi- and contralateral RGCs.

Disclosures: **N. Slavi:** None. **C. Mason:** None.

Digital Abstract Session

P188. Retinal Circuitry

Program #/Poster #: P188.03

Topic: D.07. Vision

Title: Partial Information Decomposition of the response from a Retina

Authors: ***Q.-R. LIN**[1,2], K.-H. CHEN[1,3], P.-Y. CHOU[1,4], C. CHAN[1];
[1]Inst. of Physics, Academia Sinica, Taipei, Taiwan; [2]Dept. of Physics, Natl. Taiwan Univ., Taipei, Taiwan; [3]Dept. of Mol. Med., Natl. Tsing Hua Univ., Hsinchu, Taiwan; [4]Dept. of physics, Natl. Central Univ., Taoyuan, Taiwan

Abstract: In order to survive, escape of predators or pursuit of prey, animals must make predictions to compensate delays from visual processing pathway. Previous studies revealed that predictive information of motion is encoded in spiking activities of retinal ganglion cells (RGCs). In order to study the predictive properties of a retina in a more systematic manner, stimuli in the form of a stochastic moving bar are used in experiments with retinas from bull frogs in a multi-electrode system. Trajectories of the bar are produced by Ornstein Uhlenbeck (OU) processes with different time correlations (memories) induced by a butter-worth low-pass filter with various cut-off frequencies.
We then investigated the predictive properties of single RGC by calculating the time shifted (δt) mutual information ($I(x,r;\delta t)$) between spiking output ($r(t)$) from RGCs and the bar trajectories ($x(t)$). Our measurements show that some RGCs (P-RGCs) are predictive while the others are non-predictive (NPRGCs). It is very reasonable that a P-RGC encodes both position (x) and velocity (v) of the input. So we perform partial information decomposition (PID)[1] on the mutual information between r and the combined state $\{x,v\}$ which can be written as $I(\{x,v\},r) = S + U_v + U_x + R$. U_v and U_x are the unique contribution from x and v to r, respectively. R, the redundancy, is the information that x and v can both provide. Finally, S represent synergy contribution from x and v. Then, we construct a model for P-RGC with the spiking response being a linear combination of x and v: $r=(1-\lambda)*x+\lambda*v$. By comparing the PID of our

experimental results and those of this simple model, we find that λ≈0.45 fits our data quite well (Fig.1). Implication of this simple coding strategy of the retina will also be discussed.

1. Williams PL, Beer RD. Nonnegative Decomposition of Multivariate Information. arxiv. 2010

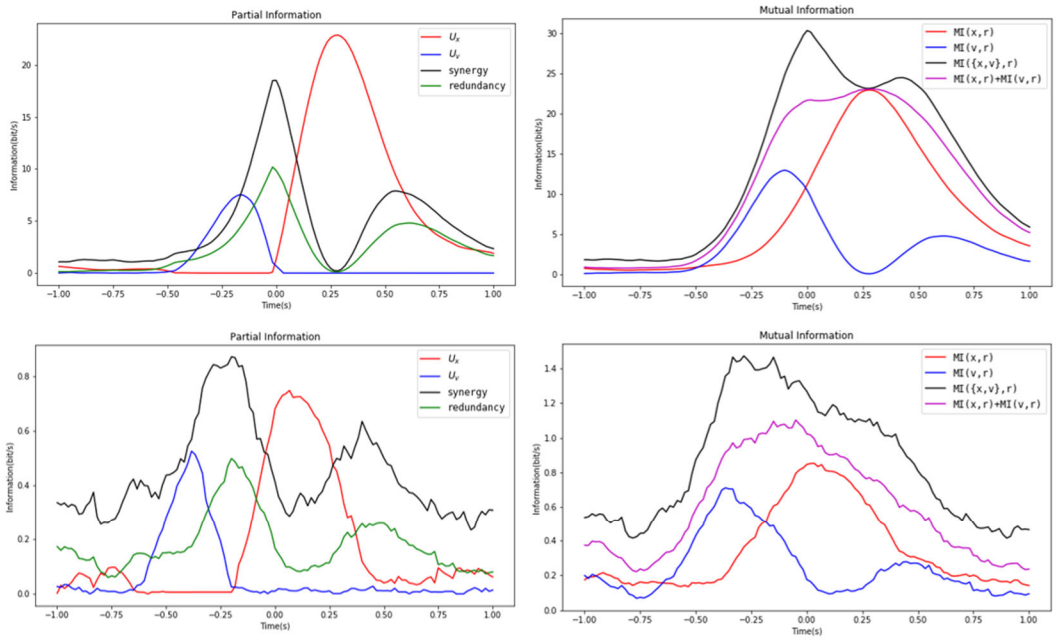

Fig. 1: A case that the (a) MI and (b) Partial Information of model ($\lambda = 0.45$) looked similar to (c)(d) those of output of a real RGC. Thus the processing time of this RGC can be around 200ms.

Disclosures: **Q. Lin:** None. **K. Chen:** None. **P. Chou:** None. **C. Chan:** None.

Digital Abstract Session

P188. Retinal Circuitry

Program #/Poster #: P188.04

Topic: D.07. Vision

Title: Anticipation and Negative Group Delay in a Retina

Authors: *P.-Y. CHOU;
Academia Sinica.

Abstract: The mechanism of negative group delay (NGD) is used to understand the anticipative capability of a retina. Experiments with retinas from bull frogs are performed to compare with the predictions of the NGD model. In particulars, whole field stochastic stimulation with various time correlations are used to probe anticipative responses from the retina. We find that the NGD model can reproduce essential features of experimental observations characterized by the cross correlations between the simulation and the retinal responses. The main finding is that the

prediction horizon of a retina depends on the correlation time of the stimulation as predicted by the NGD model. Experiments with dark and bright Gaussian light pulses further support the NGD mechanism; but only for the dark pulses indicating that the NGD effect of a retina might originate from its OFF response. Our finding suggests that sensory systems capable of using negative feedback for adaptation can give rise to anticipation as a consequence of the delay in the system.

Disclosures:

Digital Abstract Session

P188. Retinal Circuitry

Program #/Poster #: P188.05

Topic: D.07. Vision

Support: 5T32EY007031-43 (MPI)
EY028542
EY028111

Title: The spatial and temporal aspects of retinal ganglion receptive fields are dependent on ambient light conditions

Authors: *N. T. INGRAM, F. RIEKE;
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Abstract: Current studies in visual neuroscience look to understand how adaptive modulations and circuit routing affect stimulus encoding across layers of the retina. At the output of the retina, retinal ganglion cells (RGCs) are able to encode rapid stimulus fluctuations despite the relatively slow input they receive from upstream photoreceptors. They also have receptive fields that display center-surround antagonism, in which lateral inhibition driven by the surround inhibits responses generated from stimulating the center (Barlow, 1957). Previous studies in mouse show that the spatial dimensions of center versus surround depend on ambient light conditions (Hoggarth et al., 2015; Farrow et al., 2013). It is not known if all RGC subtypes undergo similar shifts, to what degree circuit mechanisms like changes in rod-cone signal routing are responsible, and if other receptive field features such as surround-dependent temporal filtering are similarly dependent on light level. To answer these questions in the primate retina, we make electrophysiological recordings from primate RGCs with stimuli that span 8 orders in magnitude in light intensity. We measure light-dependent shifts in spatial receptive fields through this entire range of stimuli and in multiple RGC subtypes. Both parasol and midget RGC receptive fields show a similar light dependence when measuring spatial features. In addition, we show for the first time, that the strength of surround-mediated temporal filtering also depends on the background light intensity. Interestingly, the effects of surround-mediated temporal filtering are greater in dim light, a condition in which the surround contributes little to the spatial receptive field. Light-dependent changes in these receptive field features are likely to have large

effects on an organism's ability to perform complex visual tasks which depend on correlations between space and time (e.g. motion detection).

Disclosures: **N.T. Ingram:** None. **F. Rieke:** None.

Digital Abstract Session

P188. Retinal Circuitry

Program #/Poster #: P188.06

Topic: D.07. Vision

Support: NIH Grant 5R01EY028111
NIH Grant 5R01EY028542

Title: Designing stimuli that minimize cone adaptation to study neural coding

Authors: ***Q. CHEN**, F. RIEKE;
Physiol. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: Classic work on how adaptation enhances the encoding of visual inputs focuses on the mechanisms accommodating day/night changes in luminance. But light levels encountered by visual neurons change dramatically as gaze shifts from one location to another within a single scene. Thus, efficient encoding of naturalistic environments requires rapid and strong adaptational mechanisms that match the statistics of visual inputs to the limited dynamic range of visual neurons. In steady-state, multiple mechanisms work in concert to maintain visual sensitivity across light levels, with adaptation in cone phototransduction playing a particularly prominent role. We lack, however, a similar understanding of the location and properties of the adaptational mechanisms that operate during dynamic natural viewing. Moreover, in primate models, whose visual system and eye movements closely recapitulates that of humans, we lack tools like genetic manipulations to reveal the operation of neural circuits. Here, we use a biophysical model that captures the responses of cone photoreceptors to a wide range of stimuli. This model constitutes a set of differential equations, describing the biochemical components of phototransduction. We verified the accuracy of this model via directly record primate cones. We then develop a reverse-engineering paradigm through optimization to design a transformed version of original stimuli to cause cones to respond as if they lacked adaptation. This novel tool allows us to determine the contribution of photoreceptor adaptation to the final output of primate retina in different visual pathways. In fact, we found that light adaption in parvocellular pathway is dominated by cone photoreceptor adaptation while magnocellular pathway engages strong post-receptoral adaption. The methodology, per se, could pave new way of physiologically and psychophysically studying both non-human primate and human visual systems.

Disclosures: **Q. Chen:** None. **F. Rieke:** None.

Digital Abstract Session

P188. Retinal Circuitry

Program #/Poster #: P188.07

Topic: D.07. Vision

Support: R01EY019498
R01EY013528
P30EY003176
K99EY030909-01A1

Title: Retinal direction selectivity maps develop independently of visual input but require retinal waves

Authors: *A. TIRIAC[1], K. BISTRONG[2], M. B. FELLER[3,2];
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Abstract: Detecting the directions in which objects move is critical for everyday behavior. In mice, directional motion detection begins in the retina where a subset of direction selective retinal ganglion cells (DSGCs) fire more action potentials in response to visual stimuli moving in one direction, called the preferred direction, than visual stimuli moving in the opposite direction, called the null direction. In adult mice, the preferred directions cluster along four directions that align along two optic flow axes—paths that visual stimuli trace across the retina as the animal moves through space (Sabbah et al., Nature 2017). Here, we used two-photon calcium imaging to confirm that in normal-reared mice, the preferred directions of DSGCs changed as a function of their location on the retina, with the axes of preferred directions of DSGCs skewed in the more ventral parts of the retina, consistent with their alignment along the axes of optic flow. Furthermore, we show that the direction selectivity map is present at eye opening and is not impacted by dark rearing. However, disruption of cholinergic retinal waves prevented the maturation of direction-selective responses along the horizontal optic flow axis but did not affect the vertical preferring responses. Hence direction selectivity maps are established prior to vision in a manner dependent on the presence of retinal waves.

Disclosures: **A. Tiriac:** None. **K. Bistrong:** None. **M.B. Feller:** None.

Digital Abstract Session

P189. Subcortical Visual Pathways

Program #/Poster #: P189.01

Topic: D.07. Vision

Support: NIH Grant EY026286

Title: Visual responses in the tree shrew superior colliculus

Authors: *E. L. SAVIER[1], S. TANABE[1], H. CHEN[1], J. CANG[2];
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Abstract: Visual signals produced by the retina are transmitted to two parallel pathways, often referred to as the primary (retina → thalamus→ primary visual cortex (V1)) and the secondary visual pathways (retina → superior colliculus (SC) → pulvinar → visual cortex). The two pathways are traditionally thought to mediate different aspects of vision, with the primary pathway for conscious vision and the secondary pathway for reflexive behaviors. However, there is increasing evidence that the secondary visual pathway is also involved in higher perceptive and cognitive functions, via their massive connections with many brain structures. Tree shrews have a well-developed SC, which makes them an ideal subject to study the role of the secondary visual pathway in visual behaviors. These animals are among the closest relatives to primates, are diurnal, have a cone-dominant retina and display exquisite visual behaviors. However, a systematic characterization of the SC visual receptive fields is lacking. We sought to characterize the visual response in tree shrew SC using multi-electrode array recordings. We performed recordings in both male and female tree shrews under awake conditions, using high density silicon probes. We tested the visual responses to drifting gratings, moving dots, sparse and dense noise. With this stimulus set, we found a surprisingly wide range of receptive fields sizes and shapes, variety of speed tuning and distribution of spatial frequency tuning. This study lays the foundation towards a better understanding of the distinct visual processing between the two visual pathways.

Disclosures: E.L. Savier: None. **S. Tanabe:** None. **J. Cang:** None. **H. Chen:** None.

Digital Abstract Session

P189. Subcortical Visual Pathways

Program #/Poster #: P189.02

Topic: D.07. Vision

Support: ARC Centre of Excellence for Integrative Brain Function (CIBF)

Title: Binocular integration in the superior colliculus

Authors: *R. BROERSEN, G. J. STUART;
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Abstract: The superior colliculus (SC) is a layered, evolutionarily conserved midbrain structure involved in different behaviours, such as saccadic eye movements, orienting movements and innate defensive behaviours. It is a major visual centre in rodents, receiving input from 85-90% of retinal ganglion cells that terminate predominantly in the superficial layers. The SC is also innervated by the ipsilateral primary visual cortex and the SC in the opposite hemisphere. While integration of visual inputs from both eyes takes place at different stages within the visual

system, the effect of binocular summation on visually-evoked neuronal responses in SC remains largely unexplored. Here we determine the characteristics of binocular responses in SC to a range of visual stimuli relevant to natural situations and dissect the neuronal circuit involved. In vivo juxtasomal recordings in urethane-anaesthetized adult male Bl6 mice were used to study neuronal responses to brief, full-field (LED) flashes, flashing dark spots as well as looming stimuli. This was combined with AAV viral injections, optogenetics and histology to allow neuronal circuit mapping. We find that binocular neurons are located across multiple layers of SC. While the vast majority of neurons were solely driven by contralateral visual input and therefore were monocular, a subset of neurons was driven by both contralateral and ipsilateral visual input and were therefore characterised as binocular (~5-25% of neurons, depending on the visual stimulus). Binocular neurons responding to LED flashes were located mainly in superficial layers, while those responding to more complex stimuli (flashing or looming spot) were located in intermediate and deeper layers. Binocular stimulation consistently resulted in larger spike responses compared to monocular stimulation, but summation was predominantly sublinear. Optogenetic activation of the opposite SC using Channelrhodopsin-2 showed that the amplitude of optogenetically evoked responses correlated with the amplitude of ipsilateral visually-evoked responses, suggesting that ipsilateral eye responses in binocular neurons originate from interhemispheric connections. Our findings indicate that while visual responses in SC are primarily driven by the contralateral eye, a significant subset of SC neurons respond to binocular stimuli. Furthermore, the data are consistent with the idea that ipsilateral eye responses in these binocular SC neurons are largely driven by interhemispheric connections from the opposite SC.

Disclosures: **R. Broersen:** None. **G.J. Stuart:** None.

Digital Abstract Session

P189. Subcortical Visual Pathways

Program #/Poster #: P189.03

Topic: D.07. Vision

Support: NSF grant (IOS-1656838)
DARPA grant (HR0011-18-2-0019, TA2)
Fondecyt 1170027

Title: Visual receptive field properties of superior colliculus neurons in a diurnal precocial rodent (Octodon degus).

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Abstract: The superior colliculus (SC) is a layered structure at the dorsal surface of the midbrain that receives a massive direct input from the retina, as well as from other sensory modalities. The SC controls visually driven behaviors such as gaze shifts, reorientation toward objects of interest, and escape or freezing responses to a looming object. Neurons from the superficial layers of the SC (sSC) are tuned to specific configurations of visual stimuli within their receptive fields (RFs). Recent studies comparing the RF properties of SC neurons in normal (NR) and dark reared (DR) rodents have challenged the general assumption, derived from studies in the visual cortex of cats and monkeys, that visual experience is necessary for RF refinement. It should be noted, however, that the rodent species studied so far (hamsters and mice) are nocturnal, altricial, and possess a poorly developed visual system. As a counterpoint to these studies, we have characterized the RF properties of SC neurons in Chilean degus, a diurnal, precocial rodent species with a well-developed visual system, as an initial step for comparing their RF properties with those of DR degus. We isolated single neurons from the sSC in anesthetized NR degus and characterized responses to four types of visual stimuli: (1) a moving white square on a dark background, (2) sinusoidal gratings with varying spatial frequencies and contrasts, (3) a black, looming circle, and (4) a stationary black circle. We sampled the anterior part of the sSC representing the frontal visual field. As we reported previously for multiunit recordings, RF sizes of single units were consistently smaller in the most superficial layers (3° - 9° in diameter in the first 500 μm) and increased in size with depth (8.3° - 40° from 500 - 1100 μm). Most units responded to moving sinusoidal gratings and displayed specific spatial frequency tuning that we classified as low-pass (with responses decaying below 50% near 0.08 cycles per degree (cpd)), band-pass (with peak frequencies between 0.04 - 0.24 cpd) or hi-pass (with peak responses above 0.08 cpd). Lastly, we found two kinds of neurons that responded to looming objects in a similar way to neurons previously described in pigeons and cats. These neurons differed in their peak firing rate with respect to the time the object reached maximum size, reducing their activity before or at this time point. When these units were stimulated with a stationary black circle they showed ON, OFF or ON-OFF responses. Our study is an important step in understanding the role of visual experience in a rodent species that has a different visual ecology and that represents a different phylogenetic group than more commonly studied species.

Disclosures: **N.I. Márquez:** None. **I. Perales:** None. **A. Deichler:** None. **P. Fernández-Aburto:** None. **J. Letelier:** None. **G. Marín:** None. **J. Mpodozis:** None. **S.L. Pallas:** None.

Digital Abstract Session

P190. Visual Cortex: Circuits

Program #/Poster #: P190.01

Topic: D.07. Vision

Support: Jane Coffin Childs Memorial Fund
Howard Hughes Medical Institute

Title: Erasing motion: The scrambling of direction selectivity in visual cortex during saccades

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Abstract: Sensory stimuli are often generated by the animal's own movements. Many sensory systems suppress these self-induced inputs and selectively respond to externally generated stimuli, thereby maintaining the perceptual stability of the external world. Rapid eye movement, called saccades, induces motion of the visual scene on the retina. Several mechanisms have been proposed that prevent the perception of such motion. Yet, how they are implemented in the circuitry of visual cortex is poorly understood. Here, we focused on the mouse primary visual cortex (V1). In V1, neurons encode the motion direction of visual stimuli. We began by comparing the response of V1 neurons to spontaneous saccades and their response to external visual stimuli designed to mimic the motion of visual scene induced by saccades, which we termed "pseudo-saccades". Many neurons showed direction selectivity for saccades (i.e., responded more to saccades moving in one direction than to those in the other direction) as well as for pseudo-saccades. Interestingly, however, there was no correlation between the direction selectivity for the two conditions. Consequently, a downstream decoder that discriminates the direction of pseudo-saccades based on the population activity in V1 is unable to discriminate the direction of visual motion induced by actual saccades. Thus, in V1, direction selectivity is scrambled during saccades, and information about the direction of motion is suppressed. Where does this scrambling originate from? We discovered that V1 receives a non-visual input from the pulvinar nucleus of the thalamus around the time of saccades. To determine whether this pulvinar input mediates the scrambling of direction selectivity, we pharmacologically silenced the pulvinar. Upon silencing this thalamic nucleus, the direction selectivity for real saccades and pseudo-saccades became correlated. Thus, the pulvinar erases saccade-induced motion of the visual scene in V1 by scrambling direction selectivity.

Disclosures: S.K. Miura: None. **M. Scanziani:** None.

Digital Abstract Session

P190. Visual Cortex: Circuits

Program #/Poster #: P190.02

Topic: D.07. Vision

Support: Jefferson Scholars Foundation

Title: Neurons in tree shrew V1 are highly selective for binocular disparity

Authors: *S. TANABE, J. CANG;
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Abstract: The visual system uses small differences between the two eye's images, i.e., binocular disparity, to construct depth information in a perceptual process called stereopsis. The neuronal responses to stereoscopic images have revealed certain general principles. For example, it is

known that the two eyes' inputs remain segregated until the primary visual cortex (V1), where neurons sensitive to binocular disparity are first seen. Despite these advances, the circuit mechanisms underlying stereopsis and their developmental processes remain largely unknown. The main reason for the lack of progress is limitations in the animal models that are currently used in binocular vision research, most notably monkeys and mice. Monkeys are not yet an ideal model for circuit and developmental studies because of the still limited technology available to primate research and long generation time. In comparison, mice provide opportunities for genetic, optogenetic, viral, and imaging techniques. Unfortunately, mice are not ideal subjects for studying stereopsis because of their low visual acuity and rudimentary binocular vision. We set out to establish a novel animal model, the tree shrew, for the neurophysiological investigation of binocular vision. Tree shrews are agile in an arboreal environment, which requires well-developed stereopsis. In our experiments, the animals were head fixed to be presented with monocular or dichoptic visual stimuli through a mirror stereoscope. We inserted multi-electrode silicon probes (64 channels spanning all cortical layers) into the binocular region of the primary visual cortex (V1). We tested the orientation tuning with monocularly presented gratings, disparity tuning with dynamic random-dot stereograms, and linear and nonlinear receptive-field properties with white noise. Many neurons were orientation tuned with gratings presented to either eye. White noise analysis recovered receptive fields that were confined to a small location ~1 deg in diameter. We found neurons that are highly selective for disparity. A subset of neurons increased firing rate from 0 to 50 spikes/s within a range smaller than 1 deg disparity. Our results suggest that the tree shrew V1 is an excellent model for dissecting circuit mechanisms of binocular combination.

Disclosures: **S. Tanabe:** None. **J. Cang:** None.

Digital Abstract Session

P190. Visual Cortex: Circuits

Program #/Poster #: P190.03

Topic: D.07. Vision

Support: NIH Grant EY028723
NIH Grant NS108683
NIH Grant EY0190005
NIH Grant EY020503-06
NIH Grant MH020002-16A
The Fiona and Sanjay Jha Chair in Neuroscience
Canadian Institute for Health Research

Title: Spontaneous traveling waves are an intrinsic feature of ongoing cortical dynamics and regulate perceptual sensitivity

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Abstract: Neocortical activity is highly variable, fluctuating from moment-to-moment, with broadly distributed spectral energy (Shadlen and Newsome, J Neurosci, 1998). In multielectrode array recordings made in area MT of the common marmoset, we find that these fluctuations are neither fully random nor synchronous across space: rather, they are often spatiotemporally organized into traveling waves. As they traverse the cortex, these waves modulate spontaneous and stimulus-evoked spiking activity and perceptual sensitivity as measured in monkeys trained to detect faint visual targets. To gain insight into the neural mechanisms underlying traveling waves, we study a large-scale spiking network model with conductance-based synapses, biologically realistic topographic connectivity and action potential propagation speeds consistent with those observed in unmyelinated horizontal fibers (Girard et al., J Neurophysiol, 2001). We find that these properties are sufficient to generate spontaneous waves across the entire range of network parameters that produce asynchronous-irregular spiking dynamics (Brunel, J Comput Neurosci, 2000; Renart et al., Science, 2010). Further, we find that neuronal participation in these waves is sparse, enabling traveling waves to coexist with asynchronous-irregular spiking activity without necessarily inducing correlations, which have been found to impair perception (Nandy et al., eLife, 2019). This sparse-wave network regime remained sensitive to feed-forward driving input and modulated the strength of stimulus-evoked responses in a manner similar to that observed in cortex. This was in contrast to smaller scale networks that produce dense spike coupling to traveling waves, which drove strong pairwise correlations and rendered the network insensitive to feed-forward driving input. We conclude that the presence of traveling waves in cortical dynamics can be explained as a natural consequence of activity propagating along unmyelinated horizontal fibers. Traveling waves, which improve perceptual sensitivity in MT (Davis et al., Nature, 2020), appear to be an intrinsic feature of cortical dynamics, and they therefore likely impact the moment-to-moment processing of information throughout the brain.

Disclosures: Z.W. Davis: None. **G.B. Benigno:** None. **T.J. Sejnowski:** None. **J.H. Reynolds:** None. **L. Muller:** None.

Digital Abstract Session

P190. Visual Cortex: Circuits

Program #/Poster #: P190.04

Topic: D.07. Vision

Support: NSF Award # 0726113
NSF Award # 1439189
NSF Award # 1734887

Title: Visual offset responses, in V1, are not modulated by neural feedback.

Authors: *N. BRUNET[1], F. BARTSCH[2], D. A. BUTTS[3], S. MARTINEZ-CONDE[4], S. L. MACKNIK[5];
[1]Millsaps Col., Jackson, MS; [2]Univ. of Maryland, College Park, MD; [3]Dept. of Biol., Univ. of Maryland at Col. Park Dept. of Biol., College Park, MD; [4]Dept. of Ophthalmology, State Univ. of New York Downstate Med. Ctr., Brooklyn, NY; [5]Dept. of Ophthalmology., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY

Abstract: How the duration of a visual stimulus affects its perceived brightness is not well understood. To uncover the neural mechanisms of duration on brightness perception, we recorded from single cells in non-human primates. We (1) located V1 neurons, (2) mapped its receptive field (RF), and (3) presented Gabor stimuli, with variable duration across trials, in the RF of the identified V1 neuron.
For each recorded cell, we recorded the entire response and analyzed both onset and termination responses to the visual stimuli. We present the electro physiological results and several models, aimed to characterize the relationship between onset and termination responses, as a function of stimulus duration. The results reveal a strong correlation between the timing of the onset and the termination responses to the visual stimulus, suggesting that it is unlikely that either response is derived by feedback signals from downstream areas.

Disclosures: N. Brunet: None. **F. Bartsch:** None. **D.A. Butts:** None. **S. Martinez-Conde:** None. **S.L. Macknik:** None.

Digital Abstract Session

P190. Visual Cortex: Circuits

Program #/Poster #: P190.05

Topic: D.07. Vision

Title: Distinct representation of prediction signals in excitatory and inhibitory populations during a visual task

Authors: *F. NAJAFI, M. GARRETT, A. PIET, D. OLLERENSHAW, I. YAVORSKA, N. PONVERT, P. GROBLEWSKI, S. MIHALAS, S. R. OLSEN;
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Abstract: Predictive coding states that the brain constantly generates and updates predictions about sensory inputs. Previous studies have shown that excitatory neurons in the visual cortex detect oddball stimuli, potentially mediated by SST and VIP inhibitory neurons, as well as the neuromodulatory system. While these studies have begun to illuminate the cell types involved in predictive coding, many questions still remain. In particular, it is unclear how predictive signals arise in each cell type. A change in an animal's internal state or in top-down and bottom-up inputs may underlie predictive signals in distinct cell types. Here, we seek to address these questions by studying the activity of distinct excitatory and inhibitory neurons in a visual task under different internal states and sensory contexts. The visual task involved repeated image

presentations interspersed with rare image omissions. To assess the role of internal state, images were presented either passively or while animals were engaged in the task. Moreover, animals received two image sets across sessions: one familiar set that mice were trained on, and another novel set that they had never seen, which allowed studying how novel context affects predictive signals in each cell type. We used Multiplane Mesoscopes to simultaneously image multiple cortical layers and areas. To characterize sensory and prediction signals in our dataset, we fit a GLM to the activity of each neuron. Our results indicate that excitatory, SST and VIP neurons are modulated by omissions. Additionally, we found that novel context strongly affects omission coding in excitatory and VIP cells, and to a lesser extent in SST cells. Next, we performed a series of population decoding analyses to investigate omission-related signals at the ensemble level. First, we used a binary classifier to decode the presence of omissions, and found that omissions are strongly represented in all populations. Next, we used multi-class classification to study if the omission-evoked signal encoded image identity. Interestingly, we observed a slow steady increase in image selectivity following omissions in excitatory and SST populations. In contrast, the omission response was not image selective in the VIP population. Our results indicate that excitatory and SST neurons may carry stimulus-selective prediction signals, while VIP neurons represent a non-selective signal following unexpected events. Moreover, excitatory neurons seem to be more influenced by novel context compared to SST neurons. Overall, our findings suggest that separate cell types encode distinct prediction signals that vary in their stimulus specificity and modulation by context.

Disclosures: **F. Najafi:** None. **M. Garrett:** None. **A. Piet:** None. **D. Ollerenshaw:** None. **I. Yavorska:** None. **N. Ponvert:** None. **P. Groblewski:** None. **S. Mihalas:** None. **S.R. Olsen:** None.

Digital Abstract Session

P190. Visual Cortex: Circuits

Program #/Poster #: P190.06

Topic: D.07. Vision

Support: NIH Grant 1U01MH114824-01

Title: Automated characterization of neuronal arbors from brightfield images in a multimodal dataset

Authors: *O. GLIKO, M. MALLORY, R. DALLEY, R. GALA, S. SORENSEN, U. SÜMBÜL;
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Abstract: Recent advances in integrated methods, such as Patch-seq, assessing gene expression, electrophysiology and neuroanatomy at the single cell level provide a multi-modal approach to neuron classification leading to deeper understanding of the brain cell type diversity. Motivated by the relatively low throughput of morphological characterization in the Patch-seq experiment

due to manual tracing, we developed a fully automated reconstruction pipeline for in-slice brightfield images of biocytin-filled neurons. The pipeline performs automated segmentation of dendritic and axonal arbors followed by post-processing to produce digital representations of neuronal geometry and topology (e.g., swc file). On a large set of manually reconstructed neurons, we apply a topology-preserving variant of the fast marching algorithm to generate volumetric labels. Using these labels as the training set and employing multiple data augmentation strategies, we train a convolutional neural network (U-Net) to produce accurate segmentations. We post-process these segmentations by removing background noise, connecting broken segments and assigning separate labels to apical and basal dendrites. The overall pipeline can produce neuron reconstructions in the swc format from raw images at a rate of 100 cells/day using 16 GPUs. We have so far processed more than 3500 inhibitory and excitatory cells from the mouse visual cortex, including 1000 neurons with manual traces used to assess reconstruction accuracy. Bringing the throughput of anatomical characterization closer to that of transcriptomic and/or electrophysiological aspects of the multi-modal Patch-seq experiment enables data-driven analyses of the anatomical correlates of gene expression and response characteristics.

Disclosures: **O. Gliko:** None. **M. Mallory:** None. **R. Dalley:** None. **R. Gala:** None. **S. Sorensen:** None. **U. Sümbül:** None.

Digital Abstract Session

P190. Visual Cortex: Circuits

Program #/Poster #: P190.07

Topic: D.07. Vision

Support: Wellcome Trust 205093
Wellcome Trust 108726
H2020-MSCA 835489
Royal Society NIF\R1\180184

Title: Recording the activity of transcriptomic cell types in mouse primary visual cortex

Authors: S. BUGEON[1], T. HAULING[1], J. DUFFIELD[1], D. NICOLOUTSOPOULOS[1], D. ORME[1], I. PRANKERD[1], Y. ISOGAI[2], M. CARANDINI[1], K. D. HARRIS[1];
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Abstract: Cortical computations rely on microcircuits that involve multiple neuronal subtypes. While cortical neurons have been classically subdivided into broad subtypes (i.e. Vip, Sst and Pvalb positive interneurons), transcriptomic analysis has revealed many more subpopulations of finely distinguished cortical neurons (Tasic et al., Nature 2018). To decipher the functional properties and network activity of these fine cell types in primary visual cortex (V1), we have applied post-hoc in situ transcriptomic analysis to cortical neurons after imaging their activity in vivo. We first recorded the activity of V1 neurons using two-photon calcium imaging in awake freely running mice, during visual stimulation. The visual stimuli comprised drifting and static

gratings, and natural scenes. We also recorded V1 neuronal activity during navigation in a virtual corridor containing visual landmarks. After these recordings, we collected consecutive brain slices which were registered to a reference z-stack from the in-vivo recording, using multiple landmarks. We then matched each individual cell between the in vivo recordings and the brain sections. These brain sections were then processed for post-hoc transcriptomic analysis using in situ sequencing (Qian et al., Nature Methods 2020), a multiplexed single RNA detection procedure. This method allows us to characterize the expression of 73 different genes at the single cell level. Based on this single cell gene expression, we then assigned a transcriptomic cell type to each recorded cell. To confirm that this procedure can reveal the functional properties of transcriptomic cell types, we reproduced findings on the size tuning of different V1 cell types, and their modulation by locomotion (Dippopa et al., Neuron 2018). Importantly, while these results were previously obtained using Cre lines to record activity of one cell class at a time, we can record the different cortical cell types in a same animal and in a same recording session. Thus, this method provides a high-throughput approach to characterize the in vivo activity of fine transcriptomic cortical cell types.

Disclosures: **S. Bugeon:** None. **T. Hauling:** None. **J. Duffield:** None. **Y. Isogai:** None. **D. Nicoloutsopoulos:** None. **D. Orme:** None. **I. Prankerd:** None. **M. Carandini:** None. **K.D. Harris:** None.

Digital Abstract Session

P191. Visual System: Neural Coding

Program #/Poster #: P191.01

Topic: D.07. Vision

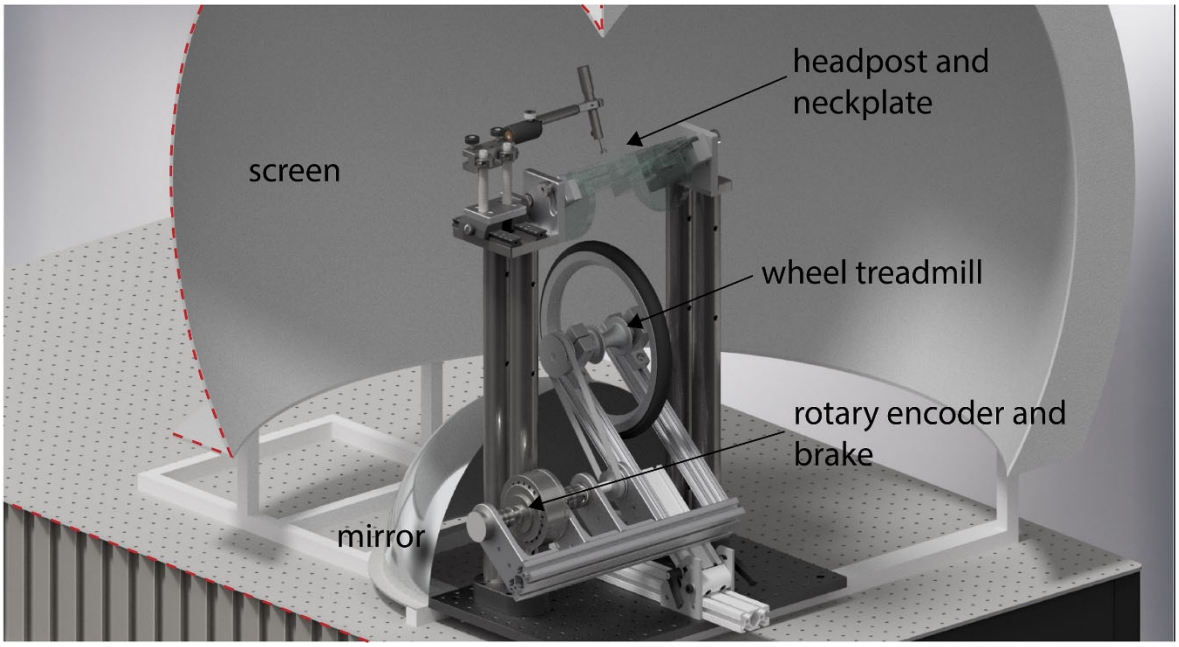
Support: BRAIN Initiative U01 UF1NS116377
AFRL FA9550-19-1-0357

Title: Electrophysiological assessment of marmoset primary visual cortex during free locomotion

Authors: *J. P. LISKA, C. BADILLO, J.-O. MUTHMANN, A. HUK;
Univ. of Texas at Austin, Austin, TX

Abstract: The emergence of the mouse model system in visual neuroscience has produced a number of novel findings in early visual cortex. One notable example is the observation of significant firing rate modulations in primary visual cortex (V1) when mice are allowed to freely locomote. While there has been continued investigation into this effect in mice, it is not known whether this phenomena is also present in the visual cortex of primates. It has traditionally been infeasible to allow awake primates to safely locomote in a laboratory environment during invasive data acquisition, but the common marmoset allows us to leverage the experimental flexibility of a rodent in an animal with the visual system of a primate. We have therefore created an immersive VR environment allowing for linear locomotion for use with the common

marmoset. A dome-shaped projection surface is used to present stimuli ranging from simple oriented gratings to immersive, naturalistic stimuli across 180+ degrees of the marmoset's visual field while it is allowed to locomote freely on a treadmill which can be yoked to control the stimulus. We have recorded activity from ensembles of V1 neurons using chronically-implanted multi-electrode arrays. Recording quality maintains high SNR even during locomotion, allowing for single unit isolation and quantification of conventional V1 tuning properties via spike sorting. V1 neurons (N=15 per session) were reliably activated by high contrast orientated gratings placed in particular locations in the virtual environment. Neuronal response patterns were also similar between nearby neurons. These results confirm the basic functional properties and organization of V1 despite being recorded in a complex naturalistic environment with ongoing locomotion behavior.



Disclosures: **J.P. Liska:** None. **C. Badillo:** None. **J. Muthmann:** None. **A. Huk:** None.

Digital Abstract Session

P191. Visual System: Neural Coding

Program #/Poster #: P191.02

Topic: D.07. Vision

Support: NIH R01 EY030226

Title: Images associated with reward resist surround suppression in macaque area V2

Authors: ***M. CARR**[1], N. WILLIAMS[2], C. R. OLSON[2];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: It is possible that images associated with reward elicit enhanced visual responses from neurons in low-order visual cortex of primates. Recent fMRI-based studies have indicated that reward-associated images elicit enhanced BOLD responses in low-order visual areas of humans (Serences, 2008). However, enhanced BOLD activation need not indicate enhanced spiking activity since the signal is driven by both excitatory and inhibitory synaptic events. Moreover, fMRI lacks the temporal resolution to distinguish enhanced visual response strength from delayed activation related to reward anticipation, as observed in mouse V1 (Shuler & Bear 2006). We set out to resolve this issue by monitoring the activity of neurons in area V2 of a rhesus macaque monkey trained through repeated passive exposure to associate certain natural images with large reward and others with small reward. On each training trial, while the monkey maintained central fixation, a single image was presented for 300 ms, following which, after a delay of 300 ms, the associated reward was delivered. Each image subtended 3° of visual angle and was centered in the lower quadrant of the visual field opposite the hemisphere destined for recording. Training involved more than a hundred exposures to each image over a period of weeks. Upon completion of training, we measured the visual responses of V2 neurons to "good" and "bad" images presented in superimposition on their classical receptive field. The timing of each trial was the same except for stimulus presentation, which lasted 600 ms. On average, good images and bad images elicited responses of indistinguishable strength. However, it seemed possible that under more naturalistic testing conditions, differences in efficacy might emerge. To test this idea, we monitored neuronal responses to displays consisting of a good or bad image centered on the neuron's classical receptive field and a reward-neutral concentric annulus with a 4° inner diameter and a 8° outer diameter. Under these conditions, we observed a marked difference between good and bad images. Surround suppression, measured as a reduction in response strength induced by the annulus, occurred robustly for bad images but was minor or absent for good images. The difference emerged around 75 ms after image onset, a latency shorter than that commonly associated with top-down effects. It persisted during sessions in which reward amount was fixed regardless of image identity and thus reflected the impact of long-term associations rather than short-term expectation. We conclude that images repeatedly paired with large reward do not elicit stronger responses in V2 but do acquire immunity to surround suppression.

Disclosures: **M. Carr:** None. **N. Williams:** None. **C.R. Olson:** None.

Digital Abstract Session

P191. Visual System: Neural Coding

Program #/Poster #: P191.03

Topic: D.07. Vision

Support: OptoVision 637638

Title: Optogenetic stimulation of Primate V1 induces activity across cortical layers and elicits a visual percept

Authors: *B. AGAYBY[1], S. RIMA[2], M. ORTIZ-RIOS[3], M. HAAG[2], M. C. SCHMID[2,1]; [1]Biosci. Inst., Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [2]Dept. of Neurosciences and Movement Sci., Univ. of Fribourg, Fribourg, Switzerland; [3]Functional Imaging Lab., German Primate Ctr. (DPZ), Göttingen, Germany

Abstract: Optogenetics has been successfully used to examine perceptual processing along the columnar architecture of the primate primary visual cortex (V1) [1–3] and to induce saccadic eye movements[4]. However, it remains to be determined how optogenetic stimulation affects activity across V1 layers and whether such activation induces a visual percept ('phosphene').To address these two questions, we injected AAV9-hSyn-ChR2-eYFP across multiple depths and sites in the opercular part of V1 of a macaque monkey. Laminar probes with an embedded optical fibre were used to record multiunit activity (MUA) across the layers of the cortex as well as for light delivery. Across 19 sessions, 354 out of 380 contacts showed a significant increase in firing rates in response to a blue light (473nm) pulse. The averaged laminar profile showed significant activation across all contacts covering the cortex in line with an expected non-specific expression of the construct. Examining the latency of the activation elicited by lower light intensities revealed that the earliest responses were located in putative layer 2/3 & L4A, which may indicate a higher expression of the opsin in these layers or could be due to the location of the optical fibre. To examine if optogenetic stimulation could induce a visual percept we trained a monkey on a two-alternative forced-choice task. The macaque reported, via a saccade-based perceptual detection, whether a visual or optogenetic stimulation was present or not (catch trials). The performance showed exceeded chance levels across all three task conditions: visual, optogenetic and catch. Interestingly, by comparing the success rate, reaction times and saccade velocities of the optogenetic trials to those of the visual trials, we were able to infer that the animal perceived a "phosphene" stimulus that is similar to a weak visual stimulus. Taken together, our results demonstrate that optogenetic stimulation could be used to induces wide-spread activation across the cortical layers of V1 demonstrating its suitability for generating phosphenes.
1.Andrei, A. R., et al. *Nat. Commun.* 10, 1–13 (2019)2.Nassi, J. J., et al. *Neuron.* 86, 1504–1517 (2015)3.Chernov, M. M., et al. *Proc. Natl. Acad. Sci.* (2018)4.Jazayeri, M., et al. *Nat. Neurosci.* 15, 1368–70 (2012).

Disclosures: B. Agayby: None. **S. Rima:** None. **M. Ortiz-Rios:** None. **M. Haag:** None. **M.C. Schmid:** None.

Digital Abstract Session

P191. Visual System: Neural Coding

Program #/Poster #: P191.04

Topic: D.07. Vision

Support: NIMH Grant R00MH115082
Whitehall Foundation

Title: Differential responding of cortical cell-types for novelty detection

Authors: *C. G. GALLIMORE, J. M. ROSS, J. HOLMES, A. D. FERRELL, J. P. HAMM; Neurosci., Georgia State Univ., Atlanta, GA

Abstract: The processing of stimuli in their spatial and temporal context is a core function necessary for an organism's survival. Contextual modulation of sensory processing is known to be present in neocortical circuits, whereby neural responses differentiate contextually novel stimuli from those which are predictable, or redundant. This allows the organism to update its internal model of the world, appropriately allocating resources for ongoing processing or goal-directed action. However, it is unclear how specific cell-types give rise to this modulation. Here, we examined the activity of various cell-types in mouse primary visual cortex (layers L2-4) during a visual oddball paradigm, in which orthogonal square-wave gratings are presented to the animal in conditions of varying contextual salience: novel ("deviant"), predictable ("redundant"), or neither. Using type-specific Ca^{2+} indicator expression (GCaMP6s), we were able to selectively monitor the activity of excitatory pyramidal cells (PYRs) and two classes of inhibitory interneurons (INs; SST, VIP) with high spatiotemporal precision using fast two-photon Ca^{2+}-imaging (28 Hz). Results revealed two underlying contextual modes of neural responses: stimulus-specific adaptation (SSA), a suppression of cortical responses to redundant stimuli, and deviance detection (DD), an augmented response to deviant stimuli, reminiscent of "prediction-error". Excitatory PYRs displayed SSA across all cortical layers observed, though only displayed DD in supragranular L2-3. This response profile was robust, and is consistent with the predictive coding framework. Inhibitory INs, however, displayed dynamics broadly consistent with their mutually inhibitory influences on one another. For example, L2/3 SSTs showed some evidence of DD, whereas those in L4 did not. And SSTs at all depths showed ongoing SSA, such that subsequent redundant gratings elicited decreasing response magnitudes. Conversely, VIPs showed no evidence of DD at any layer, and tended to ramp up their responses to redundant repetitions preceding a deviant stimulus - an inverse relationship to SSTs. Our L2/3 SST observations are consistent with the notion that their primary input source is local PYRs, as well as past work showing chemicogenetic suppression of SSTs eliminates DD in PYRs. We propose that a competitive disynaptic inhibitory motif between PYRs of opposite preferred orientation, mediated by L2/3 SSTs, gives rise to DD in cortical circuits. However, future work is needed for a more complete picture of circuit dynamics, incorporating measurements of the PV IN class, as well as teasing apart the contributions of feedback from higher cortex.

Disclosures: C.G. Gallimore: None. **J.M. Ross:** None. **J. Holmes:** None. **A.D. Ferrell:** None. **J.P. Hamm:** None.

Digital Abstract Session

P191. Visual System: Neural Coding

Program #/Poster #: P191.05

Topic: D.07. Vision

Support: ERC Grant OptoVision 637638

Title: Stimulus dependence of theta rhythmic activity in primate V1

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Abstract: A growing body of psychophysical research suggests that perceptual sampling of complex environments might occur at a theta rhythm (3-8 Hz)[1]. Electrophysiological recordings point to a neural origin of this theta-rhythmic sampling mechanism in higher level cortical areas[2,3], often associated with exerting top-down attentional influences on perception[4]. However it remains unknown whether theta oscillations can be observed in primary visual cortex (V1) and to what extent their emergence might depend on stimulus properties. To address these questions, we recorded multi-unit activity (MUA) and single unit activity (SUA) from the V1 of two macaque monkeys passively viewing a visual stimulus with variable properties. Analysis of the MUA showed that among the visually responsive electrode sites (n = 107 in Monkey 1 and n = 78 in Monkey 2), more than 50 % showed a statistically significant theta oscillation when the stimulus appeared compared to a baseline period without a stimulus. Doing the same analysis for single units (n = 38 in Monkey 1 and Monkey 2), we found that more than 80 % of the sampled visually responsive units showed a statistically significant theta oscillation. Theta power varied depending on size, contrast, and orientation of the stimulus. Within each of these stimulus property domains (e.g. size), there was usually a single stimulus value that induced the strongest theta. The present study shows that a highly stimulus dependent neuronal theta oscillation can be elicited in V1 at the earliest level of visual cortical processing. Stimulus driven theta oscillations in visual cortex might be an additional mechanism for perceptual sampling that occurs at the same frequency range.

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Disclosures: **P. Fanyiwi:** None. **B. Agayby:** None. **R. Kienitz:** None. **M. Haag:** None. **M.C. Schmid:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.01

Topic: D.07. Vision

Support: Japan Society for the Promotion of Science (JSPS) KAKENHI (Grant-in-Aid for Young Scientists (A), JP17H04684

Title: Investigating structural covariance of the human optic tract and primary visual cortex in a neuroimaging dataset

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Abstract: The relationship between the size of human brain structures within a population can inform our understanding of the organization and development of the nervous system. A previous post-mortem study demonstrated a correlation between the size of the optic tract and primary visual cortex (V1) across human individuals (Andrews et al., 1997), suggesting that the development of these structures is coordinated. In this study, we aimed to investigate covariance between structural properties of the optic tract and V1 in living human populations, by analyzing retinotopic maps from the Human Connectome Project Retinotopy dataset (N = 178; Benson et al., 2018) and diffusion MRI (dMRI) data acquired from the same human participants. We first calculated the surface area of V1 estimated by combining the fMRI dataset and a retinotopic atlas (Benson et al., 2018). We then identified the optic tract from all participants by using probabilistic tractography (Sherbondy et al., 2008) on dMRI data. For each optic tract, we measured the cross-sectional area and fractional anisotropy (FA; Basser & Pierpaoli, 1996). We initially analyzed FA estimated from dMRI data acquired with b = 2000 s/mm^2, and used dMRI data acquired with other b-values as replication datasets. We did not find a significant correlation between optic tract size (cross-sectional area) and V1 surface area (R = 0.04), in contrast with the previous post-mortem study. However, we found a small, but statistically significant negative correlation between FA of the optic tract and V1 surface area (R = -0.19, P = 0.009). The correlation was most prominent along the middle portion of the optic tract. This result was replicated in dMRI dataset acquired with different b-values (R = -0.15, P = 0.048 for b = 1000 s/mm^2; R = -0.16, P = 0.007 for 3000 s/mm^2). These results reveal a modest correspondence between tissue properties of the optic tract and the size of V1, suggesting that properties of retinal ganglion cells have some impact on the development of human V1. Further work is needed to understand why the size correlations differ from the prior post-mortem report.

Disclosures: **T. Miyata:** None. **N.C. Benson:** None. **J. Winawer:** None. **H. Takemura:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.02

Topic: D.07. Vision

Title: Testing the molecular anchors hypothesis in human and macaque visual cortex

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Abstract: The adult primate visual system comprises a series of retinotopically organized areas. These areas are present in every individual and have a stereotypical spatial organization across the cortical surface. While these areas are present at birth (Arcaro & Livingstone 2017), it has been proposed that the visual cortex develops sequentially with primary visual area V1 acting as an anchor and extrastriate areas V2, V3, and V4 self-organizing around V1 (Rosa, 2002). This hypothesis is difficult to test directly in primates given the limited tools available for manipulating early map formation. However, this hypothesis, predicts that features of extrastriate maps such as areal extent are tied to the map of V1. Here, we test this hypothesis by measuring the sizes of retinotopic maps in a large group of human and macaque subjects.

We used fMRI to map the retinotopic organization of the visual system in 53 adult humans (Wang et al. 2015) and 7 juvenile macaques (Arcaro & Livingstone, 2017). We mapped polar angle and eccentricity representations using a traveling wave paradigm and created individual cortical surface models from high-resolution anatomical MRIs. From these measurements, we identified 25 retinotopic maps in humans and 21 retinotopic maps in macaques. We measured the cortical extent of each area in each subject.

In both humans and monkeys, V1 had the largest cortical area and the sizes of extrastriate areas progressively decreased moving anterior from V1. Across subjects, the size of V1 was correlated with the sizes of V2, V3, V4, and MT in both humans and macaques. In general, correlations between V1 and more anterior areas along both dorsal and ventral visual pathways were weak in both species. Correlations in areal size between V1 and extrastriate areas were strongest for matching (e.g. dorsal V1: dorsal V3) than non-matching (e.g., dorsal V1: ventral V3) visual field representations. Together, these data provide empirical evidence in support of the molecular anchor hypothesis (Rosa 2002) and indicate that the development of extrastriate areas is directly tied to the visual field map of V1. In addition, the relatively weak correlations between V1 and anterior areas indicates that additional factors substantially contribute to the map development of higher order visual maps. Lastly, the correlation in areal size between V1 and MT is notable given the relatively large cortical distance between the two areas in both species. Given the early development of area MT (Bourne & Rosa 2006), this may reflect common thalamic constraints on development.

Disclosures: M. Martynek: None. **M.S. Livingstone:** None. **M.J. Arcaro:** None. **S. Kastner:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.03

Topic: D.07. Vision

Support: Wu Tsai Neurosciences Institute Big Ideas Project
NIH 1 R21 EY030588-01

Title: Cortex exhibits microstructural tissue growth that proceeds hierarchically within the visual processing streams during the first 6 months of human life.

Authors: *V. S. NATU[1], M. ROSENKE[1], F. R. QUERDASI[1], H. KULAR[1], H. WU[1], M. GROTHEER[1], S. BERMAN[2], A. MEZER[2], K. GRILL-SPECTOR[1];
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Abstract: The topology and chronology of postnatal human brain development have been of great interest for more than a century. Since Flechsig's seminal 1901 paper, the predominant focus has been on the maturation and myelination of white matter tracts, suggesting that projection fibers to primary sensory cortical areas are more mature at birth than those to other cortical areas. However, how cortical tissue develops postnatally is largely unknown. By combining T_1 relaxation time from quantitative MRI and mean diffusivity (MD) from diffusion MRI in 13 infants, we tracked cortical tissue development within individuals across three timepoints (newborn, 3 months, and 6 months, N=10 at each timepoint). Lower T_1 and MD indicate higher microstructural tissue density associated with more developed cortex. Our data reveal three main findings: First, primary sensory/motor areas (V1: visual, A1: auditory, S1: somatosensory, M1: motor) have lower T_1 and MD at birth than higher-level cortical areas. However, all primary areas show significant reductions in T_1 and MD in the first six months of life, illustrating profound tissue growth even after birth. Second, systematic and significant reductions in T_1 and MD from newborns to 6 month-olds occur in all visual areas of the ventral and dorsal visual streams. Strikingly, this development was heterogenous across the visual hierarchy: Earlier areas were more developed at birth (lower T_1 and MD) than higher-level areas, and also had a slower rate of T_1 and MD development as observed by a negative relationship between cortical tissue properties at birth and developmental rate. Third, a detailed look at regions that will become selective for faces and places by adulthood, reveals that face areas display at a faster rate of development than place areas, suggesting a fine-grained and functionally relevant developmental trajectory within higher-level areas. Finally, analysis of transcriptomic gene data that compares gene expression in postnatal vs. prenatal tissue samples shows strong postnatal expression of genes associated with myelination and synaptic signaling. We hypothesize that these cellular structures contribute to profound postnatal cortical tissue growth, which we observed with *in-vivo* T_1 and MD measurements. We propose a novel principle of postnatal maturation: development of cortical tissue proceeds in a fine-grained, hierarchical manner within cortical streams. We hypothesize that this hierarchical development

enables the cortical microcircuitry of lower-level areas to develop first, which, in turn, provides the necessary scaffolding for efficient signal conduction, tunability, and learning.

Disclosures: **V.S. Natu:** None. **M. Rosenke:** None. **F.R. Querdasi:** None. **H. Kular:** None. **H. Wu:** None. **M. Grotheer:** None. **S. Berman:** None. **A. Mezer:** None. **K. Grill-Spector:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.04

Topic: D.07. Vision

Support: NIH 2RO1 EY 022318-06
NSF DGE-1656518

Title: Cytoarchitecture, not function, determines a visual region's connectivity profile in childhood

Authors: ***E. KUBOTA**[1], M. GROTHEER[1], J. GOMEZ[2], V. NATU[1], D. FINZI[1], A. REZAI[1], H. KULAR[1], M. NORDT[1], K. GRILL-SPECTOR[1];
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Abstract: Ventral temporal cortex (VTC) contains regions involved in the processing of ecologically relevant stimuli that fall in consistent anatomical locations across individuals. However, the principles underlying the relationship between the topography of these domain-selective regions in VTC and brain structure are unclear. Here, we tested if white matter tracts that connect to functional regions in VTC are organized by cytoarchitecture, domain, or both, using functional MRI and diffusion MRI in 29 children (ages 5-12) and 28 adults (ages 22-29). To test these hypotheses, we quantify in each participant, the distribution of white matter fascicles ("connectivity profiles") of VTC domain-selective regions that have been previously shown to be located in different cytoarchitectonic areas. We find that across both children and adults, the connectivity profiles are similar between ROIs within the same cytoarchitectonic area, even if they are selective for different domains, e.g. face (mFus-faces) and word (mOTS-words) selective regions in cytoarchitectonic area FG4 have similar connectivity to the posterior arcuate (pAF), arcuate fasciculus (AF), and inferior longitudinal (ILF). In contrast, we find that connectivity profiles are significantly different between ROIs in different cytoarchitectonic parcellations even if they are selective for the same domain, e.g. mOTS-words in FG4 has particularly strong connections to the pAF, while pOTS-words in FG2 has particularly strong connections to the vertical occipital fascicle (VOF). This finding challenges the idea that regions selective for different domains have unique white matter connectivity profiles that support domain-specific processing. Interestingly, in adults but not children, mOTS-words has a greater percentage of fibers connecting to the AF compared to mFus-faces. This finding is important because mOTS-words is a brain region involved in reading and the AF is considered to be a language tract. Moreover, this increased connectivity is a result of mOTS-words shifting closer

to the AF from childhood to adulthood. Together, these findings suggest that large scale white matter connectivity is organized by cytoarchitecture early in development, whereas domain-specific connections emerge in response to reading experience.

Disclosures: **E. Kubota:** None. **M. Grotheer:** None. **J. Gomez:** None. **V. Natu:** None. **D. Finzi:** None. **A. Rezai:** None. **H. Kular:** None. **M. Nordt:** None. **K. Grill-Spector:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.05

Topic: D.07. Vision

Support: NIH Grant 1ZIAEY000558

Title: Human visual cortex is organized by an OFF-dominated map: evidence from multivariate analyses of magnetoencephalography (MEG) data

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Abstract: The lateral geniculate nucleus relays to visual cortex information at each retinal location about luminance increments and decrements. These ON and OFF signals converge on cortical neurons to create multiple overlapping maps, including those that represent the visual field, ocular dominance, and orientation. Single-cell electrophysiology and optical imaging in cats and tree shrews show that the topographic constraints of each map are satisfied by a precise spatial arrangement of thalamic inputs, with the OFF inputs forming the scaffolding (Kremkow et al., and Lee et al., Nature, 2016). However, it is not known whether OFF inputs also govern the organization of visual cortex in humans, or of any primate. We tested this hypothesis by: (1) simulating the impact of small eye movements on the spatial representation across the cortex of ON and OFF inputs; and (2) directly measuring the extent to which ON versus OFF signals could be decoded from the spatial pattern of responses measured with MEG. The simulations suggest that small eye movements produce less variability in the pattern of activation for OFF versus ON, implying that responses are more precise for darks compared to lights. This prediction was supported by the multivariate analyses of MEG data, which showed that the spatial pattern of responses to darks were more consistent than those to lights. Participants (N=18) were shown brief flashes (116ms; 1s ISI) of spiral-shaped stimuli (spanning 10°) of 26% luminance contrast as increments and decrements, while measuring MEG activity (Elekta Triux system, 306-channel probe). Univariate responses to darks and lights were not significantly different. The precision of the maps was assessed with representational similarity analyses. For each 5-ms time bin, we determined the extent to which the pattern of activity across the array of sensors was correlated in independent data sets. The correlation matrix was compared to two models, one in which patterns elicited by dark stimuli were more correlated than the patterns elicited by light stimuli, and one in which patterns elicited by light stimuli were more correlated. The results showed that

the spatial patterns elicited by darks were initially more correlated, with an onset latency of 75-ms (peak at 95-ms), followed by higher correlation for lights (peaks at 145 ms and 195-ms), and another peak for darks at 245-ms. The early time of the onset latency suggests the correlation structure reflects the initial OFF-dominated wave of thalamic activity recorded in visual cortex. These results provide the first evidence we are aware of showing that the topographic structure of visual cortex in humans is governed by an OFF-dominated map.

Disclosures: S.R. Singh: None. **B.R. Conway:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.06

Topic: D.07. Vision

Support: Canadian NSERC grant OPG0001978

Title: A psychophysical signature of Y like neuronal responses in human vision

Authors: *A. L. RAMIREZ HERNANDEZ[1], A. ROSENBERG[2], C. L. BAKER[3];
[1]McGill Univ., Montreal, QC, Canada; [2]Neurosci., Univ. of Wisconsin - Madison, Madison, WI; [3]Ophthalmology, McGill Vision Res. Unit, Montreal, QC, Canada

Abstract: Establishing the contributions of the different types of retinal ganglion cells (RGCs) to visual perception has been challenging. In cats, X and Y RGCs have been neurophysiologically well characterized. These cell types are similar to primate Midget and Parasol cells, respectively. Y-cells (and Parasol cells) are distinguished by a nonlinear response at higher spatial frequencies (SFs) and higher temporal frequencies (TFs), but the functional roles of these nonlinear responses in human visual perception are not well understood. Subcortical Y-like inputs are thought to mediate cortical responses to contrast modulation (CM) patterns, which consist of a high SF grating carrier whose contrast is modulated by a low SF sinewave envelope. Here, we leveraged the Y-like carrier response properties of cortical neurons to CM patterns to demonstrate a novel psychophysical approach to revealing the function of Y-like RGCs.CM as well as luminance modulation (LM) patterns were viewed monocularly by subjects with normal vision, at 2.1, 4.3, 6.4, and 8.5 degrees of eccentricity. In each stimulus trial (120 msec duration), a CM pattern or a LM pattern was presented. The CM stimuli had a contrast-reversing carrier and a drifting sinewave envelope with a SF of 0.25 cycles/deg (cpd) and a TF of 3 Hz. The psychophysical task was to report the perceived direction of motion of LM gratings or CM envelopes. Within each block of trials, SF (for LMs) or carrier SF (for CMs) was varied with a method of constant stimuli for different values of TF (LMs) or carrier TF (CMs).We found that the ability to correctly perceive envelope direction of motion with CM patterns was bandpass with carrier SF, exhibiting best performance at relatively high carrier SFs (1.5 to 3.0 cpd), and high carrier TFs (15 to 20 Hz). For CM patterns, the performance at the highest carrier TF (20 Hz) did not decrease uniformly with eccentricity. In contrast, for LM patterns, the best

performance at 1.5 to 3.0 cpd was at low TFs (5-10 Hz), and the performance decreased systematically with eccentricity. Because the linear mechanisms of RGCs respond best at lower TFs, whereas the nonlinear subunit responses of Y-like cells respond well at higher TFs, the measured psychophysical performance for CM patterns likely reflected nonlinear subunits. In addition, the good performance at SFs that are high for peripheral vision and relatively independent of eccentricity was more consistent with the responses of small nonlinear subunits than with linear mechanisms. Therefore, the performance for CM patterns with high carrier SFs and TFs likely arises from Y-like cells such as Parasol cells.

Disclosures: **A.L. Ramirez Hernandez:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Based on the reported findings, the Wisconsin Alumni Research Foundation, the technology transfer office for UW–Madison, has filed a patent application (pending) with the authors listed as inventors. **A. Rosenberg:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Based on the reported findings, the Wisconsin Alumni Research Foundation, the technology transfer office for UW–Madison, has filed a patent application (pending) with the authors listed as inventors. **C.L. Baker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Based on the reported findings, the Wisconsin Alumni Research Foundation, the technology transfer office for UW–Madison, has filed a patent application (pending) with the authors listed as inventors..

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.07

Topic: D.07. Vision

Support: NIH Grant R01EY030434
NIH Grant R01EY026881
NIH Shared Instrumentation Grant S10-RR019371

Title: Motion and Stereopsis Are Encoded by Distinct Mesoscopic Channels in Human Extrastriate Cortex

Authors: *S. NASR[1], B. KENNEDY[2], R. B. H. TOOTELL[1];
[1]Massachusetts Gen. Hospital, Harvard Med. Sch., Charlestown, MA; [2]Radiology, Martinos Ctr. for Biomed. Imaging, Charlestown, MA

Abstract: BACKGROUND: The encoding of stereopsis and motion involves much of visual extrastriate cortex in humans, including thick-type columns in V2, V3, and area V3A. Many of these sites are reported to be strongly influenced by the magnocellular stream. However, it remains unclear whether motion and stereopsis are processed diffusely within a common magnocellular-influenced channel, or instead, within smaller scale ‘sub-channels’ within it.

METHODS: We used high-resolution fMRI (1 mm isotropic), collected in a 7T scanner, to localize motion- and stereo-selective columns in the human extrastriate cortex (n = 14), based on the response to moving-vs-stationary (Tootell and Nasr, 2020) and 3D-vs-2D (Nasr and Tootell, 2018) stimuli, respectively. As a control, color-selective 'V2/V3 'thin' stripes were also localized in the same subjects, based on their response to color-vs-luminance varying stimuli (Nasr et al., 2016). Finally, we studied the functional connectivity between these columns by measuring their spontaneous fMRI fluctuations during the resting-state (eyes closed).
RESULTS: In areas V2 and V3, motion- and stereo-selective activity clusters were localized *between* the color-selective 'thin' stripes. Regions with overlapping "motion-and-stereo" selectivity (~5% of V2/V3 area) showed a relatively strong selective response to both visual cues. In contrast, regions with overlapping "motion-and-color" or "stereo-and-color" selectivity showed relatively weak selectivity to either one of the visual cues. This suggests a more gradual shift in stimulus selectivity between stereo-vs- motion selective columns, and a relatively sharper segregation between color-vs-motion and/or color-vs-stereo selective columns. In V3A, motion- and stereo-selective activity formed larger clusters compared to V2/V3 with little overlap. Color-selective clusters were rare in this region. We also found a stronger functional connectivity between columns with alike (e.g. motion- to motion-selective) rather than unlike selectivity (e.g. motion- to color-selective) in either ipsilateral or contralateral hemispheres.
CONCLUSION: Within a well-described magnocellular-influenced stream, our evidence suggests the existence of a mesoscopic 'sub-stream', which responds selectively to motion or stereopsis.

Disclosures: S. Nasr: None. **B. Kennedy:** None. **R.B.H. Tootell:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.08

Topic: D.07. Vision

Support: Max Planck Society, University of Tuebingen

Title: Depth perception by anti-correlated random-dot-stereograms in central visual field

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Abstract: In a random dot stereogram (RDS), object surfaces in a three-dimensional scene are generated by images presented to left and right eyes that comprise interocularly corresponding random black and white dots. The spatial disparities between the corresponding dots determine the depth of the object surfaces. When a black dot in one monocular image corresponds to a (contrast-polarity reversed) white dot in the other, the RDS is called anti-correlated. Neurons in the primary visual cortex (V1) respond to such RDSs as if their preferred disparities become non-proferred and vice versa, thereby reversing the disparity signs reported to higher visual

areas. Humans can perceive such reversed depths in peripheral but not in central vision (Zhaoping & Ackermann 2018). According to the central-peripheral dichotomy in recognition (Zhaoping 2017, 2019), V1 signals are fed forward to higher visual areas to suggest initial perceptual hypotheses about scenes; in central but not in peripheral vision, top-down feedback from higher to lower visual cortical areas aid recognition using analysis-by-synthsis, so that the would-be visual input for each hypothesis (e.g., a depth order) is fed back to V1 to verify whether it matches the actual visual input, and the hypothesis yielding a good or poor match is boosted or suppressed, respectively, this is termed the Feedforward-Feedback-Verify-reWeight (FFVW) process for recognition. Accordingly, V1's reversed depth signals are perceived peripherally, but not centrally since, by FFVW, they disagree with the would-be inputs expected by the brain from past experience.
In this work, I show that the typically invisible reversed depth signals can influence depth perception in central vision in RDSs containing polarity-matched dots, polarity-reversed dots, and noise dots which are inter-ocularly independent. To report whether a central disk is in front or behind the surrounding ring in a noisy RDS, observers can see the defined depth order by the polarity-matched dots more (or less) clearly when the depth order in the reversed depth signals by the polarity-reversed dots is the same as, or opposite to, the defined depth order. Accordingly, the threshold signal-to-noise level needed in a noisy RDS to see depth can be lowered or raised respectively by adding congruent or incongruent reversed depth signals. The reversed depth signals are more perceptually more influential in dynamic RDSs with brief stereo-frames to make top-down feedback in FFVW less effective. Hence, the feedforward reversed depth signals from V1 can influence percept in central vision when the feedback veto can be reduced by brief inputs or mitigated by normal depth signals.

Disclosures:

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.09

Topic: D.07. Vision

Support: NIH Grant DC014589
NIH Grant NS098981

Title: Event-related phase synchronization propagates as waves in human ventral visual cortex

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Abstract: Electrophysiological studies have revealed that visual stimuli evoke spreading waves of cortical activity in primary visual cortex, a presumed manifestation of the intrinsic connectivity of hierarchically organized early visual cortex. However, it unknown if this

phenomenon occurs in higher-order visual cortex, where inputs from early visual cortex integrate with semantic, attentional and memory inputs. To probe the organizational principles of higher visual cortex we performed intracranial recordings in 90 individuals, with >2,000 electrodes in ventral visual cortex, while they performed visual tasks of varying visual, cognitive and attentional complexity (word reading, face naming and scene classification). We tracked the spatiotemporal progression of changes in theta (4 - 8 Hz) inter-trial phase coherence (ITC), theta power, event related potentials (ERPs) and gamma (70 - 150 Hz) power across the cortical surface. Strong phase modulation was noted almost ubiquitously across ventral occipitotemporal cortex; from occipital pole to anterior fusiform in all tested visual tasks. Visual onsets and offsets induce posterior-to-anterior travelling waves of phase and power modulation across the entire ventral occipitotemporal cortex. Spread of ITC and gamma power was well correlated with spatial location (>20% variance explained) and demonstrated propagation velocities of 1-1.1 m/s for ITC and 0.4-0.5 m/s for gamma power. Phase modulation, but not oscillatory power, was strongly predictive of the ERP amplitude at each electrode, suggesting that ERP is at least partially a manifestation of phase reset. Importantly, attentional modulation, within task, resulted in a dissociation between ITC and ERP, with attentional modulation showing unique patterns of modulation of the time course of ITC, ERP, theta and gamma power. In conclusion, this is novel evidence for the existence of posterior-to-anterior travelling waves of theta phase modulation and gamma power, spreading from calcarine cortex to visual associative areas in fusiform cortex. We provide multiple strands of evidence to reveal that while phase, power and ERP are correlated, they index distinct processes in the visual system. We propose this theta-centric travelling wave could represent sequential cortico-cortical integration across the ventral visual pathway.

Disclosures: O. Woolnough: None. **K.J. Forseth:** None. **P.S. Rollo:** None. **Z.J. Roccaforte:** None. **N. Tandon:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.10

Topic: D.07. Vision

Support: Fellowship from the George E. Hewitt Foundation for Medical Research
NIH Grant K99EY031795
NIH Grant P30-EY0190005

Title: Laminar processing of border ownership in primate extrastriate visual cortex

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Abstract: Gestalt psychologists have known for over a century that the primate visual system compulsorily assigns contrast edges to objects, i.e. edges are perceived as being owned by foreground objects. Early primate extrastriate visual cortex contains neurons that encode border

ownership, providing border ownership signals which occur even when the visual stimulus features that distinguish foreground object from background fall well outside the neuron's classical receptive field (Zhou et al., 2000). It is unresolved whether the information that establishes border ownership selectivity is mediated through feedforward, horizontal or feedback pathways (e.g. Zhaoping, 2005; Sakai and Nishimura, 2006; Zhu et al., 2020). These pathways have distinct laminar connectivity patterns. Here we mapped the timing of border ownership signals across cortical layers, using 32-channel laminar probes. We recorded responses from well-isolated units in extrastriate visual area V4 in two behaving macaques. By replacing the native dura with a transparent artificial dura we were able to position the probes orthogonally to the cortical surface. We used current source density analysis of stimulus-evoked local field potentials to identify the granular (input) layer as the current sink with the shortest latency, and locate the units recorded from to supragranular, granular or infragranular layers. We then measured spiking activity evoked by square stimuli presented on a uniform background. The stimuli were positioned such that only one border of the object fell within the classical receptive field of each neuron. Border ownership was thus defined by stimulus features outside of the classical receptive field. We quantified border ownership selectivity using the reliability metric introduced by Zhou et al. (2000). Our data confirm earlier reports of a dominant presence of border ownership signals in V4. In contrast to the hypothesis that border ownership signals in V4 are inherited from V2, we find that border ownership selectivity rises first in the infragranular layer, before it appears in the granular and the supragranular layers (bootstrap latency analysis $p<0.05$). Our data thus suggest that border ownership signals are not inherited in a feedforward manner from upstream areas. Rather, they appear either to be established in V4 deep layers, or they may arrive through cortical feedback from downstream visual areas. Deep layers include neurons that project to upstream cortical areas, raising the possibility that these early border ownership signals in deep layers are part of the cortical feedback projecting to upstream areas.

Disclosures: T.P. Franken: None. **J.H. Reynolds:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.11

Topic: D.07. Vision

Title: A model for the origin of ocular dominance columns in primary visual cortex

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Abstract: Introduction. Ocular dominance columns are a prominent feature of carnivore and primate primary visual cortex. Their visual function (if any) and origin are, however, little understood. We modelled ocular dominance columns to explore their contribution to binocular vision. **Methods.** We extended an existing monocular model of the cat's upstream visual system (Nguyen, Freeman, 2019) by adding a second eye. Both eyes supplied the cortex with multiple

channels, each of which comprised a photoreceptor, bipolar cell, ganglion cell and lateral geniculate nucleus cell. Each neuron was represented by a differential equation, and all equations were simultaneously integrated to calculate responses. Ganglion cells were distributed across the retina so as to reproduce the spatial statistics of real arrays (Wässle et al., 1981). The geniculocortical synaptic strength of each channel developed with a Hebbian process that reduced destructive interference between neighbouring on- and off-channels, and thereby produced orientation-selective cortical neurons. Critically, the starting values of these weights was set proportional to the interocular difference of local retinal ganglion cell densities. **Results.** Ocular dominance was assessed for each cortical neuron by drifting an optimally oriented sinusoidal grating across its receptive field. A neuron's ocular dominance index was calculated as the normalised interocular difference in the fundamental Fourier component of impulse rate, with values ranging from pure contralateral to pure ipsilateral drive. The ocular dominance index was mapped across the simulated patch of visual field; not surprisingly, the map closely replicated interocular differences in retinal ganglion cell density. More importantly, the map was reminiscent of measurements in cats (Löwel et al., 1988). In particular, the periodicity of this map was 1.1 mm, which is within the empirical range. Other cortical properties in the model also tallied well with experiment: the interocular difference in preferred orientation was small, with a standard deviation of 8 deg; interocular differences in receptive field location were variable, leading to preferred stimulus locations nearer than, on, or beyond the fixation plane. **Discussion.** There is debate whether ocular dominance columns result from molecular interactions between geniculate axons and cortical cells, or from activity-based changes in innervation. We have avoided this debate by assuming that the columns depend, in both cases, on the relative densities of afferents from the two eyes. This assumption leads to a model that reproduces several of the well-known properties of binocular vision.

Disclosures: A. Somaratna: None. **A.W. Freeman:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.12

Topic: D.07. Vision

Support: NIMH Intramural Research Program
NIH BRAIN U01 NS108683

Title: Performance in even a simple visual task depends on mouse secondary visual areas

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Abstract: Primary visual cortex (V1) in the mouse projects to numerous brain areas, including several secondary visual areas, frontal cortex, and basal ganglia. While it has been demonstrated that optogenetic silencing of V1 strongly impairs visually-guided behavior, it is not known which

downstream areas are required for visual behaviors. Here we trained mice to perform a contrast-increment change detection task. For this task, substantial information is available in V1 to allow a perceptual decision to be made -- V1 neurons show contrast-dependent changes in firing rate. Therefore, it could be that animals perform this task by using V1 information directly and bypassing secondary visual areas, or alternatively, secondary areas might be used even in this simple contrast-step task. Via optogenetic silencing of visual responses in V1 and secondary visual areas, we found that secondary areas' activity is required. First, we verified that inhibition of V1 at even low light intensities greatly affects performance during this task (mean 100% crossing point; the light required to increase contrast threshold by 100%: 0.42 mW/mm^2, bootstrap 95% CI 0.30 - 0.55 mW/mm^2, N = 9). Inhibition of control locations outside visual areas produced significantly weaker effects (mean 100% crossing point 4.2 mW/mm^2, bootstrap 95% CI 2.1 - 6.8 mW/mm^2, N = 5). Inhibition of areas lateral to V1 (LM, AL, RL) produced effects that were, in some cases, even larger than those seen with inhibition of activity in V1 (mean 100% crossing point 0.37 mW/mm^2, bootstrap 95% CI 0.20 - 0.57 mW/mm^2, N = 7). Inhibition of area PM produced effects that were significantly smaller than those seen during V1 inhibition (mean 100% crossing point 1.87 mW/mm^2, bootstrap 95% CI 0.65 - 3.08 mW/mm^2). In vivo electrophysiology and widefield GCaMP imaging showed that, although inhibiting secondary visual areas could produce some feedback effects in V1, the principal effect was profound suppression at the location of the optogenetic light. The results show that pathways through secondary visual areas are necessary for even perhaps the simplest visual behaviors that involve V1.

Disclosures: H.C. Goldbach: None. **B. Akitake:** None. **C.E. Leedy:** None. **M.H. Histed:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.13

Topic: D.07. Vision

Support: DFG RTG 2175 ("Perception in Context and its Neural Basis")

Title: Orientation discrimination in mice is influenced by the history of recent trials.

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Abstract: Finding regularities in the environment and utilising them is important in this complex, yet structured world. Perceptual decisions, therefore, not only depend on current sensory input, but are also influenced by previous experience.
To investigate history effects during visual perceptual decision-making, we trained head-fixed

mice to perform a lick-left/lick-right orientation discrimination task. We used logistic regression to estimate the contributions of task-relevant (current stimulus) and task-irrelevant factors (past stimuli, past choices) on the animals' decisions.
Mice learned the task and reached stable performance in 6-8 weeks. Examining model weights during learning, we found that progress in learning was paralleled by increasing weights assigned to the current stimulus ($r = 0.87$). After learning, average performance was $67.5\% \pm 0.9\%$. To better understand the overall sub-optimal performance, we focused on response times (RTs), and separated trials with short or long RTs, using as boundary each animal's median RT - 0.5 s. Performance was at chance level for trials with short RTs ($50.7\% \pm 2.8\%$) but better for trials with long RTs ($71.8\% \pm 0.4\%$). To account for variability related to response time, we included RT as a predictor, which improved model performance. Model weights for current stimulus were negligible for trials with short RTs, suggesting that on these trials, animals failed to use relevant sensory evidence to guide decisions. Irrespective of RT, significant weights were assigned to past stimulus and choice, indicating that history effects influence decisions across all trials.
To investigate how mice trade off stimulus-related and contextual information, we introduced blocks of trials with stimulus-specific imbalances of reward. Here, mice showed a consistent bias towards the response side associated with larger reward. Under these conditions, the weight assigned to the current stimulus decreased compared to blocks with balanced rewards. This suggests that mice integrate information regarding expected reward size into their visually driven choice.
We conclude that, even for simple visual stimuli well above threshold, perceptual decision-making in mice is influenced not only by the current visual stimulus but also by the history of past trials and the context of reward. In addition, not accounting for trial-to-trial variability in the speed of the response will likely underestimate behavioural performance.

Disclosures: **S. Khanal:** None. **L. Meyerolbersleben:** None. **S. Katzner:** None. **T. Wachtler:** None. **L. Busse:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.14

Topic: D.07. Vision

Support: NIH R01NS104949

Title: The spatial structure of feedforward information in mouse primary visual cortex

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Abstract: Location-sensitive and motion-sensitive units are two major functional types of feedforward projections from lateral geniculate nucleus (LGN) to primary visual cortex (V1) in

mouse. The distribution of these inputs in cortical depth remains under debate. We systematically mapped the spatial organizations of location-sensitive and motion-sensitive LGN inputs in V1 of awake mice by imaging their axonal calcium activities. Although both types distributed similarly across cortical depth, we found notable organizations specific to each type: the motion-sensitive axons showed a depth-dependent bias in motion direction while the location-sensitive axons showed a depth-independent OFF dominance. By grouping boutons into axon segments, we showed the motion-sensitive axons had greater horizontal bouton spread than location-sensitive axons. While, within each type, the bouton spread was relatively uniform, indicating the spatial organization was determined by the axon distribution. Overall, our results suggest a new model of receptive biases and laminar structure of thalamic inputs to V1.

Disclosures: **J. Zhuang:** None. **N. Ouellette:** None. **R. Larsen:** None. **K. Takasaki:** None. **T. Daigle:** None. **B. Tasic:** None. **J. Waters:** None. **H. Zeng:** None. **R. Reid:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.15

Topic: D.07. Vision

Support: NIMH Grant MH063912
NEI Grant EY022577

Title: Functional interlaminar connectivity in mouse primary visual cortex

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Abstract: One of the defining characteristics of the cerebral cortex is its organization into 6 layers, each distinguishable by a unique composition of cell types and representing a fundamental component of sensory information-processing. Most of what we know about functional interlaminar connectivity is from paired recordings in slice preparations which, due to cut axons, dramatically underestimate the rate of interlaminar connections. This leaves us with an incomplete understanding of how cortical circuits transform sensory information across layers. In this study, we use an optogenetic approach to bypass the issue of cut axons and comprehensively characterize relative synaptic input strength between cortical layers 2/3, 4, 5, and 6. We use transgenic mouse lines and viral vectors to restrict Channelrhodopsin-2 expression to excitatory cells within a single layer in mouse primary visual cortex. We then pair optogenetic stimulation of axon terminals (including live, cut axons) with *ex vivo* intracellular recordings in neurons across cortical layers and measure the monosynaptic input strength from the stimulated cell population to the recorded cell. This enables us to compare postsynaptic responses between cells recorded in different layers and thereby quantify the proportion of excitatory input from each cortical layer to other layers. Preliminary results suggest input strength from a given layer

to a particular cell depends strongly on both the input layer and the laminar location of the recipient cell. Consistent with previous research, L4 (the primary layer receiving sensory input relayed from thalamus) provides most of its input to L2/3, and L2/3 provides strong input both within L2/3 and to L5. We also find that interlaminar input strength varies by cell type; while extratelencephalic (ET) neurons in L5 primarily provide input to other cells in L5, L5 intratelencephalic (IT) neurons provide the majority of their input to L2/3. Interestingly, inputs from L6 ET neurons are evenly distributed between L4, L5, and L6. We also expect functional input strength to differ between excitatory cells and inhibitory cells and are further examining how each layer-specific population provides input to excitatory versus inhibitory cells within a particular layer. Together, our results provide a more complete picture of the relative distribution of excitatory synaptic input from each layer to other layers within a single cortical region.

Disclosures: M.A. Rossa: None. **E.M. Callaway:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.16

Topic: D.07. Vision

Support: NEI Grant R01EY011787
NIMH Grant R01MH115900

Title: Long-term stability of neuronal ensembles in mouse visual cortex during spontaneous and visually-evoked activity

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Abstract: Neuronal ensembles, i.e. coactive groups of neurons, are thought to participate in the internal representations of memories, perceptions, and mental states. New insights from experiments *in vivo* demonstrate their causal role in perception and behavior, but it is still unknown how stable they are over time. We performed two-photon volumetric calcium imaging of layer 2/3 of visual cortex of awake mature adult mice (TIGRE2.0 GCaMP6s/f, n = 6) ranging from 3 - 6 months in age. We imaged and tracked over weeks the activity of the same neurons in response to visual stimuli and spontaneous activity. Less than 20% of neurons remained active across weeks. Analyzing them, we found both stable ensembles, lasting up to 46 days, and transient ones, observed during one single session. Most visually-evoked ensembles were stable, whereas spontaneous ensembles were both stable and transient. Stable and transient ensembles had similar number of neurons and functional network density, but stable ensembles were more robust and more than 60% of their neurons still belonged to the same ensemble after weeks. These core cells had stronger functional connectivity than neurons that stopped belonging to the ensemble. Our results demonstrate that spontaneous and evoked neuronal ensembles can last

weeks, providing a neuronal mechanism for long-lasting representation of perceptual states or memories.

Disclosures: J. Pérez-Ortega: None. **T. Alejandre-García:** None. **R. Yuste:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.17

Topic: D.07. Vision

Support: BMBF, FKZ: 01GQ1002
MPG M.FE.A.KYBE0004
DFG, GRF: 276693517 – SFB 1233

Title: Probing chromatic responses of mouse retinal ganglion cells

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Abstract: Color is an important feature in guiding visual behavior in animals. Mice are dichromatic; they express short (S) and medium (M) wavelength-sensitive opsins. While true S-cones are distributed relatively uniformly across the mouse retina, M-cones co-express S-opsin with increasing co-expression gradient towards the ventral retina [1]. This results in a green-sensitive dorsal and UV-sensitive ventral retina. Despite this uneven opsin distribution, behavioral studies have demonstrated that mice can discriminate colors [2] in the upper visual field [3]. Here, we investigate retinal correlates of this behavior by analyzing chromatic processing in mouse retinal ganglion cells (RGCs).
To systematically examine how RGCs encode chromatic information, we used two-photon calcium imaging with visual stimulation. First, we employed a set of artificial stimuli such as a UV and green center-surround flicker stimulus to analyze chromatic preference of RGC receptive fields. Our study [4] on > 8,000 recorded ganglion cell layer cells confirmed that the dorso-ventral opsin expression gradient determines the spectral sensitivity of RGC center responses. Interestingly, we found that the surround chromatic preference of ventral RGCs was systematically shifted towards green, resulting in color-opponent center-surround antagonism. This stands in line with behavioral experiments showing that color vision in mice seems more prominent in the upper visual field [3].
Next, we investigated how chromatic information is processed in RGCs with more naturalistic stimuli. For that, we used “mouse natural movies” capturing the two color channels relevant for mice and the natural visual statistics of their environment more closely than artificial stimuli. We

probed RGC responses by displaying sequences from upper and lower visual field of these movies. To estimate spatial and temporal RGC receptive fields and to model RGC responses, we implemented a convolutional neural network (CNN) [5]. The CNN's architecture is inspired by the tiling of RGC types: features learned in the core are shared among all cells, and a cell-specific readout determines the combination of features and location in space. This approach enabled to predict RGC responses to naturalistic stimuli, capture their functional diversity and generate optimal stimuli (most exciting inputs, MEIs). Additionally, analyzing chromatic preferences reflected in spatial and temporal structures of MEIs allows to investigate how RGCs encode chromatic information present in the natural environment.
[1] Röhlich et al. 1994 [2] Jacobs et al. 2004 [3] Denman et al. 2018 [4] Szatko, Korympidou et al. 2020 [5] Klindt et al. 2017

Disclosures: **K.P. Szatko:** None. **L. Höfling:** None. **C. Behrens:** None. **Y. Qiu:** None. **P. Berens:** None. **M. Bethge:** None. **A. Ecker:** None. **T. Euler:** None. **K. Franke:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.18

Topic: D.07. Vision

Support: Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektnummer 276693517 – SFB 1233

Title: Mouse retinal circuit specializations reflect natural environment statistics

Authors: ***Y. QIU**[1,2,3], Z. ZHAO[1,2], D. KLINDT[1,2,3], K. SZATKO[1,2,3,4], M. KAUTZKY[5,6], F. SCHAEFFEL[2], K. RIFAI[2,7], K. FRANKE[1,2,4], L. BUSSE[5,8], T. EULER[1,2,4];
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Abstract: Pressures for survival make sensory circuits adapted to a species' natural habitat and its behavioral challenges [1]. Thus, to advance our understanding of the visual system, it is essential to consider an animal's specific visual environment, by capturing natural scenes, characterizing their statistical regularities, and using them to probe visual computations. Mice, a prominent model system for vision research, have salient visual specializations, i.e. being dichromatic with enhanced sensitivity to green and UV in the dorsal and ventral retina, respectively. However, the characteristics of their visual environment that likely have driven these adaptations are rarely considered. Here, we built a gimbal-stabilized, UV-green sensitive

camera to record natural scenes from the habitat of mice. Analysis of the spectral channels revealed that chromatic contrast greatly diverged in the upper but not in the lower visual field. Models that were trained to encode the natural scenes suggest that this environmental difference might have driven the emergence of superior chromatic opponency in the ventral mouse retina, supporting color discrimination in the upper visual field [2,3]. In addition, we found that the upper visual field was biased towards dark contrasts implied in aerial predator detection, in particular for the UV channel, and this bias was paralleled by a higher frequency of large-receptive field, light-offset sensitive (Off) ganglion cells in the ventral retina. Together, our findings lend further support to the idea that, during evolution, natural scene statistics shaped retinal circuit processing [4]. The recorded footage is meant as a resource for mouse vision research and will be made publicly available. **References** [1] Simoncelli EP and Olshausen BA. Natural Image Statistics and Neural Representation. Annu Rev Neurosci 2001; 24(1):1193-1216. [2] Denman DJ, Luviano JA, Ollerenshaw DR, Cross S, Williams D, Buice MA, Olsen SR, Reid RC. Mouse color and wavelength-specific luminance contrast sensitivity are non-uniform across visual space. eLife 2018;7:e31209. [3] Szatko KP*, Korympidou MM*, Ran Y, Berens P, Dalkara D, Schubert S, Euler T, Franke K# (2020) Neural circuits in the mouse retina support color vision in the upper visual field. Nature Communications 11(1):3481, 10.1038/s41467-020-17113-8. [4] Baden T, Euler T, Berens P (2019) Understanding the retinal basis of vision across species. Nat Rev Neurosci 10.1038/s41583-019-0242-1.

Disclosures: **Y. Qiu:** None. **Z. Zhao:** None. **D. Klindt:** None. **K. Szatko:** None. **M. Kautzky:** None. **F. Schaeffel:** None. **K. Rifai:** None. **K. Franke:** None. **L. Busse:** None. **T. Euler:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.19

Topic: D.07. Vision

Support: NIH Grant EY013588
NIH Grant EY012576
NIH Grant MH082174

Title: Temporal contrast modulates burst activity in the lateral geniculate nucleus

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Abstract: Bursting is a ubiquitous feature of thalamic neurons and is well documented for visual neurons in the lateral geniculate nucleus (LGN). While bursts are often associated with states of drowsiness, they are also known to convey visual information to cortex. The occurrence of thalamic bursts depends on the inactivation state of T-type Ca^{2+} channels, which become de-inactivated and primed to evoke bursts following periods of increased membrane

hyperpolarization. Given the time/voltage relationship that underlies the production of bursts, it is reasonable to assume that bursts are influenced by the temporal contrast of visual stimuli. We therefore recorded the spiking activity of LGN neurons in the anesthetized cat while presenting drifting sine-wave gratings that varied in contrast. Spikes were categorized as tonic or bursts using established criteria, and comparisons were made between these categories under different contrast conditions. For high-contrast stimuli, we predicted the null phase of the stimulus would cause a deeper and more sustained hyperpolarization of relay cells than would occur with low-contrast stimuli, and that this difference would affect burst activity. Results show that burst rate increased significantly with high-contrast stimuli compared with low-contrast stimuli, as did the tonic spike rate; however, a more detailed analysis confirmed that the increase in burst rate was not simply due to an overall increase in firing rate. Further, bursts were more reliable and more temporally precise for the preferred phase of the stimulus cycle during high-contrast stimulation than during low-contrast stimulation. These results support the hypothesis that stimulus contrast and the biophysical properties underlying the state of T-type Ca^{2+} channels interact to influence the reliability and precision of geniculate bursts, presumably to facilitate stimulus detection. More subtle aspects of stimulus discrimination are best conveyed by tonic spikes.

Disclosures: **A.N. Sanchez:** None. **H.J. Alitto:** None. **W. Usrey:** None.

Digital Abstract Session

P192. Architecture, Development, and Function of Visual Circuits

Program #/Poster #: P192.20

Topic: D.07. Vision

Support: ARC FF FT180100466
Aus Gov. RTP

Title: Target facilitation precedes competitive selection in a dragonfly visual neuron

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Abstract: Animals that rely on pursuit predation to feed must be able to identify and track moving targets throughout a highly cluttered world, often amongst swarms of distractions. Despite this computational complexity, dragonflies are highly successful aerial predators, whose capture success is unperturbed by larger swarms of prey. How is the dragonfly nervous system able to achieve such success under such complex conditions? We have recorded intracellularly from an identified neuron in the dragonfly optic lobe, known as 'Centrifugal Small-Target Motion Detector 1,' ('*CSTMD1*'). Previously, CSTMD1 has been shown to display complex winner-takes-all selective attention enabling it to selectively single-out a chosen target from a pair. Additionally, CSTMD1 exhibits a predictive gain modulation, both enhancement (termed

‘facilitation’) and surround suppression which is thought to drive the neuronal response to a target to saturation in order to minimise the effect of distraction. However, CSTMD1 also exhibits dynamic switching between competing target stimului. In a total of 20 wild-caught, male dragonflies (*Hemicordulia sp.)* we tested whether all targets are facilitated, before one is selected; or whether a single selected target is then facilitated. By analysing the neuronal response to targets that were preceded by either an attended or non-attended target, we observed that in either case the tested probe displayed facilitatory gain enhancement. This reveals that neuronal facilitation is a lower-order computation applied to all targets, before selection is committed. By driving responses towards saturation when the target is within the receptive field for some time, facilitation blocks out transiently salient distractions. Thus, behaviourally important stimuli that persist over several hundred milliseconds (target trajectories) are given the competitive boost to drive selection and switching.

Disclosures: B.H. Lancer: None. **B.J.E. Evans:** None. **S.D. Wiederman:** None. **J.M. Fabian:** None. **D.C. O'Carroll:** None.

Digital Abstract Session

P193. Cross-Modal Processing: Neural Circuitry and Development

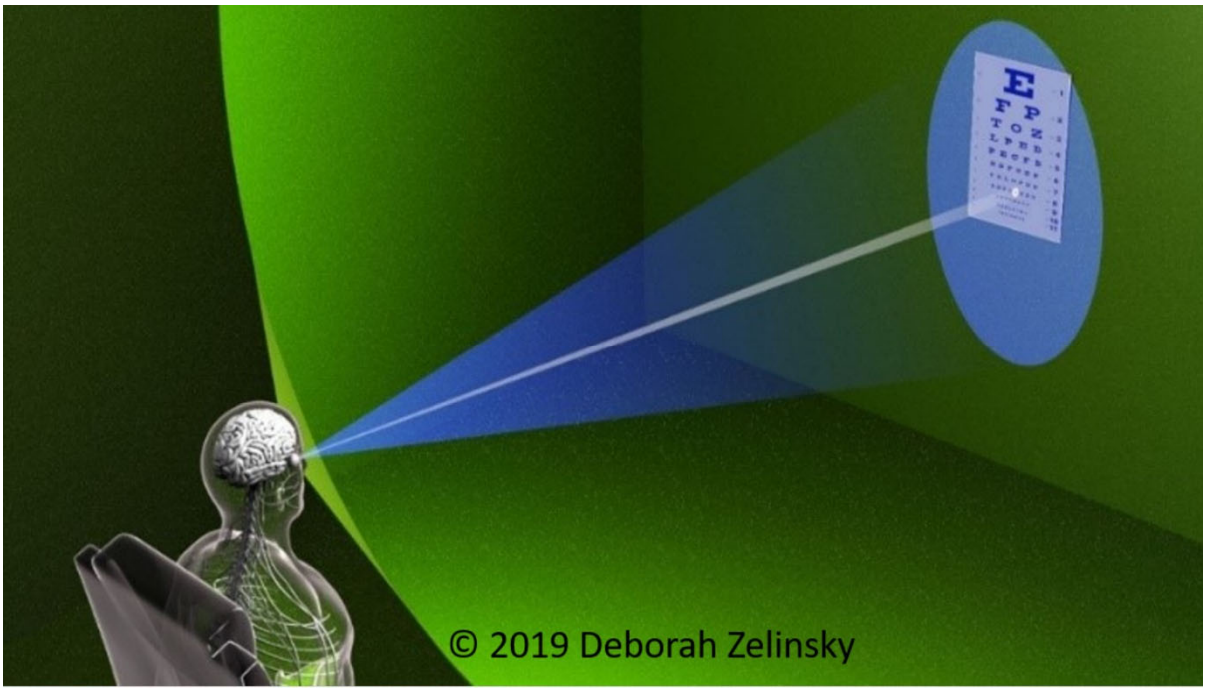
Program #/Poster #: P193.01

Topic: D.09. Multisensory Integration

Title: From Moment to Memory: The Life Span of a Visual Signal

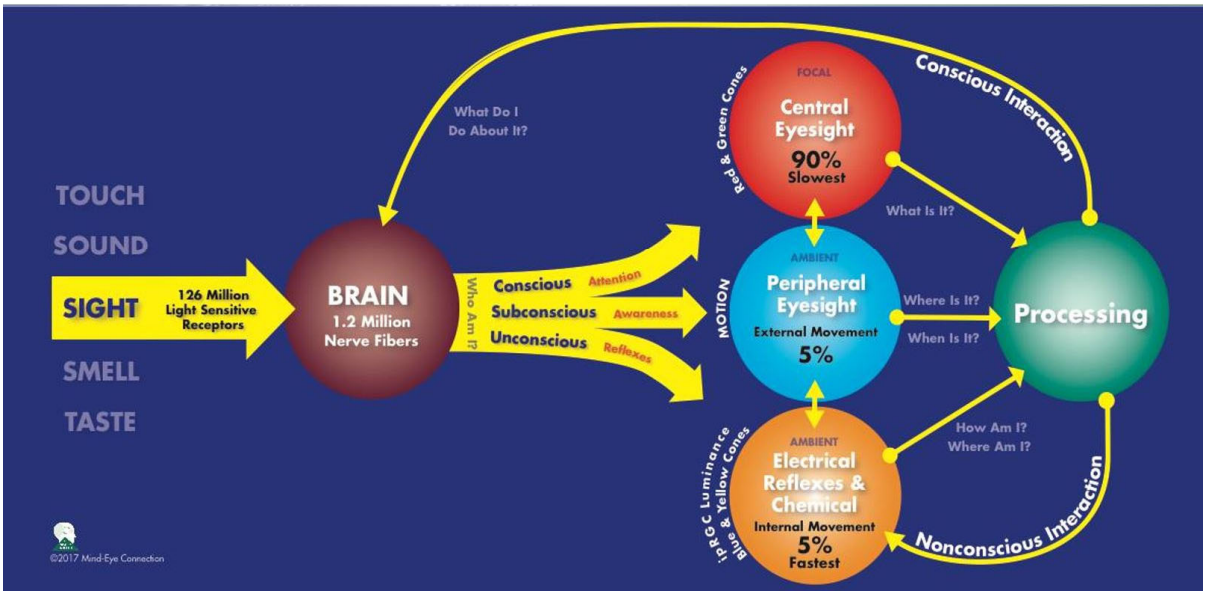
Authors: *D. G. ZELINSKY;
The Mind-Eye Connection.

Abstract: Retinal tissue is comprised of brain tissue and is considered part of the central nervous system as an interface between internal and external environments. It is nourished by blood flow from the inside, while processing light input from the outside. In order to pay attention to a target, 94% of the surroundings has to be tuned out via processes requiring coordination among many chemical and electrical systems.



The life of a visual signal starts by activation of one of 126 million light-sensitive receptors involved in retinal circuitry. After being filtered into 1.2 million exiting signals, each signal travels through a specific pathway, including fast "highways" to and from the limbic system & brainstem, as well as slower pathways to and from the cortex. The 94% of peripheral eyesight is represented by 200 thousand ganglion axons; the other 6% representing central eyesight is represented by 1 million axons. Optometry is beginning to use individualized Brainwear™ (eyeglasses designed for the 94%, rather than the 6%) to selectively activate brain circuitry. This novel use of retinal stimulation for neuromodulation is an important upgrade to the current, outdated eye examination.

21st Century Optometric Viewpoint



Sensory systems are integrated -not separate- so the future optometrist is envisioned as a primary healthcare provider who can selectively stimulate neuroendocrine functions while enhancing subconscious awareness of surrounding space, forming a link between orientation, navigation and identification skills in addition to memory.

Disclosures:

Digital Abstract Session

P193. Cross-Modal Processing: Neural Circuitry and Development

Program #/Poster #: P193.02

Topic: D.09. Multisensory Integration

Support: EMBO ALTF 740-2019
Wellcome Trust 205093

Title: Visual cortex is hardly auditory

Authors: *C. BIMBARD, T. SIT, A. LEBEDEVA, P. COEN, K. HARRIS, M. CARANDINI; Univ. Col. London, London, United Kingdom

Abstract: Sensory cortices are increasingly thought to encode multisensory information. For instance, mouse primary visual cortex (V1) appears to be influenced by auditory inputs, which have been suggested to provide global inhibition or loudness- and frequency-specific information. However, sounds can also evoke unsolicited behavioral responses, and such behavioral responses are now known to elicit neural activity across the brain. Thus, multisensory

studies may suffer from a potential confound between acoustic-related and movement-related activity. We measured the neuronal responses to natural movies and natural sounds in V1 using chronically implanted Neuropixels probes, thus recording the activity of hundreds of units. At the same time, we filmed the behavior of the mice. We replicated previous results showing that sounds could evoke neural activity in V1. Though sound-evoked responses were weak compared to video-evoked responses, we could significantly decode sound identity from population activity. However, we also observed that in response to the sounds the mice performed stereotypical, unsolicited movements. These movements induced activity in the V1 population activity. Removing it significantly reduced the responses to sounds. We conclude that a significant part of sound-evoked responses in V1 comes from indirect behavioral responses, and that a careful characterization of auditory inputs to sensory areas requires controlling sound-induced changes in behavioral state.

Disclosures: C. Bimbard: None. **T. Sit:** None. **A. Lebedeva:** None. **P. Coen:** None. **K. Harris:** None. **M. Carandini:** None.

Digital Abstract Session

P193. Cross-Modal Processing: Neural Circuitry and Development

Program #/Poster #: P193.03

Topic: D.09. Multisensory Integration

Title: Behavioral and neuronal effects of predictability in multi-sensory objects

Authors: *O. D. GILDAY, A. MIZRAHI;
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Abstract: Perception is constantly shaped by non-sensory effects such as expectation, attention and context. Experimental studies have shown that such effects also modulate activity in sensory cortical neurons. Based on the theory of predictive processing such modulation is a result of top-down innervation from higher cortical regions. Evidence for such top-down effects, and specifically the effect of expectation, and its relation to perception, however, is scarce. In order to investigate these questions, we designed a behavioral task for mice that is a derivative of the "Go-No-Go" task. In this paradigm the "Go" and "No-Go" cues are auditory, and the extent to which their presentation is predictable is modulated by preceding olfactory cues. We show that although mice correctly use the auditory cues to maximize their rewards and minimize their punishments, their behavior is affected by the prior probabilities formed by the preceding olfactory cues. We also show examples of activity in the Auditory Cortex (ACx) of awake behaving mice, that although is not responsive to odor, is nonetheless modulated by the predictability of sounds that follow olfactory cues. Given that olfactory information is not coded in ACx, we propose that this modulation is a result of a top-down innervation of ACx, and suggest this behavioral paradigm as a fertile ground for research of predictability in multi-sensory objects.

Disclosures: **O.D. Gilday:** None. **A. Mizrahi:** None.

Digital Abstract Session

P194. Plasticity of The Visual Cortex

Program #/Poster #: P194.01

Topic: D.07. Vision

Support: NIH Grant EY028219
NIH Grant EY007023

Title: Developmental changes in eye-specific inputs to dendritic spines of neurons in mouse visual cortex

Authors: ***K. R. TSIMRING**[1], P. K. IP[2], J. C. ZEPEDA[1], K. JENKS[1], D. H. YUN[1], T. KU[3], K. CHUNG[1], M. SUR[1];
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Abstract: Neuronal circuits in the primary visual cortex undergo significant reorganization during the critical period. In mice, this reorganization corresponds with an experience-dependent convergence of ipsilateral and contralateral eye inputs onto neurons in the binocular region of the primary visual cortex (bV1). How synaptic inputs onto bV1 neurons, reflected in the responses of individual spines, change across development remains unknown. We compared the functional properties of synapses on L2/3 bV1 neurons between critical period and adulthood, using two-photon calcium imaging of dendritic spines in awake mice during monocular and binocular presentations of visual stimuli. We find that adult neurons have a significantly higher fraction of spines with a stronger bias to either the ipsilateral or contralateral eye than during the critical period, when spines are more binocular. Some spines in adult neurons that exhibit a preference towards one eye during monocular stimulation show reduced responses to binocular stimulation, indicating that these spines are either directly or indirectly suppressed by the non-preferred eye. To determine how dendritic spine responses evolve over development, we imaged the same spines and dendritic segments at different time points. Our preliminary findings show that some visually responsive spines shift their relative responses to each eye and develop binocular suppression, indicating dynamic changes in eye-specific drive onto individual spines. These changes can arise from presynaptic inputs onto spines or can be constructed at individual spines, potentially reflecting changes in excitatory inputs from one eye and development of inhibitory inputs from the other eye. To further probe mechanisms of binocular suppression in spines, our ongoing work employs a novel tissue expansion technology, Magnified Analysis of the Proteome (MAP), that allows super-resolution measurement of the synaptic composition of dendritic spines from L2/3 bV1 neurons. We hypothesize that the enrichment and distribution of inhibitory and excitatory synapses onto spines could provide a mechanistic explanation for the emergence of inter-ocular suppression across development.

Disclosures: **K.R. Tsimring:** None. **P.K. Ip:** None. **J.C. Zepeda:** None. **K. Jenks:** None. **D.H. Yun:** None. **T. Ku:** None. **K. Chung:** None. **M. Sur:** None.

Digital Abstract Session

P194. Plasticity of The Visual Cortex

Program #/Poster #: P194.02

Topic: D.07. Vision

Support: NIH Grant 1F31EY031602-01
NIH Grant EY025613

Title: High-throughput analysis of layer specificity of plasticity in visual cortex after monocular deprivation

Authors: ***J. BOTTORFF**, G. TURRIGIANO;
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Abstract: Hebbian and homeostatic forms of plasticity are crucial for efficient learning and information storage, and experimental and theoretical evidence suggest they may be segregated temporally to avoid possible interference. Monocular deprivation (MD) is a well-established protocol that induces a biphasic change in firing rates of excitatory neurons in the deprived hemisphere of rodent monocular visual cortex (V1m), while neural activity in the control hemisphere remains unchanged. In deprived neurons, Hebbian mechanisms cause a depression of firing rates in the first few days after MD, while homeostatic mechanisms return this activity to baseline in the next few days; further, our lab has shown that the homeostatic rebound is gated by wake behavioral states. However, the cellular and molecular mechanisms, including many aspects of cell-type specificity, underlying these phenomena are unknown. We developed a method using viral expression of CaMPARI2, a calcium and UV-dependent photoconvertible fluorescent protein, and *ex vivo* imaging in critical period rats to study layer and cell-type specific activity changes after MD (Trojanowski et al. 2019, biorxiv). By photoconverting CaMPARI2 *ex vivo* at different times after MD and measuring the ratio of converted (red) to unconverted (green) CaMPARI2 in deprived vs. control hemisphere V1m, we can quantify the time course of activity changes in neural subgroups after MD. We found that this method is sensitive enough to detect the known early drop and later rebound in deprived activity after MD. After 3 days MD, red/green ratios of excitatory, deprived neurons were significantly lower than control, but after 6 days MD, the ratios in each hemisphere were indistinguishable (MD3: $p = 1x10^{-12}$; MD6: $p = 0.83$, Mann Whitney-U test; $n > 800$ cells from 5-6 animals per condition). Interestingly, these dynamics were layer-dependent; on MD3, the median red/green ratio of deprived cells in cortical layer 2/3 (L2/3) dropped to 75% of control, in L4 the median deprived ratio was 64% of control, but in L5/6, the median deprived ratio remained at 97% of control. Thus, we can track layer and cell type-specific changes in activity after MD, and we found that the established biphasic changes in deprived activity do not occur in the same way in L5/6. These results suggest that CaMPARI2 is a valuable, high-throughput tool with which to further

dissect the cellular and molecular mechanisms regulating distinct neural dynamics after MD. This will also enable investigation of mechanisms underlying the observed wake-specific gating of the homeostatic rebound via manipulations of behavioral state-specific neuromodulators during discrete phases after MD.

Disclosures: J. Bottorff: None. **G. Turrigiano:** None.

Digital Abstract Session

P194. Plasticity of The Visual Cortex

Program #/Poster #: P194.03

Topic: D.07. Vision

Support: NIH Grant EY023037-01

Title: Visual recognition is heralded in V1 by shifts in local oscillatory activity and inhibitory networks

Authors: *D. J. HAYDEN[1], D. P. MONTGOMERY[1], S. F. COOKE[2], M. F. BEAR[1];
[1]MIT, Cambridge, MA; [2]King's Col. London, London, United Kingdom

Abstract: Filtering out familiar, irrelevant stimuli is a major step to ease the computational burden on the cerebral cortex. Inhibition is a candidate mechanism in this filtration process. Oscillations in the cortical local field potential (LFP) serve as markers of the engagement of different inhibitory neurons. In awake mice, we find pronounced changes in LFP oscillatory activity present in layer 4 of V1 with progressive stimulus familiarity. Over days of repeated stimulus presentation, low frequency (alpha/beta) power increases while high frequency (gamma) power decreases. This high frequency activity re-emerges when a novel stimulus is shown. Thus, high frequency power is a marker of novelty while low frequency power signifies familiarity. Consistent with their role in the generation of gamma oscillations, two-photon imaging of parvalbumin-expressing inhibitory neurons reveals disengagement with familiar stimuli and reactivation to novelty, whereas somatostatin-expressing inhibitory neurons respond to familiarity but disengage to novelty, indicating a contribution to the emergence of lower frequency oscillations. We also reveal that stimulus familiarity and novelty have differential effects on oscillations and cell activity over a shorter timescale of seconds. Taken together with previously published results, we propose a model in which two interneuron circuits compete to drive familiarity or novelty encoding.

Disclosures: D.J. Hayden: None. **D.P. Montgomery:** None. **S.F. Cooke:** None. **M.F. Bear:** None.

Digital Abstract Session

P194. Plasticity of The Visual Cortex

Program #/Poster #: P194.04

Topic: D.07. Vision

Support: NIH R21NS115036
NIH R01EY024678

Title: Integration of new skills does not perturb preexisting function in primary visual cortex

Authors: *B. B. JEON[1], S. M. CHASE[1], S. J. KUHLMAN[2];
[1]Biomed. Engin., [2]Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The ability to learn and practice new skills applies to the computation of abstract properties such as geometric form and neuroprosthetic control, as well as physical actions. We trained adult mice to successfully use an optical brain-computer interface (BCI) device so that we could assess the extent to which abstract skill learning disrupts previously acquired cortical function. We found that animals used multiple neural strategies to gain volitional control of the BCI device, and continued to do so after additional training. Despite significant engagement of local neural activity during BCI performance, tuning properties and stimulus encoding were not perturbed by new skill acquisition.Successful integration of new skills into an existing network requires balancing maintenance of perception and action with the acquisition of new function. New skill learning, whether it is perceptual, motor, or abstract, involves changes in cellular physiology that are distributed across the brain (Koralek et al., 2012; Allen et al., 2017; Neely et al., 2018; Roth et al., 2020). Locally, up to 50% of the neurons within a circuit are recruited during task-specific behaviors, and it is observed that synaptic plasticity is pervasive among the activated neurons, particularly in the early phases of training (Wang et al., 2016; Roth et al., 2020). The extent to which these widespread adaptive changes disrupt or otherwise impact existing circuit function is not known. Although addressing this issue is fundamental to understanding the biological constraints on learning, it is challenging to investigate because it requires identifying the neurons responsible for improved performance and monitoring these same neurons longitudinally throughout learning, as well as monitoring existing network function before and after new skill acquisition. We met these challenges by combining 2-photon calcium imaging with a behavioral paradigm in which mice were trained to perform a *de novo* task wherein they earned a reward by modulating the activity of a selected set of sensory neurons in a user-defined pattern. Stimulus tuning and discriminability were quantified before and after skill acquisition to assess maintenance of visual function. The use of a genetically encoded sensor, which was expressed specifically in excitatory neurons, allowed the same neurons to be tracked throughout training.These results indicate that flexible selection of neural strategies during goal-directed practice may facilitate the integration of new skills with existing function.

Disclosures: B.B. Jeon: None. **S.M. Chase:** None. **S.J. Kuhlman:** None.

Digital Abstract Session

P194. Plasticity of The Visual Cortex

Program #/Poster #: P194.05

Topic: D.07. Vision

Support: NIH R01EY024678

Title: Development of natural scene representation in primary visual cortex continues past the peak of the critical period for ocular dominance plasticity

Authors: T. FUCHS, B. JEON, ***S. J. KUHLMAN**;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The development of the visual system is known to be shaped by early-life experience. To identify response properties that contribute to enhanced natural scene representation, we recently performed calcium imaging of excitatory neurons in the primary visual cortex (V1) of awake mice raised in three different conditions (standard-reared, dark-reared, and delayed-visual experience) and compared neuronal responses to a small set of highly similar natural scene images relative to simpler grating stimuli that varied in orientation and spatial frequency. We assessed population selectivity in V1 using decoding methods and found that natural scene discriminability increased by 75% between the ages of 4 to 6 weeks. Both natural scene and grating discriminability were higher in standard-reared animals compared to those raised in the dark. This increase in discriminability was accompanied by a reduction in the number of neurons that responded to low-spatial frequency gratings. Light exposure restricted to a 2-4 week window during adulthood did not induce improvements in natural scene nor in grating stimulus discriminability. Our results demonstrate that early visual experience enhances natural scene discriminability by directly increasing responsiveness to natural scene features (Kowalewski et al. 2020, Current Biology in press).
Notably, these results establish that natural scene discriminability continues to develop past the peak of the critical period for ocular dominance plasticity, and might not rely on the refinement of low-level feature representations. We hypothesize that natural scene representation in V1 becomes sparser with visual experience, and that visual experience continues to play a role in shaping natural scene responsiveness past the critical period for ocular dominance plasticity. To determine whether lifetime sparseness and population sparseness continue to develop past this critical period, we expanded our natural scene stimulus set to include 60 natural scenes and are currently testing whether visual experience is both required and sufficient to improve natural scene discriminability specifically during the ages of 4-6 weeks.

Disclosures: S.J. Kuhlman: None. **T. Fuchs:** None. **B. Jeon:** None.

Digital Abstract Session

P194. Plasticity of The Visual Cortex

Program #/Poster #: P194.06

Topic: D.01. Sensory Disorders

Support: NIH Grant 5U01EY025858-04

Title: Examining changes in spontaneous activity and functional connectivity in the human visual system following deprivation and increased use.

Authors: *L. L. FLEMING, P. DEMIRAYAK, M. DEFENDERFER, K. M. VISSCHER; Neurobio., Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: It is well known that different types of experiences, such as increased use and decreased use due to sensory deprivation, can have measurable impacts on brain structure and function. Adaptation to different types of experiences like deprivation and increased use may involve multiple, simultaneous modes of plasticity. As a result, an outstanding question in the field is how different forms of plasticity may operate in response to different types of experiences. Here, we examine experience-dependent plasticity in macular degeneration (MD), a disease that allows for the observation of the separate effects of both sensory deprivation and increased use in the adult brain. MD is characterized by retinal lesions often resulting in bilateral central vision loss. This forces individuals to rely on peripheral vision for everyday visual tasks. As such, MD serves a model for examining the relationship between experience and neural plasticity. Here, we use resting-state fMRI to measure functional outcomes of experience-dependent plasticity in MD. We focused on cortical representations of three parts of the visual field: the area of deprivation (lesion projection zone, LPZ), the area of increased use (preferred retinal locus, PRL), and a control region that remains relatively intact but is not preferentially used (un-preferred retinal locus, URL). Using resting-state data obtained during complete darkness with eyes open, we examined functional connectivity between these regions in early visual cortex and specialized regions that fall later in the visual system hierarchy. We found that the PRL region in MD patients showed greater functional connectivity to fusiform face area, a region that is selectively responsive to faces. Surprisingly, functional connectivity to middle temporal area, a region selectively responsive to motion, displayed stronger connections toward both the PRL and URL. This finding suggests that using peripheral vision for motion detection causes an increase in connectivity to peripheral regions overall. These findings are consistent with the notion that changes in functional connectivity reflect a history of repeated co-activation been brain regions (i.e. – Hebbian plasticity). Additionally, we examined the amplitude of spontaneous activity during complete darkness. Spontaneous activity in the PRL was lower in MD patients, suggesting a possible homeostatic change that results from increased daily use of that region. Together, these results provide evidence that different outcomes of neural plasticity in the human brain can be differentially impacted by different types of long-term changes in experience.

Disclosures: L.L. Fleming: None. **K.M. Visscher:** None. **P. Demirayak:** None. **M. Defenderfer:** None.

Digital Abstract Session

P195. Eye Movements and Perception

Program #/Poster #: P195.01

Topic: D.08. Visual Sensory-motor Processing

Support: BB/S006605/1

Title: Population receptive fields in human visual cortex during active vision

Authors: *J. H. FABIUS, K. MORAVKOVA, A. FRACASSO;
Univ. of Glasgow, Glasgow, United Kingdom

Abstract: To adequately localize sensory events, the brain must have an accurate representation of the receptor topography. In the visual system this is achieved with an organization that preserves the retinotopic topography of photoreceptors. However, because receptors can move with respect to other parts of the body, the mere topography of receptors is not sufficient for localization of external objects. In the case of vision, eye movements change the orientation of the entire retina with respect to the head and body. A computational solution to this problem is a modulation of response gain based on receptor orientation (e.g. based on eye position). In theory, gain fields allow for the transition from a purely retinotopic to an eye position invariant representation of visual space. Evidence for gain fields in the visual system comes primarily from neurophysiological studies with non-human primates. Still, it is not clear how and to what extent the gain field mechanism is implemented in the human visual hierarchy. Here, we measure high resolution functional MRI data while participants actively made guided eye movements. In our analysis, we first estimated population receptive fields (pRF) using data from a standard moving-bar paradigm. Next, we assessed to what extent these pRF estimates can account for the data from the eye movement paradigm. Finally, we will estimate population gain fields on top of pRFs, testing whether eye-position based gain modulation increases the explained variance of the predicted contrast-based pRF response. Moreover, we will assess how reliably eye-position can be decoded from different areas along the visual hierarchy. These results will shed new light on how receptor position modulates sensory processing in the human brain, and our methods provide a new way of access to the computational code behind that modulation, potentially accessing the large-scale organization of gain-field properties in human neocortex.

Disclosures: J.H. Fabius: None. **K. Moravkova:** None. **A. Fracasso:** None.

Digital Abstract Session

P195. Eye Movements and Perception

Program #/Poster #: P195.02

Topic: D.08. Visual Sensory-motor Processing

Support: ISF Individual Research Grant 1485/18 to SG

Title: Oculomotor-related measures but not distance visual acuity are predictive of reading ability in first graders

Authors: *A. PORTNOY[1], S. GILAIE-DOTAN[1,2];
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Abstract: Vision screenings in junior schools around the globe are often limited to distance visual acuity (dVA). We hypothesize that dVA is not crucial for comfortable and efficient acquisition of effective reading skills and that a basic prerequisite for successful reading is that the ocular sensory-motor functions involved (fixation, saccades, accommodation and fusional vergences) perform in flawless harmony with effortless automaticity. Oculomotor dysfunction (OMD) is an umbrella term that includes abnormalities in comfortable and accurate control of the oculomotor system (fixation, pursuits and saccades). We speculated that OMD has a profound effect on all reading acquisition, all the more so with regard to transparent orthographies (Hebrew, Arabic). In order to assess these in the general population, we retrospectively assessed the results of an extensive optometric screening examination of an ordinary class of 28 first graders (17 boys and 11 girls) and compared them to independently obtained standardized reading evaluations (blind to the optometric results). Good or normal dVA was not indicative of reading performance (speed or accuracy), while poorer dVA was correlated with better reading speed and accuracy. As we hypothesized, performance on optometric OMD tests were predictive of reading speed and accuracy. Specifically, performance on optometric oculomotor tests (such as DEM or NSUCO) were predictive of reading test performance according to the standardized national reading norms. Our results suggest that there is a need to reevaluate which visual skills should be evaluated when assessing visual readiness in preparation for scholastic achievements such as reading acquisition. We suggest including OMD assessments and possibly other visual skills unrelated to acuity as part of a paradigm shift in the screening of vision skills in early readers. Furthermore, potential treatment protocols for those pupils struggling to acquire efficient reading skills should possibly be reassessed.

Disclosures: A. Portnoy: None. **S. Gilaie-Dotan:** None.

Digital Abstract Session

P195. Eye Movements and Perception

Program #/Poster #: P195.03

Topic: D.08. Visual Sensory-motor Processing

Support: ISF Individual Research Grant 1485/18 to SGD

Title: Investigating perception of facial expression emotional valence at parafoveal locations

Authors: *V. AKSELEVICH[1], S. GILAIE-DOTAN[1,2];
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Abstract: Facial emotion perception is typically studied at central visual field, and peripheral investigations mainly focus on locations to the right or left of fixation. Faces, however, can appear in different locations of the visual field. Furthermore, a recent study shows that parametric investigations of visual functions at parafoveal locations can be informative about the underlying mechanisms. Thus we have examined how emotional valence and eccentricity

modulate emotion perception. A group of 28 participants kept fixation while categorizing emotional valence of face photos with positive, negative, or neutral valence. Images were presented for 200 ms at fixation or at one of 8 parafoveal locations (2°, 4°) across the visual field. Since mouth regions may provide distinctive facial expression cues, we used open-mouth photos for positive and negative valence. Eye movements were monitored. As expected, performance accuracy decreased and RT increased with growing eccentricity. Furthermore, a significant effect of emotion was found with highest performance for positive emotions and lowest for negative ones, and error analysis revealed that this was not a result of response biases towards a certain valence. An interaction between valence and eccentricity was evident by negative emotions being the most influenced by eccentricity (accuracies of 83.68% at centre decreased to 52.14% at 4°) and positive emotions the least (mean accuracy of 93.87% at centre and 76.9% at 4°). Additionally, we found that performance within-valence type (but not across-valence types) was correlated across eccentricities, suggesting that these may be supported by dissociated mechanisms. Our results suggest that perception of different types of emotional valence may have different sensitivities which peripheral vision may shed a light on.

Disclosures: V. Akselevich: None. **S. Gilaie-Dotan:** None.

Digital Abstract Session

P195. Eye Movements and Perception

Program #/Poster #: P195.04

Topic: D.08. Visual Sensory-motor Processing

Support: ARL/DSO W911NF-10-2-0022 (Subaward from DCS Corp: APX02-N013)

Title: Presaccadic visual stimulus affects postsaccadic neural activity during realistic detection task

Authors: *A. D. STANKOV[1], J. TOURYAN[2], S. GORDON[3], J. KI[1], L. C. PARRA[1];
[1]City Col. of New York, New York, NY; [2]CCDC Army Res. Lab., Adelphi, MD; [3]DCS Corp., Alexandria, VA

Abstract: Little is known about visual processing during free viewing in realistic dynamic environments. Free viewing is characterized by frequent saccades, which generate large neural responses that have been linked to enhanced visual processing during the subsequent fixations. While visual input is inhibited during the saccade, we do know that the content of fixation prior to a saccade can modulate post-saccading processing. To better understand these processes in a realistic setting we study here saccades and neural responses elicited by the appearance of visual targets in a realistic virtual environment. While subjects are being driven through a 3D virtual town they were asked to detect targets that appear on the road. We found that the presence of a target elicits saccades with stronger saccade-evoked potentials at 125ms and 195ms as well as capturing potentials associated with button presses 250-750ms after the start of the saccade. Both times (125ms and 195ms post saccade) are modulated by the visibility of the target, albeit the

former being marginal in comparison to the former, suggesting that it is related to target processing and not the saccade itself. Its spatial distribution over central scalp electrodes is reminiscent of the classic P300 reorienting response. When targets appeared in peripheral vision, they elicited larger eye movements as compared to targets in central vision. However, contrary to our expectations, targets elicited similar saccade-related responses regardless of where they appeared in the visual field and resulted in similar reaction times. Together these results suggest that during natural viewing neural processing of the visual stimulus, including reorienting, starts before a saccade and continues throughout the saccade, unencumbered by saccadic inhibition.

Disclosures: **A.D. Stankov:** None. **J. Touryan:** None. **S. Gordon:** None. **J. Ki:** None. **L.C. Parra:** None.

Digital Abstract Session

P195. Eye Movements and Perception

Program #/Poster #: P195.05

Topic: D.08. Visual Sensory-motor Processing

Support: NSERC CREATE Brain-in-Action Program
Queen Elizabeth II Graduate Scholarship in Science & Technology
NSERC Discovery Grant
Canada Research Chair Program

Title: An fMRI investigation into the cortical correlates for transsaccadic perception of object orientation vs. shape

Authors: *B. BALTARETU[1], W. S. STEVENS[1], E. FREUD[1], J. CRAWFORD[2];
[2]Ctr. for Vision Res., [1]York Univ., Toronto, ON, Canada

Abstract: Recently, Dunkley et al. (*Cortex*, 2016) showed modulations in parietal cortex (i.e., supramarginal gyrus, SMG) for transsaccadic changes in object orientation. However, this result did not generalize to other features, such as spatial frequency (Baltaretu et al., *J Vis*, 2016). We hypothesized that the fundamental difference between these results was the detection of transsaccadic changes in object orientation vs. shape. To test this, we used a double-dissociation fMRI task. Participants fixated a small cross 15.4° left or right of centre, where an object was subsequently presented (rectangle, barrel-shaped, or hourglass-shaped), oriented at ±45° from vertical. After this, the fixation cross either remained in the same position (Fixation condition) or shifted to the other side (Saccade condition). Then, either the *same object* would appear at the *orthogonal orientation* (Orientation change condition) or *one of the other two objects* would appear at the *same initial orientation* (Shape change condition). Button press was used to indicate whether object orientation or shape had changed across the two stimulus presentations in a given trial. Results indicate that cortical modulations were larger for saccades (than fixations) when orientation changed in medial early occipital regions (e.g., cuneus), extending into later occipitotemporal regions. Activation for changes in object shape was larger for saccades (than

fixations) in regions similar to those for orientation changes, but were also found in medial parietal regions (e.g., precuneus and superior parietal lobule). When looking for a feature change (Orientation > Shape) specifically in cortical regions showing saccade-related (Saccade > Fixation) preferences, we found modulations within medial occipital/occipitotemporal cortex, also extending dorsally toward the parietal cortex border. These preliminary results suggest that differences in object features that occur across saccades may also appear at the cortical level (predominantly within medial occipital cortex, extending in both directions toward parietal and temporal cortex).

Disclosures: B. Baltaretu: None. **W.S. Stevens:** None. **E. Freud:** None. **J. Crawford:** None.

Digital Abstract Session

P195. Eye Movements and Perception

Program #/Poster #: P195.06

Topic: D.08. Visual Sensory-motor Processing

Support: NSERC Discovery Grant
VISTA
OGS

Title: Functional brain network of transsaccadic integration: Evidence from fMRI and graph theory analysis

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Abstract: While the brain regions involved in transsaccadic integration have been established, the networks comprised of these regions are not well understood. We formed functional brain networks from functional magnetic resonance imaging (fMRI) data collected during a task designed to dissociate saccade activity from object feature activity and evaluated the network properties between conditions. Participants (N=17) maintained fixation on a cross either at the left or right of center for 7.5 s, after which one of three objects was presented at one of two orientations (45º clockwise or counterclockwise from vertical) in the center of the display for 3 s. Following the initial presentation period, the fixation cross either remained in the same location or was displayed at the opposing side of the center (fixation vs. saccade conditions). Subsequently, an object was once again presented at the center location for 3 s; the two objects were the either the same with the second presentation at the contrary orientation (orientation change condition), or the objects were different while the orientation remained the same (identity change condition). Participants were asked to indicate which change had occurred (orientation vs. identity). Graph theory analysis was applied to identify clustering coefficient, local efficiency, global efficiency, and small world propensity. Pairwise t-tests indicated significantly higher values for clustering coefficient, local efficiency, and global efficiency for saccades relative to fixation, suggesting enhanced segregation and integration of the identified network

during saccades. These enhanced network properties suggest increased speed of information distribution during saccades. No significant differences in these measures were found for orientation vs. identity conditions, indicating that the analyzed network specializes in transsaccadic integration and is not involved in discrimination of object orientation and identity.

Disclosures: G. Tomou: None. **B. Baltaretu:** None. **A. Ghaderi:** None. **J. Crawford:** None.

Digital Abstract Session

P196. Sensorimotor Transformation: Behavior and Whole Animal

Program #/Poster #: P196.02

Topic: D.08. Visual Sensory-motor Processing

Support: Haverford College Velay Fellowship

Title: Ap2s1 is required for modulation of visually guided behavior in zebrafish larvae

Authors: *H. DOLL[1], A. LABORDE[2], M. ORGER[2], R. JAIN[1];
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Abstract: Given that many neuropsychiatric conditions have a significant genetic component, investigating the underlying mechanisms of behavior selection could illuminate pathogenesis. In humans, sequence variants in *ap2s1* have been linked to cognitive disruption and autism spectrum disorder, among other conditions. *ap2s1* is a subunit of the AP-2 Complex, which is involved in clathrin-mediated endocytosis. We previously identified the gene *ap2s1* to be involved in acoustic behavior modulation through a forward genetic screen in zebrafish larvae. We also observed a strong expression pattern in components of the visual system, suggesting the gene could modulate behavior in other modalities, such as the visual system. Elucidating the role that *ap2s1* plays in visually-guided behavior will help paint a clearer picture of where and how the gene impacts complex behaviors. Here, we used motion tracking and bout-based tracking in several behavioral assays to examine visual acuity and visually-guided behaviors in *ap2s1* mutant zebrafish. Though *ap2s1* mutations enhance escape responses to acoustic stimuli, the mutants failed to perform escape responses to threatening visual stimuli. However, the mutants did respond to visual stimuli in the whole-field lighting changes and optomotor (OMR) stimuli, strongly indicating that the mutants are not completely blind. Mutants displayed altered kinematics during these assays and unexpectedly showed enhanced accuracy in responding to OMR stimuli as compared to non-mutant siblings. Overall, we found a complex and unexpected role of *ap2s1* in the visual system which could lead us to better understand the gene's role in human behavior.

Disclosures: H. Doll: None. **R. Jain:** None. **M. Orger:** None. **A. Laborde:** None.

Digital Abstract Session

P197. Representation of Faces and Bodies

Program #/Poster #: P197.01

Topic: D.07. Vision

Support: DFG grant NO 1448/1-1
NIH grant 2RO1 EY 022318
NIH training grant 5T32EY020485
NSF Graduate Research Development Program (DGE-114747)
Ruth L. Kirschstein National Research Service Award (F31EY027201)

Title: Cortical recycling in high-level visual cortex during childhood development

Authors: *M. NORDT[1], J. GOMEZ[2], V. NATU[1], A. A. REZAI[1], D. FINZI[1], H. KULAR[1], K. GRILL-SPECTOR[1];
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Abstract: Human ventral temporal cortex (VTC) contains category-selective regions that respond preferentially to ecologically-relevant categories such as faces, bodies, places, and words and are causally involved in the perception of these categories. How do these regions develop during childhood? Here we used functional MRI to measure longitudinal development of category-selectivity in VTC in individual school-age children (n=29, initial ages: 5-12 years). During fMRI participants viewed images of faces (child/adult), body parts (limbs/bodies), characters (words/numbers), objects (cars/string instruments), and places (houses/corridors) while performing an oddball task. Using data from 128 functional sessions collected over the course of 5 years, we surprisingly find both increases and decreases of category-selectivity during childhood development: From young childhood to the teens, face- and word-selective regions in VTC expand and increase in their respective category-selectivity, but limb-selective regions in VTC shrink and lose their preference for limbs (Fig 1). Critically, as a child develops, increases in their face- and word-selectivity are directly linked to decreases in limb-selectivity. These data show that during childhood limb-selectivity in VTC is repurposed into word- and face-selectivity providing the first empirical evidence for cortical recycling during childhood development. These results suggest a rethinking of prevailing hypotheses that cortical development involves sculpting of new representations upon general-purpose cortex. Instead, our results propose a new hypothesis that during development VTC representations adjust to changes in the salience and social relevance of visual inputs, which has important implications for both typical and atypical brain development.

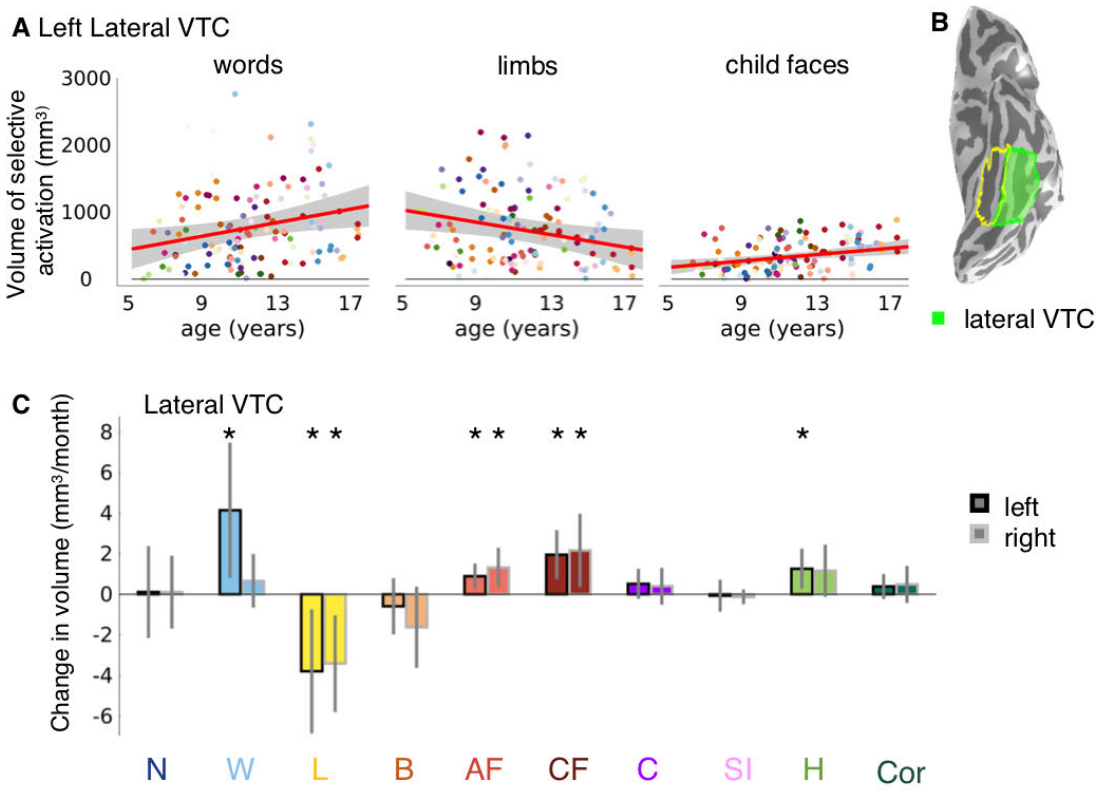

Fig 1. Developmental increases and decreases in category-selective activation in lateral VTC.

(A) Volume of word-, limb- and child-face-selective activation by age. Each dot is a session and colored by participants (n=29, 128 sessions). *Red line:* Linear mixed model (LMM) prediction of category-selective activation by age. Shaded *gray:* 95% confidence interval (CI). **(B)** Lateral VTC outlined in green on the inflated cortical surface of a 5-year-old. **(C)** LMM slopes: change in category-selective activation per month. *Error bars:* 95% CI. *Asterisks*: significant after FDR-correction ($p<0.05$). *Acronyms:* N: numbers; W: words; L: limbs; B: bodies; AF: adult faces; CF: child faces, C: Cars, SI: String instruments, H: Houses, Cor: Corridors.

Disclosures: **M. Nordt:** None. **J. Gomez:** None. **V. Natu:** None. **A.A. Rezai:** None. **D. Finzi:** None. **H. Kular:** None. **K. Grill-Spector:** None.

Digital Abstract Session

P197. Representation of Faces and Bodies

Program #/Poster #: P197.02

Topic: D.07. Vision

Support: Austrian Science Fund (FWF) Grant W1262-B29
Vienna Science and Technology Fund (WWTF), the City of Vienna and ithuba Capital AG project CS18-012

Title: Face, body and object representation in the human and canine occipito-temporal cortex

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Abstract: A large body of research revealed neural representations for faces, bodies, and objects in the human brain over the last years. However, much less is known on how our (social) environment shapes the neural bases of face, body and object processing. The emerging field of canine neuroscience allows us to close this gap non-invasively by studying a longstanding, close companion of humans. Here, we test and compare the neural underpinnings of face, everyday objects and body processing in pet dogs (*Canis familiaris*) and humans for the first time. Fifteen dogs (11 females, 4-11 years) and 26 humans (14 females, 19-28 years) underwent MRI scanning. All dogs received extensive training; they were awake and unrestrained during MRI. Across two runs, both dogs and humans viewed faces (F) and bodies (B) of dogs and humans, everyday objects (O), and a visual control (scrambled images; S). Univariate results in dogs, revealed partially overlapping face and body sensitive caudal temporal regions compared to scrambled images (B > S; F > S), but we only found a specialized sub-region for bodies (B > F + O + S) not faces (F > B + O + S). We did not replicate previously reported differences due to species in dogs. In line with previous research, univariate results in humans replicated specialized processing regions for faces (F > B + O + S) and bodies (B > F + O + S) in occipito-temporal regions. Comparing species, human (> dog) stimuli led to increased activation in similar ventral temporal regions, vice versa (dog > human) we found increased activation in occipital regions (e.g., V1). Investigating the neural representation in dogs (representational similarity analysis: whole-brain, searchlight), the results indicated increased similarity for all object categories (F x B x O > S) in caudal and ventral temporal regions, the occipital lobe and the frontal lobe. Within these regions, we partly observed increased similarity for animate compared to non-animate objects (F x B > O). Finally, we found sub-clusters for dog (> human) bodies in a part of the specialized region for body processing along with the insula, piriform lobe and caudal temporal regions. In humans, we again replicated previous findings. In sum, our results indicate object representations and sensitivity in occipito-temporal regions of both species, while dogs seem to specialize more on bodies, not faces, different from humans. Taken

together, we provide first comparative insights into categorical object representations yielding an evolutionary perspective on how such neural representations are shaped by the environment.

Disclosures: **M. Boch:** None. **I.C. Wagner:** None. **S. Karl:** None. **L. Huber:** None. **C. Lamm:** None.

Digital Abstract Session

P198. Representation of Objects and Scenes

Program #/Poster #: P198.01

Topic: D.07. Vision

Support: ERC under the European Union's Horizon 2020 research and innovation programme (grant agreement No 788535)

Title: Self-supervised natural image reconstruction and rich semantic classification from fMRI

Authors: ***G. GAZIV**[1], R. BELIY[1], N. GRANOT[1], A. HOOGI[1], F. STRAPPINI[1], T. GOLAN[2], M. IRANI[1];
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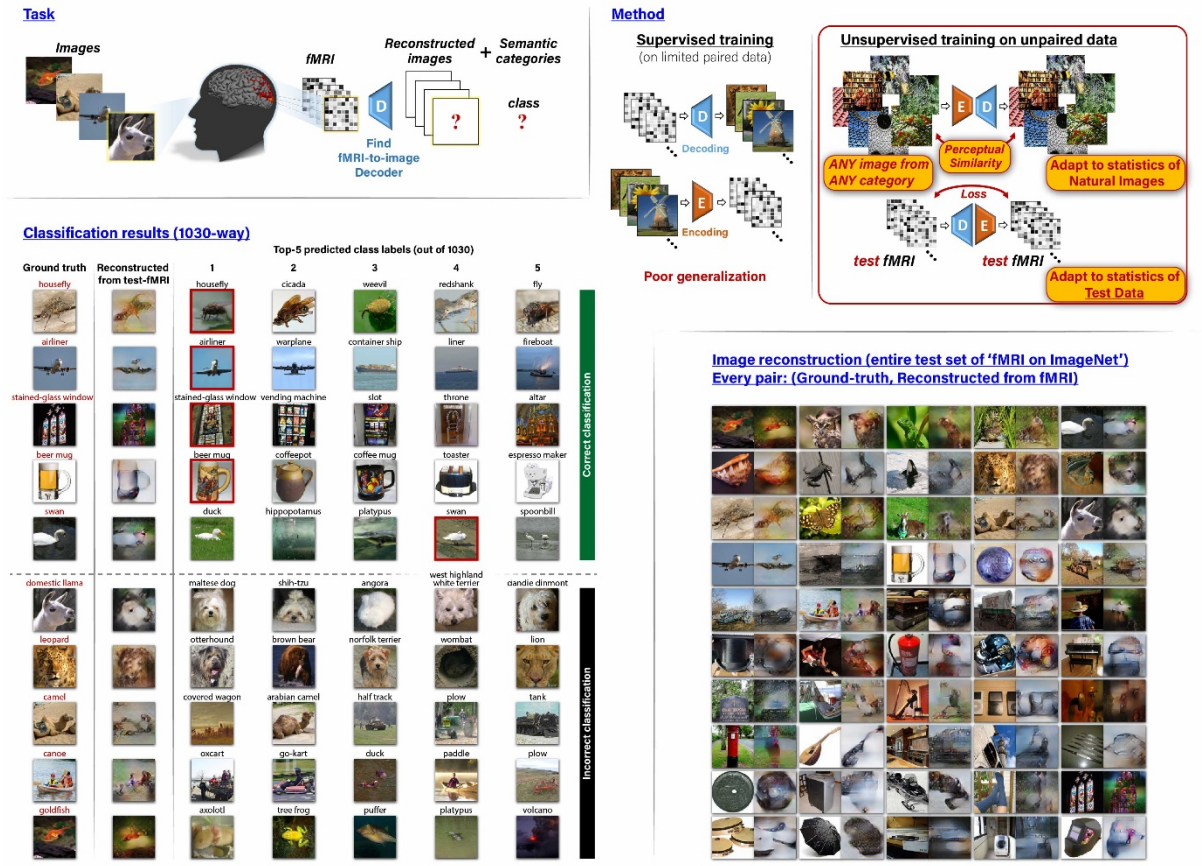
Abstract: Reconstructing seen natural images from a subject's evoked fMRI response and decoding their semantic class is a milestone towards brain-machine interfaces and for the study of consciousness. Unfortunately, acquiring sufficient (Image,fMRI) "paired" training data to span the huge space of natural images and their semantic classes is prohibitive, resulting in limited generalization power of today's decoders. We present a novel self-supervised approach that overcomes the inherent lack of training data, simultaneously for both tasks -- image reconstruction and large-scale semantic classification. Specifically, we impose cycle-consistency using two networks, Encoder (E) & Decoder (D), and train on additional "unpaired" data from the image and the fMRI domains. Concatenating those two networks back-to-back, E-D, allows for unsupervised training on unpaired images (i.e., images without any fMRI recordings) - e.g., 50,000 natural images from 1000 ImageNet semantic classes in our experiments. Such self-supervision adapts the network to the statistics of novel images and their diverse novel categories. Similarly, reversely concatenating our two networks, D-E, allows for unsupervised training on additional unpaired fMRI samples (i.e., fMRI recordings without images). Moreover, imposing high-level perceptual reconstruction criteria within the self-supervision on unpaired images results in a leap improvement over top existing methods, achieving unprecedented image-reconstruction from fMRI of never-before-seen images (evaluated by image metrics and human testing), and large-scale semantic classification (1000 diverse classes) of categories that are never-before-seen during network training (exceeding chance level accuracy by more than 100-fold). To our best knowledge, such large scale semantic classification (1000 classes) from fMRI data has never been exhibited before. We further visualize the receptive field underlying our decoder and show the emergence of classic retinotopic organization. These results support the biological plausibility of our model.



Disclosures: **G. Gaziv:** None. **R. Beliy:** None. **N. Granot:** None. **A. Hoogi:** None. **F. Strappini:** None. **T. Golan:** None. **M. Irani:** None.

Digital Abstract Session

P198. Representation of Objects and Scenes

Program #/Poster #: P198.02

Topic: D.07. Vision

Support: Deutsche Forschungsgemeinschaft (DFG) grants (CI241/1-1, CI241/3-1)
European Research Council Starting Grant (ERC-2018-StG 803370)

Title: Unraveling the temporal cortical dynamics of indoor scene navigation using behavioral and deep neural network models

Authors: ***M. P. BALODE**[1,2], K. DWIVEDI[2,3], G. ROIG[3], R. M. CICHY[2];
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Abstract: Humans have to process scene information rapidly in order to navigate through their immediate environment. How does the rapid cascade of neural processing elicited by viewing a scene enable navigational planning? We addressed this question by comparing human electroencephalography (EEG) responses with computational models representing distinct levels of scene processing and a behavioral model capturing navigational planning. We recorded EEG (N = 16, 7 females, mean age 28.9 ± SD 5.6) while participants viewed 50 indoor scene images and engaged in a navigational task during which they had to respond whether the exit path displayed on the screen corresponded to any of the exit paths from the previous trial (Fig. 1A). As for the computational models, we used activations from 18 deep neural networks (DNN) (Zamir, 2018) optimized for different visual tasks that were grouped into 3 categories (2D, 3D, semantic) corresponding to low-, mid- and high-level scene features, respectively. To model navigational planning we used the navigational affordance map (NAM) model (Bonner < Epstein, 2018) that was constructed from participants' responses in a behavioral task in which they had to draw possible navigational paths on the same set of images.We computed the unique variance of EEG data explained by each of the models by comparing the Representational Dissimilarity Matrices (RDMs) of EEG for every 10 ms from -200 to +800 ms relative to image onset (Fig. 1B) with RDMs of DNN (Fig. 1C) and NAM (Fig. 1D) data.The results revealed a different temporal pattern of unique variance for each of the models (Fig. 1E). Navigational affordance representations peaked significantly (t-test, FDR-corrected, $p < 0.05$) later (NAM: 326 ms) than high- (semantic: 177 ms), mid- (3D: 154 ms) and low- (2D: 126 ms) level visual representations (Fig. 1F).Taken together, our findings describe the time course with which the processing of different scene features precedes navigational affordance representations, suggesting that the processing of 2D, 3D, and semantic scene features facilitate navigation planning.

Figure 1

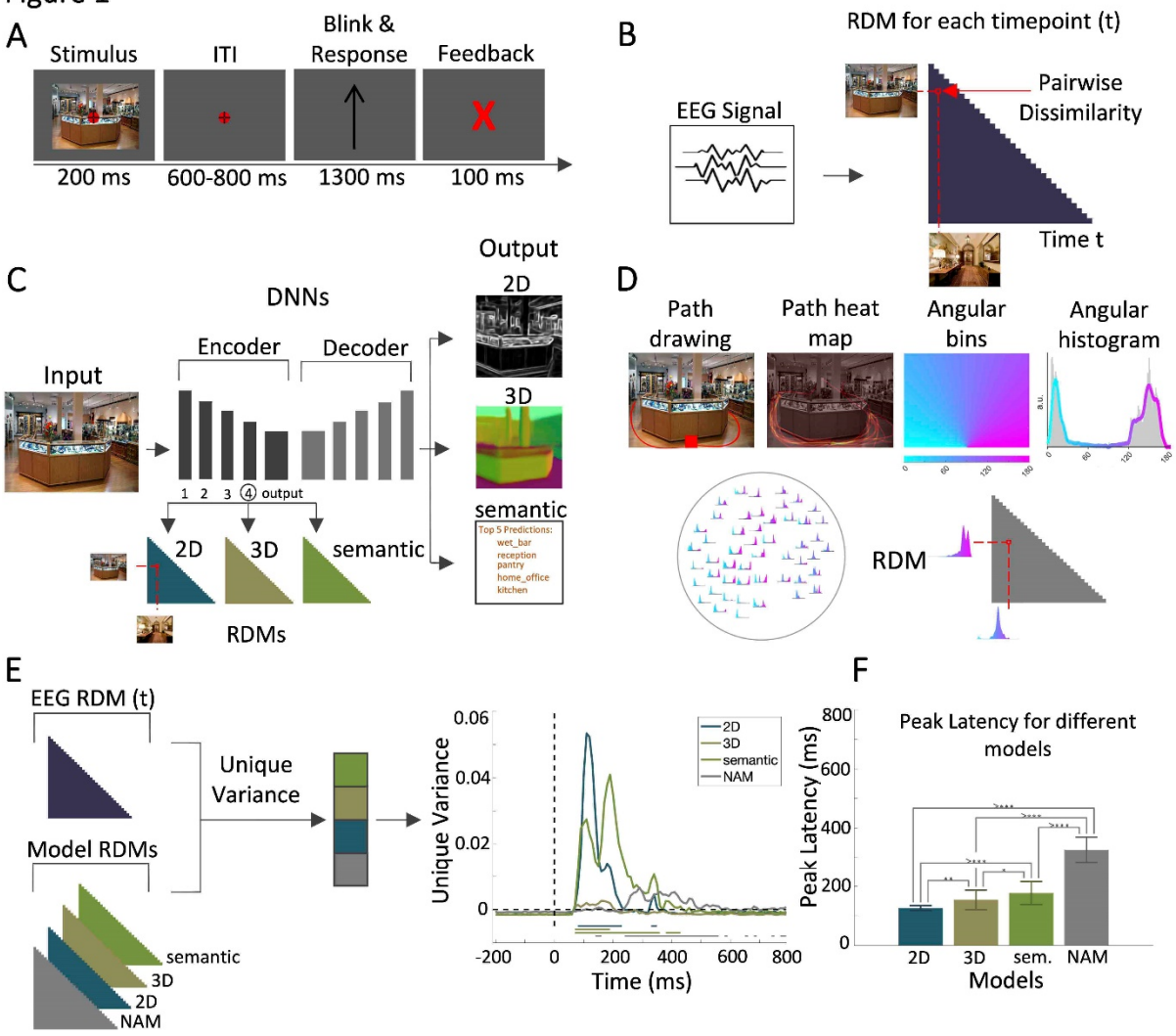

Figure 1: A) EEG paradigm. The participants viewed 50 images of indoor scenes and were asked to mentally plan possible exit paths through the scenes. After a varying number of image trials the participants had to respond whether the exit path displayed on the screen corresponded to any of the exit paths from the previous trial. **B) EEG RDMs.** We computed the RDMs for each EEG time point (every 10 ms from -200 to +800 ms with respect to image onset) using pairwise decoding accuracy. **C) DNN RDMs.** We calculated the RDMs from the 4th encoder block of 2D, 3D and semantic DNNs, corresponding to low-, mid- and high-level scene features, respectively. **D) NAM model and RDM** (Bonner and Epstein, 2018). **E) Unique Variance.** We calculated the unique EEG variance explained by each of the models revealing different temporal activation patterns. Lines below the plots indicate significant times using t-test (FDR corrected $p < 0.05$). **F) Peak latencies of different models.** 2D: 126 ms, 3D: 154 ms, semantic: 177 ms, NAM: 326 ms. Error bars indicate standard deviation for 16 subjects. Stars above bars indicate significant differences across different models (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, t-test FDR corrected).

Disclosures: M.P. Balode: None. **K. Dwivedi:** None. **G. Roig:** None. **R.M. Cichy:** None.

Digital Abstract Session

P198. Representation of Objects and Scenes

Program #/Poster #: P198.03

Topic: D.07. Vision

Support: NIH Grant EY014924
NIH Grant EY029759

Title: Robust Representation of Natural Scenes within Columns of Primary Visual Cortex

Authors: *X. CHEN[1], K. BAI[3], S. ZHU[4], R. XIA[1], N. KONG[2], S. WEINGARTNER[4], A. NORCIA[2], T. MOORE[4,1];
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Abstract: The visual system is believed to have adapted to the statistical properties of the natural environment. However, the stage at which sensitivity to naturalistic structure emerges during visual processing is controversial. To address this, we recorded in macaque V1 using high-density electrode arrays (Neuropixels probes, IMEC) and investigated the neural representation of images in macaque primary visual cortex (V1) using three sets of images that varied in their higher order image statistics: natural scenes (NA), synthetic natural texture (ST) images, and phase-scrambled synthetic noise (SN) images. We measured the response from hundreds of V1 neurons across the columnar structure of V1. We first examined the sensitivity of single neurons for natural images and found that V1 neurons responded more vigorously to NA than to SN and ST images within the first 50 ms of the visual response. This sensitivity to natural image structure was robustly observed throughout all cortical layers, including neurons in the input layer 4C. We next examined the population coding of V1 neurons for natural scenes. We found that V1 neurons showed high population sparseness and high lifetime sparseness for natural scenes. Moreover, individual neurons were less synchronized with the overall population activity for images containing more natural statistical components. In addition, the neural ensembles showed higher discriminability for natural scenes. Lastly, we examined the activity patterns of an ImageNet-trained deep convolutional neural network model (DCNN) in response to the same set of stimuli. This analysis shows that higher sensitivity to natural scenes also emerges early in this feedforward artificial visual system. Taken together, these results revealed a distinct coding preference for natural images starting at the earliest stages of visual cortex. In addition to feedback modulation from higher visual cortex, these results suggest a feed forward sensitivity to natural scenes during cortical visual processing.

Disclosures: X. Chen: None. **K. Bai:** None. **S. Zhu:** None. **R. Xia:** None. **N. Kong:** None. **S. Weingartner:** None. **A. Norcia:** None. **T. Moore:** None.

Digital Abstract Session

P198. Representation of Objects and Scenes

Program #/Poster #: P198.04

Topic: D.07. Vision

Title: Toward a benchmark test that selectively measures shape recognition competence

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[1]Neurosci. Grad. Program, [2]Biomed. Engin., USC, Los Angeles, CA

Abstract: Deep networks (DNs) trained on object recognition tasks achieve high performance on standard benchmarks, but unlike biological vision systems, which rely heavily on shape information for classification, DNs mainly depend on local color and texture features. An ongoing impediment to creating a more human-like recognition system that generalizes based on shape similarity is the lack of a benchmark that specifically tests shape recognition competence. We are developing such a benchmark, where performance is measured by ordering similarly matching scores for each object view in the database against all other object views (our database currently contains ~12,000 total views). A correct match is scored when for a given object view, the closest (eligible) matching view of the same object has a higher matching score than that of any view of any other object in the database. Task difficulty is controlled through the use of "exclusions" - the removal from contention during matching of any view of the same object that is "too similar", whether in viewpoint, contrast, or other viewing parameters. Our first task was to challenge a state-of-the-art pre-trained DN using this benchmarking approach. On a database containing only 10 objects (~600 views each), shape-matching performance of a Resnet 50 pre-trained on ImageNet was already poor under an exclusion condition involving a only a contrast reversal and a modest change in 3D viewpoint. Error rate began at 25% for contrast reversal alone, and climbed rapidly as the viewpoint exclusion increased. Our near term goal is to increase task difficulty by growing the database to 100 objects, which will significantly increase the density of distractors during matching, and to implement several more types of exclusions designed to frustrate OR systems that lack a strong internal shape representation.

Disclosures: J.W. Nam: None. **A.S. Rios:** None. **B.W. Mel:** None.

Digital Abstract Session

P198. Representation of Objects and Scenes

Program #/Poster #: P198.05

Topic: D.07. Vision

Support: NIH Grant 1R01MH111439

Title: Neural responses to scene cuts in movies reflect semantic processing in higher order cortical lobes

Authors: *M. NENTWICH[1], M. LESZCZYNSKI[2,3], B. E. RUSS[3], S. BICKEL[4], A. MEHTA[4], C. E. SCHROEDER[3,2], L. C. PARRA[1];
[1]Biomed. Engin., The City Col. of New York, New York, NY; [2]Departments of Psychiatry, Neurol. and Neurosurg., Columbia Univ. Col. of Physicians and Surgeons, New York, NY; [3]Ctr. for Biomed. Imaging and Neuromodulation, Nathan S. Kline Inst. for Psychiatric Res., Orangeburg, NY; [4]Donald and Barbara Zucker Sch. of Med. at Hofstra/Northwell, Northwell

Hlth. Syst., Feinstein Inst. for Med. Res. and Departments of Neurosurg. and Neurol., Manhasset, NY

Abstract: Naturalistic stimuli offer a unique opportunity to study the interaction of low level visual stimulus features and higher level features of narratives. Specifically, scene cuts in commercially produced movies are designed to guide the narrative of a story and are accompanied by strong changes in visual contrast. Here, we investigate the neural responses to scene cuts that reflect a change of camera position ('camera'), in contrast to the responses to scene cuts that are related to semantic changes in the narrative of a movie ('semantic'). We analyzed electrophysiological data from over 4400 intracranial electrodes from 19 patients with medically refractory epilepsy. The patients watched a total 53 min of short clips of commercially produced movies, ranging from animations to documentaries, while simultaneous eye tracking and intracranial EEG data was recorded. We find that a larger share of electrodes respond to 'semantic' scene cuts than to 'camera' scene cuts. While electrodes in the occipital cortex respond indiscriminately to any type of scene cuts, electrodes in the parietal, temporal and frontal cortex show more specific responses to either 'camera view' or 'semantic' scene cuts. This effect was seen in the broadband local field potential as well as in the power of particular frequencies: theta (4-7 Hz), alpha (8-14 Hz), beta (15-30 Hz) and broadband high-frequency amplitude (70-150 Hz). Additionally, we observe that both types of scene cuts elicit an increase in saccade rates. The saccade rates are not significantly different after 'camera view' or 'semantic' scene cuts. In conclusion, we find a more widespread response to scene cuts that represent semantic changes of the narrative, as compared to simple changes in camera view position. This contrast increases in higher order cortical lobes and is independent of saccades that cluster after scene cuts.

Disclosures: M. Nentwich: None. **M. Leszczynski:** None. **B.E. Russ:** None. **S. Bickel:** None. **A. Mehta:** None. **C.E. Schroeder:** None. **L.C. Parra:** None.

Digital Abstract Session

P198. Representation of Objects and Scenes

Program #/Poster #: P198.06

Topic: D.07. Vision

Support: CIHR grant 366062

Title: Fmri adaptation and multi-voxel pattern classification support different conclusions about hippocampal pattern separation

Authors: *K. M. FERKO, A. KHAN, S. KÖHLER;
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Abstract: Pattern separation is a neural computation thought to underlie our ability to form distinct memories of similar events. It involves transforming overlapping inputs into less overlapping outputs. Research on pattern separation has primarily focused on transformations of

representations between entorhinal cortex and the dentate gyrus (DG) of the hippocampus. Evidence for pattern separation in human neuroimaging research has primarily come from fMRI adaptation (fMRIa) paradigms, in which repetition suppression and novelty signals are used to make inferences about pattern separation for activity in DG associated with targets and similar lures. Multivariate pattern-analysis (MVPA) techniques provide a more direct way to measure the separability of patterns of activity in DG for similar items. There is evidence suggesting that similar scenes matched for degree of novelty can be classified based on activity patterns in DG. At present it is unclear whether comparable separation can be observed for objects. Given that many extant findings obtained with fMRIa in DG have involved visual presentation of objects it is important to determine whether pattern-based analyses confirm the role of DG in pattern separation for this stimulus class. In the present study, we directly compared fMRIa and MVPA classification in combination with ultra-high resolution image acquisition. Hippocampal subfields and other medial temporal-lobe regions were segmented with ASHS, including a region that captured DG/CA3. Participants (N=23) performed a modified 1-Back paradigm with similar exemplars from multiple real-world categories (e.g, apples, flowers) that required identification of repetition at the exemplar or at the category level. fMRIa analyses of activity revealed evidence for pattern separation between objects from the same category in DG/CA3 and perirhinal cortex (PrC), a structure that has been implicated in object discrimination in many prior studies. Specifically, similar objects that followed exemplars from the same category elicited higher levels of activity (i.e. less adaptation) in both regions when compared with exact repetitions of the same objects. In contrast, MVPA classification revealed separable patterns of activity for exemplars from the same category only in PrC, but not in DG/CA3, when novelty status of exemplars was matched. In other words, fMRIa on DG/CA3 revealed evidence of the kind that is typically interpreted as pattern separation whereas MVPA did not. By contrast, in PrC findings from both methods converged. The divergence of conclusions drawn from both methods about DG in the same data question the notion that they get at the same computation.

Disclosures: **K.M. Ferko:** None. **A. Khan:** None. **S. Köhler:** None.

Digital Abstract Session

P198. Representation of Objects and Scenes

Program #/Poster #: P198.07

Topic: D.07. Vision

Support: This work was funded by a Max Planck Research Group grant to MNH.

Title: Feature-reweighted RSA: a general purpose method for increasing the fit between vision models and brain data?

Authors: *P. KANIUTH, M. N. HEBART;
Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: Representational Similarity Analysis (RSA) has emerged as a popular tool in cognitive neuroscience to compare representations of different modalities, species, and models (e.g., brains or deep neural networks, DNNs). RSA works by comparing representational dissimilarity matrices (RDM), which characterize the dissimilarity between all pairs of conditions (e.g., visual stimuli). However, classical RSA assumes that individual features of measured representations or models (e.g., brain voxels, DNN units) all contribute equally to the final similarity estimate. This is a strong assumption and contrasts with other common methods, such as multivariate decoding, that allow for a linear reweighting of individual features. Recent developments counteract this by reweighting features of DNN models for use in RSA, and indeed find improved performance (Jozwik et al., 2017; Peterson et al., 2018; Storrs et al., 2020). Here, we systematically evaluated the degree to which this feature-reweighted RSA (FR-RSA) can serve as a general purpose method for improving the sensitivity of measured representations. To this end, we used three publicly available datasets of real-world object images (84, 92, and 118 conditions, respectively), testing the correspondence between DNNs, behavior, fMRI, and MEG for MEG-fMRI fusion. For all datasets and models, we found that FR-RSA consistently increased the correspondence of pairs of RDMs, sometimes doubling the amount of shared variance. Sanity checks and the fitting algorithm's design ensured that this was not due to overfitting. RSA is commonly deployed to estimate how well different models capture how the brain represents relations between stimuli or conditions. FR-RSA could become a general purpose method that further refines this process, ultimately facilitating the selection between competing models.

Disclosures: P. Kaniuth: None. **M.N. Hebart:** None.

Digital Abstract Session

P199. Sensorimotor Transformation: Neuroprocessing

Program #/Poster #: P199.01

Topic: D.08. Visual Sensory-motor Processing

Support: R01 EY028219
PIIF
A*STAR National Science Scholarship

Title: Behavioral state-dependent modulation of medial Lateral Posterior (LP) thalamic visual input to the Anterior Cingulate Cortex (ACC)

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Abstract: Selective attention is a critical cognitive process to adaptively highlight task-relevant stimuli while gating indiscriminate stimulus responsivity. Functional connectivity between the reciprocally connected pulvinar and frontal areas like the anterior cingulate cortex (ACC) is associated with impaired sensory filtering and over-responsiveness to stimuli. We sought to

examine how the lateral posterior (LP) thalamic nucleus, the rodent homologue of the pulvinar, may exert behavior state-dependent gating of its visual-associated input to ACC. Using two-photon calcium imaging of LP axons in ACC of head-fixed mice presented with visual stimuli, we demonstrate that visual responsiveness and feature tuning to the same stimuli are highly correlated with spontaneous fluctuations in behavioral state as well as direction of self-motion. We also find dissociable contributions of pupil-linked arousal and locomotion. As ACC simultaneously receives visual input from primary visual cortex and other higher visual areas, state-dependent gating by LP could in turn influence the visual information integrated at ACC or bias ACC-dependent top-down modulation of visual inputs.
To identify neural sources that may convey such behavioral states, we performed monosynaptic rabies tracing of brain-wide inputs to LP-ACC projections and its top-down complement, ACC-LP projections. This revealed a convergence of multi-sensory and motor input upon LP-ACC neurons, in contrast to ACC-LP neurons, which mainly receive visual and frontal inputs. In addition, the most prominent subcortical input source to LP-ACC neurons is the superior colliculus (SC) — particularly from the intermediate and deep layers which do not receive direct retinal input. With the shared involvement of ACC, LP and SC in visuomotor functions, orienting and coordinating attentional priority, LP can thus serve as one of the pathways for feedback from SC to ACC.

Disclosures: **Y.N. Leow:** None. **B. Zhou:** None. **M. Sur:** None.

Digital Abstract Session

P199. Sensorimotor Transformation: Neuroprocessing

Program #/Poster #: P199.02

Topic: D.08. Visual Sensory-motor Processing

Support: NSERC Grant
CIHR IRSC Grant
VISTA: Science to Applications

Title: Robust visuomotor transformations in the multi-unit activity of the monkey frontal cortex

Authors: ***S. SEO**[1], V. BHARMAURIA[1], A. SCHÜTZ[2], F. BREMMER[2], X. YAN[1], H. WANG[1], J. D. CRAWFORD[1];
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Abstract: Neurons work in coordination and form networks with each other to encode a stimulus or behavior. Therefore, there is an increasing trend toward analyzing the activity of several simultaneously recorded neurons. Multiunit activity (MU), which refers to the average firing of neurons recorded within the close proximity of a microelectrode is being increasingly studied in neuroscience as it contains accurate estimations of neural populations dynamics related to a stimulus or task [1]. Here we examined the spatial structure of simultaneously recorded MU activity from the frontal (FEF) and supplementary eye fields (SEF). We employed a cue-conflict

memory-guided saccade task (where a target-fixed landmark shifted during the delay) to record spiking activity (visual, memory, and motor) in head-unrestrained monkeys (*Macaca mulatta)*. Then, we isolated the single unit (SU) and MU [by simply rethresholding[2]] activity from the raw recorded signals. Using a model fitting approach on the SU and MU response fields (RF), along a target (T) to gaze (G) continuum, we then calculated if the MU activity contains the same information compared with the SU activity. In both areas, we found that the MU possesses similar visual-to-motor transformation as the SU activity [3,4]. Specifically, the MU visual (FEF = 88; SEF = 45) and motor (FEF = 90; SEF = 49) activities also encoded the target-in-eye (Te) and future gaze-in-eye (Ge) coordinates, respectively. Along the spatiotemporal domain that spanned the visual-memory-motor activity, we noticed a progressive transition from T to G coding. Finally, the FEF MU activity of motor neurons better predicted the gaze than SU activity ($p < 0.05$) implying the robust transformation in population signals. We conclude that: 1) neurons work in coordination for a common gaze goal, and 2) MU activity is a reliable marker of visuomotor transformations with potential for translational purposes in medical settings.
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Disclosures: **S. Seo:** None. **V. Bharmauria:** None. **A. Schütz:** None. **F. Bremmer:** None. **X. Yan:** None. **H. Wang:** None. **J.D. Crawford:** None.

Digital Abstract Session

P199. Sensorimotor Transformation: Neuroprocessing

Program #/Poster #: P199.03

Topic: D.08. Visual Sensory-motor Processing

Support: Grant # MOP-130444)
VISTA-Science to Applications

Title: Predictive integration of visual landmarks in the neural activity of the monkey frontal cortex

Authors: *V. BHARMAURIA[1], A. SCHÜTZ[2], H. WANG[1], X. YAN[1], F. BREMMER[2], J. D. CRAWFORD[1];
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Abstract: A major goal of the brain is to predict future events and it does so by constantly updating internal models of the environmental inputs, thus keeping us prepared for future action. Can the brain incorporate visual landmarks predictively in the gaze system and what are the neural underpinnings thereof? To investigate this question, we recorded the behavior and concurrent neural activity from two head unrestrained monkeys (*Macaca mullata*) in two important gaze areas, the frontal (FEF) and supplementary eye fields (SEF), during a cue-conflict delayed memory task. In this task, the cue-conflict was realized by a quasi-predictably shifting landmark (it shifted after a mask) during delay. Recently, in this task, in these two areas we documented that the landmark shift influenced the memory and motor activity [1,2]. In this study, we first analyzed the behavior and then all spatially tuned neurons recorded in the FEF (n = 147) and SEF (n=68). Due to the cue-conflict and quasi-predictable nature of the paradigm, we assumed the behavior as well as the neuronal activity to be driven by three components — 1) egocentric and allocentric memory/information, 2) prediction and 3) reward. To investigate this hypothesis, we modelled the behavior as the result of a random process based on these three components. We found that the behavior was best described by integration of all three components. We then fit neural response fields (RFs) against a target-to-gaze (T-G) continuum in eye-coordinates and tracked their evolution in the spatiotemporal domain (from visual response onset until the mask-off/landmark-on). A sudden predictive shift (before mask-on) toward gaze was noticed in the SEF but not the FEF responses. Collectively, these results along with our previous findings [1,2] suggest that in the gaze system the SEF provides control signals to the FEF for the final gaze command. First, the SEF detects the quasi-predictive information (while weighing the future reward) in its neural activity and then relays it to the FEF for incorporating the final gaze command through closely knit visual-visuomotor-motor circuitries between both areas with opposing roles.REFERENCES1.Bharmauria, V. *et al.* Spatiotemporal coding in the macaque supplementary eye fields: landmark influence in the target-to-gaze transformation. *bioRxiv* 2020.06.25.172031 (2020). doi:10.1101/2020.06.25.1720312.Bharmauria, V. *et al.* Integration of eye-centered and landmark-centered codes in frontal eye field gaze responses. *Cereb. Cortex* **bhaa090**, https://doi.org/10.1093/cercor/bhaa090 (2020).

Disclosures: V. Bharmauria: None. **A. Schütz:** None. **H. Wang:** None. **X. Yan:** None. **F. Bremmer:** None. **J.D. Crawford:** None.

Digital Abstract Session

P199. Sensorimotor Transformation: Neuroprocessing

Program #/Poster #: P199.04

Topic: D.08. Visual Sensory-motor Processing

Support: NIH Grant EY007023
NIH Grant EY028219
NIH Grant R00 MH112855
NIH Grant F31 EY031259

Title: Prefrontal cortical inhibition of innate orienting responses of the superior colliculus in perceptual choice

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Abstract: The natural world contains stimuli as varied and complex as the possible actions we can take to interact with them. While reflexive processes are sufficient for selecting responses to passively salient stimuli, competing environmental cues necessitate a means for selective control over perceptual choices in accordance with internal goals. While the prefrontal cortex has been broadly implicated in modulating perceptual processes, it is yet unclear how its control signals selectively drive or suppress the activity of downstream structures to obtain specific behavioral outcomes. Emerging evidence finds that one subdivision of the prefrontal cortex, the anterior cingulate cortex (ACC), plays a critical role in coordinating visually driven behaviors. Its projections to the superior colliculus (SC), a key midbrain region well known for its role in visuomotor action selection, provide a candidate pathway to investigate circuit mechanisms for the top-down modulation of goal-oriented choices. We have established a task that requires mice to move a trackball left or right to report the location of a visual cue. Using the DeepLabCut algorithm, we have identified an analogous forepaw-to-body relationship during spontaneous turning in freely moving mice and ball rotations in head-fixed mice. These findings support the validity of this task design as a model to study orienting behavior in head-fixed mice. Using this task, we previously found a role for the ACC and SC in mediating visuomotor responses to individually presented stimuli. We have now examined the relative contributions of these structures to perceptual decisions and action selection. Mice were simultaneously presented with a target and distractor cue of differing luminance, necessitating a categorical judgement and response. This behavioral design allowed us to identify the contributions of the ACC, SC, and ACC-SC projections to specific components of the resulting psychometric function. Our optogenetics results so far suggest that the SC and ACC play opposing but complementary roles in this task. We find that the SC promotes contraversive orienting in both trained and untrained mice, without affecting the likelihood of a response. While areal inactivations reveal a complex role for the ACC in several aspects of perceptual decision-making, we find that the ACC-SC projection specifically inhibits the innate function of the SC during this behavior to bias action selection in a stimulus-dependent manner. Our findings suggest that the prefrontal cortex exerts top-down inhibitory control over collicular orienting circuits to facilitate the selection of specific actions mediating perceptual choice.

Disclosures: K. Cruz: None. **R. Huda:** None. **A. Sullins:** None. **M. Sur:** None.

Digital Abstract Session

P200. Visual Motion

Program #/Poster #: P200.01

Topic: D.07. Vision

Title: Feedback and Feedforward Model in Motion Anticipation

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Abstract: To produce timely responses, animals must conquer delays from visual processing pathway by predicting motion. Previous studies [1] revealed that predictive information of motion is encoded in spiking activities of retinal ganglion cells (RGCs) early in the visual path. In order to study the predictive properties of a retina in a more systematic manner, stimuli in the form of a stochastic moving bar are used in experiments with retinas from bull frogs in a multi-electrode system. Trajectories of the bar are produced by Ornstein-Uhlenbeck (OU) processes with different time correlations (memories) induced by a butter-worth low-pass filter with various cut-off frequencies. We then investigated the predictive properties of single RGC by calculating the time shifted (δt) mutual information (MI(x,r;δt)) between spiking output (r(t)) from RGCs and the bar trajectories (x(t)). Intuitively, the peak position of MI(δt) is typically negative when considering the processing delay of the retina. Our measured peak positions of MI(δt) for some RGCs were characterized by both positive and negative peak position under low-pass OU (LPOU) stimulus. This finding indicates that some RGCs (P-RGCs) are predictive while the others are non-predictive (NP-RGCs). For LPOU with various correlation times, the MI peaks from the P-RGCs are positively correlated with the correlation times of the stimuli while those from the NP-RGCs are always around a fixed negative number (-50ms). In order to further understand the mechanism of prediction, we develop a negative group delay model which is based on Voss's [2] paper to generate anticipative responses. We extend our model to one dimension and use the same stimulation condition as we use in experiments. The model indicates that delayed negative feedback is crucial for producing MI(x,r;δt) similar to those observed in experiments. Besides, we also show feedforward inhibition can also generate similar prediction dynamics. To sum up, we presume horizontal cells' feedback inhibition and amacrine cells' feedforward inhibition may participate in this prediction phenomenon. Reference:1. Palmer SE, Marre O, Berry MJ, Bialek W. Predictive information in a sensory population. PNAS. 2015, 112(22).2.Voss, Henning U. "Signal prediction by anticipatory relaxation dynamics." Physical Review E 93.3 (2016): 030201.

Disclosures: **K. Chen:** None. **Q. Lin:** None. **C. Chan:** None.

Digital Abstract Session

P200. Visual Motion

Program #/Poster #: P200.02

Topic: D.07. Vision

Title: Modeling facilitation and suppression of motion perception in humans in the single moving stimulus design for two possible opposite motion directions

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Abstract: Human perception of a single moving pattern is related to the motion sensitive area V5. Specifically, size and contrast of the stimulus give counter intuitive results (Tadin et al., Nature, 2003, 424:321-315): at low contrast increasing size decreases the amount of stimulus presentation for correct direction discrimination, while at high contrast when increasing size people need longer presentations. It is attributed to the center-surround structure of motion sensitive neurons coding direction, contrast, and size. Models were proposed in the literature but their theoretical foundations and derivations are unsatisfactory (Tzvetanov, 2018, biorxiv-465807). Here, these foundations are clearly stated, i.e. the low-level neuronal responses coding all stimulus dimensions (direction, contrast (c), size (s), presentation time (t_{stim})) with two possible variants of excitatory/inhibitory pooling (subtractive or divisive inhibition), and the decision stage part (signal detection theory and drift-diffusion model). They are written as (Equation 1) with R_0-noise level, E()-excitatory and I()-inhibitory drives, S()-transducer, σ-constant, N()-normal distribution of unit variance, A-DDM's decision boundary height, and R^+() and R^-() the neuronal population responses with opposite preferred directions of motion. With a simple assumption (Equ.2), perceptual thresholds are derived for each case (Equ.3; $C_{decision}$-constant). The equations show that, if the mathematical functions for excitatory and inhibitory drives are the same for both low-level variants, these two variants can predict exactly the same thresholds. Thus, this experimental design cannot dissociate between the two theoretical assumptions. In addition, experimental data allow to discard some model parametrization as unrealistic. By using published functions for $E(c,s)$ and $I(c,s)$, it is demonstrated how the equations can be used to extract quantitative parameters from published psychophysics data, and how they correctly match corresponding neurophysiological reports of motion sensitive neurons.

$$(Equ.1)\begin{cases} R_{sub}(c,s,t_{stim}) = R_0 + S\Big(E(s,c,t_{stim}) - I(s,c,t_{stim})\Big) \\ R_{div}(c,s,t_{stim}) = R_0 + E(s,c,t_{stim})/\big(\sigma + I(s,c,t_{stim})\big) \\ P_{SDT}(+|c,s,t_{stim}) = \int_0^{+\infty} N\left((R^+ - R^-)\,/\left(\sqrt{Var(R^+) + Var(R^-)}\right)\right) dx \\ P_{DDM}(+|c,s,t_{stim}) = 1/\left(1 + \exp\left(-2A(R^+ - R^-)\right)\right) \end{cases}$$

$$(Equ.2)\begin{cases} R(t_{stim}) \propto t_{stim}^n/(t_{stim}^n + t_{50}^n) \\ E(s,c,t_{stim}) = R(t_{stim}) \times E(s,c) \\ I(s,c,t_{stim}) = R(t_{stim}) \times I(s,c) \end{cases}$$

$$(Equ.3)\begin{cases} \text{Subtractive inh.} : t_{stim}^{thr} = t_{50}\left(\frac{C_{Decision}}{E(c,s) - I(c,s) - C_{Decision}}\right)^{1/n} \\ \text{Divisive inh.} : t_{stim}^{thr} = t_{50}\left(\frac{\sigma C_{Decision}}{R_{exc}(c,s) - C_{Decision} R_{inh}(c,s) - \sigma C_{Decision}}\right)^{1/n} \end{cases}$$

Disclosures:

Digital Abstract Session

P200. Visual Motion

Program #/Poster #: P200.03

Topic: D.07. Vision

Support: NEI grant RO1EY018839
NIH grant T32NS099578
NIH grant 1R01EY027023-01A1
NIH grant 5 R90 DA 033461-08
Center Core Grant for Vision Research P30 EY01730
NIH/ORIP Grant P51 OD010425 to the WaNPRC

Title: A single neuron correlate for long-range motion in ventral visual area V4

Authors: *A. BIGELOW, T. NAMIMA, T. KIM, W. BAIR, A. PASUPATHY;
Univ. of Washington, Seattle, WA

Abstract: When a stimulus jumps intermittently across the visual field with large spatial (dX) and temporal (dT) steps, it induces a strong illusory motion percept known as long-range apparent motion. However, direction selective neurons in visual cortical areas V1 and MT are strikingly insensitive to the direction of this long-range motion. Psychophysical studies have argued that long-range apparent motion perception relies not on dorsal stream areas but instead on ventral stream areas dedicated to higher-order object tracking with large windows of spatiotemporal integration. Such an object-tracking process may be critical for the perception of object motion behind occluders, commonly seen in natural vision. To test the hypothesis that ventral stream areas are involved in processing long-range motion, we studied the responses of single neurons in primate area V4, an intermediate stage in the ventral visual pathway, known to be strongly inter-connected with dorsal stream visual areas MT and LIP. Our stimulus set included Gabor (sinusoidal) and Julesz (binary white noise) patches that were intermittently displaced across the visual display (dX > 0.5 deg; dT = 100 ms) in 8 directions at 45-degree increments to induce long-range motion percepts. The Gabor stimuli were either static or included local drift in the same or opposite direction as the long-range motion signal. We found that ~30% of V4 neurons (33/115), showed tuning for long-range apparent motion using a standard direction index (DI) metric (DI $\geq$ 0.5). When a range of step sizes were tested, spanning short to long-range motion, a larger fraction of V4 neurons (35/83; ~42%) exhibit direction selectivity. Many of these neurons are surprisingly insensitive to drifting grating stimuli presented within a static aperture. In control experiments, we found that tuning for long-range motion direction was consistent across positions within a single RF and consistent between equiluminant colored bars and Julesz patches, both being second order motion stimuli. To further characterize V4 direction selectivity in terms of spatiotemporal integration, we varied dT while keeping dX constant, i.e., systematically increasing the stimulus speed. We found compelling evidence in a subset of neurons that motion direction selectivity emerges based on integration over a fixed duration of time. Our results identify the first single neuron correlate of long-range

motion in the primate visual system and they support the hypothesis of a complementary motion system in the ventral stream that relies on second-order cues and large spatiotemporal windows for tracking objects in natural vision.

Disclosures: **A. Bigelow:** None. **T. Namima:** None. **T. Kim:** None. **W. Bair:** None. **A. Pasupathy:** None.

Digital Abstract Session

P200. Visual Motion

Program #/Poster #: P200.04

Topic: D.08. Visual Sensory-motor Processing

Support: NIH Grant NS103774

Title: Visual processing circuits in a basal chordate

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Abstract: To date, there are only two completed connectomes; the most recent one being from the tunicate Ciona tadpole larvae. The simple Ciona larval central nervous system (CNS) has a common structure with those of vertebrates, its close chordate relatives, and although it only has 177 neurons, it elicits complex behaviors. Ciona larvae have two distinct photomotor behaviors, negative phototaxis and a looming shadow response. These two behaviors are controlled by two distinct neural circuits arising from the group I and group II photoreceptors (PR-I and PR-II), respectively. The objective of this study is to further assess the minimum visual circuits predicted from the connectome with behavioral assays, mutant lines, and pharmacology. The PR-Is are glutamatergic and detect the direction of light. The PR-Is project to two groups of interconnected relay neurons (RNs): the cholinergic prRNs, which project to secondary interneurons and then to motor neurons, and to the GABAergic pr-AMG RNs. The PR-IIs are GABAergic and are responsible for detecting changes in ambient light. They project to pr-AMG RNs to evoke swimming, likely through a disinhibitory pathway. We find that the magnitude of the looming shadow and negative phototaxis responses, as measured by swim time, follows the rules of fold-change detection (FCD), rather than absolute light levels. The response to light-dimming exhibits other characteristics of FCD, including absolute adaptation, and fold-change-dependent response times. We hypothesize that all, or most, of the fold-change transformation of visual input occurs at the level of the RNs, as the interconnections between the excitatory prRN and the inhibitory pr-AMG RN appear to match known FCD circuits. The PR-I circuit forms an incoherent feedforward loop, while a nonlinear integral feedback loop is present in the PR-II circuit. As further evidence for the processing roles of these circuits, we observed that pharmacological alteration of the modulator neurons in the circuit resulted in disrupting FCD,

but not the overall response. The proposed FCD circuits are found in the posterior brain vesicle (pBV), a brain region we hypothesize has homology to the vertebrate midbrain. The Ciona pBV expresses a suite of genes associated with the vertebrate midbrain, and is situated anteriorly of a region of the brain previously hypothesized to be homologous to the vertebrate hindbrain. Functionally, the pBV appears to be similar to the vertebrate midbrain as it is the primary recipient of input from the photoreceptors. In summary, the simple Ciona larval CNS may provide insight into ancestral chordate sensory processing circuits and the evolution of the midbrain.

Disclosures: **C. Borba:** None. **M.J. Kourakis:** None. **S. Schwennicke:** None. **E. Newman-Smith:** None. **W.C. Smith:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.01

Topic: E.01. Eye Movements

Support: H2020 European Research Council (imove 755745)
Human Frontier Science Program (CDA 00056)
Israel Science Foundation (38017)

Title: Frontal eye field activity is correlated beyond the similarity in the response to behavior

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Abstract: Neuronal activity, as well as behavior, varies even when attempting to repeat the same action. These trial-by-trial fluctuations in firing rates are correlated between neurons. The fraction of correlated activity in motor areas that contributes to behavioral variability depends on the properties of the decoding of the neural state into action. We used a novel method to estimate the contribution of correlations in the frontal eye field (FEF) to pursuit eye-movements in monkeys. We applied a sequence of shuffles to the neuronal response. In the first shuffle in the sequence, we match each trial with the most behaviorally similar trial. In each step in the sequence, we loosened the constraints, allowing trials to be matched with increasingly distant trials. We then calculate the correlation between the activity of one of the neurons with the activity from a second neuron shuffled according to the behaver similarity. We found that although neurons in the FEF covaried in behaviorally similar trials, the correlations were strongly reduced when applying even the most constrained shuffle. Thus, only a small fraction of FEF correlations affect the behavior. Model simulations validate our approach, showing that our method can estimate the effect of correlations on behavior. Furthermore, our method has relatively modest assumptions and is general for different FEF model. We use the simulations to show that the reduction in correlation could be caused by downstream noise or the decoder of FEF activity.

Disclosures: **N. Larry:** None. **M. Joshua:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.02

Topic: E.01. Eye Movements

Support: NSF Grant 1656882

Title: Signals from parietal area 5 to superior colliculus for coordination of gaze with strides

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Abstract: The parietal cortex receives both visual and movement-related information, and is involved in the control of limb movements, including accurate steps during locomotion. The activity of parietal area 5 neurons projecting to the pyramidal tract changes when the animal steps over an obstacle (Andujar et al. 2010), and lesions in the area compromise step accuracy (Lajoie & Drew 2007). Accurate steps on a complex terrain rely on visual information about it, and gaze behavior is coordinated with strides (Matthis et al. 2018; Zubair et al. 2019). The spinal locomotor CPG plays a significant role in the coordination (Combes et al. 2008), and vestibular signals resulting from head movement during locomotion also contribute. The contribution of the brain's visual and motor centers to the coordination of the gaze with strides remains poorly understood. In this study, we investigated the activity of parietal area 5 neurons projecting to the rostral pole of the superior colliculus, the midbrain center for gaze control, as the cat walked on a flat surface and horizontally placed ladder. Walking on the flat surface does not require vision, while accurate stepping on the ladder does. Comparing neuronal activity between these two tasks allows identification of components of the activity that are related to gaze control and visual processing vs. limb movement. We investigated the activity of 30 neurons projecting to the superior colliculus (a5-SCs) and thus presumably contributing to the gaze control; the activity of 33 pyramidal tract projecting neurons (a5-PTNs), which presumably contributing to the control of limbs; and the activity of 130 unidentified neurons (noIDs), all recorded in cortical layer V of the same or neighboring microelectrode tracks through the most rostral area of the suprasylvian gyrus. We found that, as reported earlier for area 5 noIDs (Beloozerova & Sirota 2003), the activity of the majority of a5-SCs and a5-PTNs was step-related. On the flat surface, the depth of the activity modulation was higher in a5-PTNs. Upon transition to the ladder, most neurons in all groups changed one or more of: the discharge rate, depth of its modulation, stride phase distribution. The a5-SCs, however, changed the distribution of the activity much more often than the a5-PTNs, and as a group, increased the depth of the activity modulation. This indicated that the activity of a5-SCs on the ladder was significantly influenced by visual information. We concluded that a5-SC neurons may contribute to the coordination of gaze with limb locomotor

movements by conveying to the superior colliculus visual information from the cortex that is superimposed on the locomotion rhythm-related discharge.

Disclosures: W.U. Nilaweera: None. **I.N. Beloozerova:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.03

Topic: E.01. Eye Movements

Support: Vista

Title: Mechanisms for integrating allocentric and egocentric information for goal-directed movements: a neural network approach

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Abstract: Numerous studies have shown that allocentric and egocentric information are combined for goal-directed movements toward visual targets. Specifically, behavioural studies suggested that this combination follows Bayesian integration; e.g. decreasing the stability of visual landmarks decreased the reliance on allocentric information (Byrne et al., 2010; Klinghammer et al., 2017). Additionally, neural recordings found landmark-specific coding in supplementary and frontal eye fields when monkeys performed saccades toward a remembered visual target (Schütz et al., 2020; Bharmauria et al., 2020). Nevertheless, the intrinsic coordinate representation and underlying processes for this combination remain a puzzle, mainly due to inadequacy of current theoretical models to explain data at different levels (i.e. behaviour, single neuron and distributed networks). In response, we aim to build a theoretical framework to tackle this challenge. In particular, we propose a physiologically inspired neural network with two major components. First, a Convolutional Neural Network (CNN) is used to extract the allocentric and target information: Our CNN performs repeated (2 layers) convolution, rectifications, and normalization to first extract low-level features from input images. With the features extracted, the challenge is to combine them to generate an abstract representation (allocentric cues and target position). We address this challenge by training a feature pooling layer at the end of our CNN network. Second, a Multi-Layer Perceptron network (MLP) is used to combine allocentric (extracted feature maps from the CNN) and egocentric information (initial gaze position fed to the network as an additional input): Our MLP consists of three fully connected hidden layers. These three layers incrementally transform the allocentric and egocentric representations into an integrated motor response. Finally, following the MLP, an additional layer transforms motor responses into final gaze positions. We were able to train these

physiologically inspired networks to achieve good correspondence with a test dataset (MLP: R^2 = 0.94). Additionally, the activity of hidden layer units in the MLP was similar to experimentally recorded neural response fields in monkeys (Bharmauria et al. 2020) and was modulated by varying landmark positions. These results suggest that our framework provides a suitable tool to study the underlying mechanisms of allocentric and egocentric integration.

Disclosures: P. Abedi Khoozani: None. **V. Bharmauria:** None. **A. Schütz:** None. **R. Wildes:** None. **D. Crawford:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.04

Topic: E.01. Eye Movements

Support: Giorgio Sanna Memorial Scholarship
Helen Bader Age & Community Scholarship
University of Wisconsin-Milwaukee Student Research Award

Title: Visual feedback and attentional deficits are associated with altered visual strategy during a pinch force-steadiness task in some older adults

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Abstract: Introduction. Visual information processing and associated eye movements may play a critical role in age-related dexterous impairments, yet no comprehensive analysis to date has evaluated the effect of visual strategies and attentional capabilities on changes in manual dexterity. The purpose of this study was to determine the relations among visual strategies associated with altered visual feedback, attentional deficits, and decreased force steadiness in older adults. **Methods.** Eye movements were recorded from 21 young (age 20 - 38 years; 11 female, 10 male) and 21 older (age 65 - 90 years; 11 female, 10 male) adults during a submaximal pinch force-steadiness task while viewing force feedback with higher and lower gain and while performing a visuospatial dual task. Participant's force appeared as a line that moved left to right across the screen relative to a horizontal target line. Performance on standardized tests of attention (The Test of Everyday Attention and Trail Making Test) was also measured. **Results.** All participants gazed near the target line and used a series of saccades from left to right during the higher and lower gain conditions without the visuospatial task. Older adults made fewer saccades than young adults (23.6 ± 4.4 and 21.0 ± 2.9 saccades, respectively) and all made fewer saccades during the higher vs. lower gain conditions (23.7 ± 3.5 and 20.9 ± 4.0 saccades, respectively). Most participants used the same visual strategy when simultaneously performing the dual task though a subset of older adults (n = 7) used an altered visual strategy;

gaze did not stay near the target line and did not travel exclusively left to right. Performance on the standardized measures of attention was impaired in this subset compared to older adults who did not use the altered visual strategy. **Conclusions**. Results demonstrate that the amount of visual feedback influences visual strategy during force-steadiness tasks in older adults and reveal unique eye movement patterns in a subset of older adults when allocating attention across multiple tasks. Given that this subset demonstrated impaired performance on measures of attention, this may be a maladaptive visual strategy used by older adults performing near their attentional capacity. Future work could examine why some older adults use an altered visual strategy and whether this could serve as an indicator of motor and/or cognitive impairments in older adults and other patient populations.

Disclosures: B. Heintz Walters: None. **W.E. Huddleston:** None. **K. O'Connor:** None. **J. Wang:** None. **M. Hoeger Bement:** None. **K.G. Keenan:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.05

Topic: E.01. Eye Movements

Support: JSPS Grant 20H04286 to YH
JSPS Grant 18KK0286 to YH

Title: Evaluation of vocabulary acquisition by pupillary response

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Abstract: Vocabulary learning is one of the most crucial elements for second language (L2) acquisition. A general method for measuring a leaner's vocabulary size and learning achievement is a form-meaning matching test. Recent studies demonstrated that pupil sizes of L2 learners change during retrieval of paired associations from Hungarian to English (Pajkossy et al, 2019). This line of evidence suggests that the pupil can be used to evaluate vocabulary comprehension of L2 learners as an alternative to general exams. Presently, we pursued this possibility by measuring pupillary responses of L2 learners when they were learning new words. Fourteen L2 learners (12 males, 2 females) with normal or corrected vision participated in this study. Their first and second languages were Japanese and English, respectively. We selected 30 English nouns whose meanings were unknown and Japanese meanings can be written with two Japanese words. The experiment consisted of 3 parts: before (Control), during (Study), and after learning (Test) that were repeated 5 times. In Control trials, the 30 English words were presented for 3 sec in a random order on a PC monitor. Participants were asked to look at the word without blinking. A 3-sec gap was given between successive word presentations during which blinking was allowed. In Study trials, each of the 30 Japanese words was presented in random order for 3 sec, followed by the corresponding English word for 3 sec. Participants were instructed to memorize

the Japanese meaning of the English word. A 3-sec gap was given between Japanese and English word presentations. In Test trials, the 30 English words were randomly presented for 3 sec during which participants tried to retrieve their Japanese meanings. After this period, they answered verbally. A 5-sec gap was given between successive word presentations. We measured participants' pupil diameters using EyeSeeCam (EyeSeeTech, Germany) at a sampling rate of 220 Hz while English or Japanese words were presented for 3 sec in all the three parts. The results demonstrate that significantly greater pupil dilation was observed while participants viewed the English words they were failed to remember than those successfully remembered in both the Study and Test phases. Interestingly, in Control trials, greater dilation responses were observed while the participants were seeing the English words whose meanings they acquired with less repetition (< 2 times) than for those that took more repetition (> 4 times) to learn, before even knowing their Japanese meanings. These results support the idea that quantitative evaluation of pupillary responses may provide useful information on L2 learners vocabulary comprehension.

Disclosures: **T. Hirata:** None. **K. Mishima:** None. **K. Fukaya:** None. **Y. Hirata:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.06

Topic: E.02. Cerebellum

Support: NIH NS088567
Roy J. Carver Charitable Trust Bipolar Disorder Research Program of Excellence

Title: Internally and externally driven oscillations in anterior cingulate, amygdala and cerebellum during acquisition of trace eyeblink conditioning in rats

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Abstract: Eyeblink conditioning is a simple form of associative learning where a conditioned stimulus (CS), e.g., a tone) is repeatedly paired with an unconditioned stimulus (US, e.g., a periorbital shock) to yield a cerebellum-dependent conditioned response. Cerebellar learning during trace eyeblink conditioning, when there is a temporal gap between the CS and US, requires mossy input from the CS and persistent mossy fiber input from the forebrain. The amygdala has also been shown to modulate cerebellar learning during eyeblink conditioning, through modulating mossy fiber inputs necessary for learning. The necessity of the serial circuit from forebrain to cerebellum for acquisition and expression of trace eyeblink conditioned responses has been well-established, however, dynamic network-based interactions with this serial circuit during learning have not been investigated. The current experiment used simultaneous tetrode recordings in anterior cingulate (right), amygdala (right) and anterior interpositus nucleus (left) during acquisition of trace eyeblink conditioning (250 ms tone/500 ms

trace interval) to investigate the evolution of dynamic interactions among these necessary structures during forebrain dependent cerebellar learning. Spectral analysis revealed changes in externally (CS/US) and internally (conditioned response) driven oscillations during the learning process. When aligned to external events (CS onset), spectral power increased across multiple frequencies during acquisition. Aligning oscillations to conditioned response onset revealed a large increase in power across multiple frequencies in parallel to the increase in behavioral responding across sessions. Aligning oscillations to the US also suggests a dynamic interaction throughout the learning process. These results suggest network based interactions in oscillatory activity may be facilitating the serial circuit from forebrain to cerebellum necessary for trace conditioning.

Disclosures: H.E. Halverson: None. **J. Kim:** None. **J.H. Freeman:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.07

Topic: E.02. Cerebellum

Support: JSPS Grant 20H04286 to YH
JSPS Grant 18KK0286 to YH

Title: Distributed memory consolidation of vestibulo-ocular reflex gains acquired by a frequency competitive visual-vestibular mismatch paradigm

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Abstract: The vestibulo-ocular reflex (VOR) stabilizes our vision during head movements by counter-rotating the eyes in the orbit. It has been a popular model system to study cerebellar roles in motor control and learning. VOR gain, defined as eye velocity divided by head velocity in the dark, can be modified by applying a visual-vestibular mismatch paradigm in which head and visual pattern stimulations are rotated at the same frequency in either the same (gain-down) or opposite (gain-up) directions. In both cats (Kassardjian et al., 2005) and monkeys (Anzai et al., 2010) it has been shown that after a short-term (<2 hours) VOR gain-down training the modified memory is temporally stored in the cerebellum and long-term (>3 days) training is consolidated in the vestibular nucleus. By using short-term frequency competitive visual-vestibular mismatch paradigms in which head and visual pattern rotations were presented in-phase at one frequency and anti-phase at the other it was demonstrated in both monkeys (Hirata et al., 2002) and goldfish (Soga et al., 2020) that VOR gains could be modified in opposite directions. Computationally, this form of motor memory requires frequency dependent nonlinear signal transformations that are possible in the cerebellar cortical neural network (Inagaki and Hirata, 2017), but such consolidation for long-term memory might not occur in the vestibular

nucleus with an elementary network structure than the cerebellum. Herein, we explored long-term memory of oppositely modified VOR gains by training 12 goldfish for 2 hours a day successively for 7 days with a frequency competitive paradigm of gain-up at 0.5Hz and gain-down at 0.1Hz. We found that most of the VOR gains at both frequencies were modified after 2-hour daily trainings, but were lost by the next day; however, fractions remained and accumulated gradually for 7 days resulting in significant simultaneous gain-up at 0.5Hz and gain-down at 0.1Hz. We conducted cerebellectomy on day-7 after 2-hour daily training in 6 animals, and found the increased gain-up at 0.5Hz to decrease back to the daily pre-training value. The modified gain-down increase at 0.1Hz was not only lost, but also it exceeded the daily pre-training and day-1 values settling close to the cerebellectomized gain-up at 0.5Hz. These results indicate that long-term memory of frequency competitive VOR motor learning can be consolidated with the increased VOR gain stored outside, and that decreased stored within, the cerebellum. Thus, the gain-down changes are actually much larger because they need to counteract the gain-up shift stored outside of the cerebellum.

Disclosures: **R. Baker:** None. **T. Ikeda:** None. **Y. Hirata:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.08

Topic: E.02. Cerebellum

Support: NIH R01 DC004154
NIH R01 NS072406
Stanford Interdisciplinary Graduate Fellowship

Title: Heterogeneous neural correlates of short-term oculomotor learning in cerebellar Purkinje cells

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Abstract: The oculomotor cerebellum provides a powerful system for analyzing how information processing in a neural circuit changes over the course of learning. During adaptive calibration of the gain of the vestibulo-ocular reflex (VOR), observed changes in the response of Purkinje cells in the cerebellar flocculus are thought to contribute to the learned increase or decrease in the eye movement response to a vestibular stimulus. Such changes have generally been studied over the course of hours to days, yet more recent work has shown that this neural correlate of learning may be observed over time scales as short as a single trial. We analyzed the changes in Purkinje cell responses in two rhesus monkeys during brief training periods of 60-90 seconds, focusing on the horizontal gaze velocity Purkinje cells (HGVPs), which have previously been implicated in VOR learning. 45 Purkinje cells were characterized as HGVPs based on their

responses during sinusoidal smooth pursuit and VOR cancellation: these cells increased their firing rate during smooth pursuit when the eyes moved ipsiversively and the head remained fixed, and during VOR cancellation when the head moved ipsiversively while the eyes remained nearly stationary on a target that moved with the head. To induce learning, vestibular stimuli consisting of pulses of rapid acceleration of the head to a constant velocity were paired with a visual stimulus that moved exactly with the head (VOR-decrease training) or exactly opposite the head (VOR-increase training). We analyzed spiking during the first 100 ms after the onset of acceleration in each trial, the period before visual feedback influences the eye movement response. We found unexpected heterogeneity in the baseline (pre-learning) responses of the HGVPs, with some cells increasing their firing and others decreasing their firing in response to the same visual-vestibular training stimulus. This suggests that there are subclasses of Purkinje cells with different signal content within the classically defined population of HGVPs, whose responses are not well-captured by the standard classification method. Additionally, cells that exhibited different baseline responses to a visual-vestibular training stimulus showed different learned changes in this response over the course of 60-90 sec of training. Contrary to the hypothesis that HGVP cells contribute to oculomotor performance and learning in a uniform fashion, our results suggest that HGVP cells carry different signals within the VOR circuit, as individual cells systematically respond differently even under the same training conditions.

Disclosures: A. Kirjner: None. **B.J. Bhasin:** None. **J.L. Raymond:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.09

Topic: E.05. Brain-Machine Interface

Support: DARPA & ARO Grant W911NF-17-1-0022
AS: Bridge-to-Doctorate

Title: Assessment of functional benefits afforded by a sensory-enabled prosthesis to an individual with upper-limb amputation

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Abstract: Commercially-available prostheses are limited in their ability to provide users with direct sensory feedback, which results in increased demands on vision to perform daily tasks and poor embodiment of the prosthesis. To address these limitations, we have developed the Neural Enabled Prosthetic Hand (NEPH) system to provide task-relevant sensations to upper-limb prosthesis users. The system delivers stimulation via implanted electrodes/electronics that are wirelessly controlled by prosthesis-mounted sensors and electronics. With sensory feedback,

users may be afforded the benefit of graded control to confidently perform daily functional tasks and an improved ability to manipulate objects with reduced attentional demands. The first subject enrolled in the first-in-human clinical trial of the NEPH system (NCT 03432325) underwent surgery to install the implanted components, was fit with the external components, has undergone a series of tests, and has been using the system in real-world environments for more than two years. We used the Southampton Hand Assessment Procedure (SHAP) to compare the subject's performance of functional tasks under conditions with and without stimulation. The SHAP consists of three categories of functional tasks: 6 lightweight object grasping tasks, 6 heavyweight object grasping tasks and 14 activities of daily living (ADL) tasks. A self-timed assessment was conducted in which the subject performed each task using the NEPH system; once with stimulation-induced sensory-feedback and once without at three time points in the period of 18 months post-surgery. One ADL task (cutting while holding the knife with the prosthesis) was found to be extremely difficult for the subject to complete and was excluded from all statistical analyses. To assess the effects of stimulation on each day, we used a paired t-test on the completion times for each task. To investigate the effects within each task group and time post-surgery, we used a two-way repeated measures ANOVA with time (session number) and stimulation condition as factors. Results indicated that, across the full set of tasks, completion times were statistically lower with stimulation than without stimulation in the session at 18 months ($p=0.012$). Additionally, for the set of lightweight objects, performance was marginally improved ($p = 0.076$) with sensation, and performance marginally improved over the study time period ($p = 0.057$). These results suggest that the SHAP can be used to evaluate the benefits of sensory feedback to prosthesis users, however further investigation with more subjects is required to determine the true benefits afforded by a sensory-enabled prosthesis.

Disclosures: **A. Shell:** None. **D. Aguilar:** None. **B. Hillen:** None. **L. Rincon Gonzalez:** None. **J.J. Abbas:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Arizona State University. **R. Jung:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Florida International University.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.10

Topic: E.05. Brain-Machine Interface

Support: DARPA Contract N66001-15-C-4017
NSF Award CHS 1901236
NIH Award TL1TR002540
NIH Award UL1TR002538
NSF Award NCS-FO ECCS-1533649

Title: Combination training and latching filter improve activities of daily living and user experience with a bionic arm

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Abstract: This work compares continuous bionic arm control using performance-based and user-focused outcomes in real-world tasks. Advanced prostheses can restore function and improve quality of life for individuals with amputations. Unfortunately, most commercial control strategies do not fully utilize the rich control information from residual nerves and musculature. Continuous decoders can provide more intuitive prosthesis control from multi-channel neural or electromyographic recordings. Three components influence continuous decoder performance: the data used to train the algorithm, the algorithm, and smoothing filters on the algorithm's output. As individual groups often focus on a single decoder, very few studies compare different decoders using otherwise similar experimental conditions. Additionally, control improvements are often demonstrated with offline data or in virtual settings, which do not necessarily translate to real-world usage.

We completed a two-phase head-to-head comparison of 12 continuous decoders using activities of daily living. In phase one, we compared two training types and a smoothing filter with three algorithms (modified Kalman filter, multi-layer perceptron, and convolutional neural network) in a clothespin relocation task. We compared training types that included data with only individual digit and wrist movements vs. combination movements (e.g., simultaneous grasp and wrist flexion). We also compared raw vs. nonlinearly smoothed algorithm outputs. In phase two, we compared the three algorithms in fragile egg, zipping, pouring, and folding tasks using the combination training and smoothing found most beneficial in phase one. In both phases, we collected objective, performance-based (e.g., success rate) and subjective, user-focused (e.g., preference) measures.

Phase one showed that combination training improved prosthesis control accuracy and speed, and that the nonlinear smoothing improved accuracy but generally reduced speed. In phase two, user-focused metrics favored the convolutional neural network and modified Kalman filter, whereas performance-based metrics were generally similar among all algorithms.

These results confirm that state-of-the-art algorithms, whether linear or nonlinear in nature, functionally benefit from training on more complex data, and demonstrate that smoothing the output improves control. These studies will be used to select a decoder for a long-term take-home trial with implanted neuromyoelectric devices. Overall, clinical considerations may favor the mKF as it is similar in performance, faster to train, and computationally less expensive than neural networks.

Disclosures: M. Paskett: None. **M.R. Brinton:** None. **T.C. Hansen:** None. **J.A. George:** None. **T.S. Davis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); WO2018026842A1. **C.C. Duncan:** None. **G.A. Clark:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); WO2018026842A1, WO2018023026A1, 8359083, 8639312.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.11

Topic: E.05. Brain-Machine Interface

Support: NSF CHS Grant 1901236
NSF Grant 1533649
NIH 1 DP5 OD 029571-01
VA RR&D CDA1 1IK1RX002011-01A2

Title: A Self-Aware Bionic Hand that Autonomously Detects Nearby Objects and Dexterously Grasps Them with Minimal Force

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Abstract: Multiarticulate bionic hands are now capable of recreating the endogenous movements and grip patterns of the human hand. However, it is difficult to intuitively control the many degrees of freedom associated with these bionic hands, and up to half of upper-limb amputees abandon their prostheses due to limited and/or unintuitive control. One approach towards more dexterous and intuitive control is to create an autonomous self-aware bionic hand that can synergistically aid a human with complex tasks. To that end, we have developed a bionic hand that can automatically detect and grasp nearby objects with minimal force. We embedded four digits (D1 - D4) of the TASKA Hand (TASKA Prosthetics, Christchurch, New Zealand) with infrared and pressure sensors. The infrared sensors can detect objects within 5 cm and the pressure sensors can detect up to 50 N. Each digit is self-aware such that each digit independently and autonomously maximizes non-zero infrared intensity (i.e. proximity) while minimizing non-zero pressure. The ability to grasp nearby objects with minimal force uniquely enables the self-aware hand to autonomously complete dexterous tasks such as holding a fragile object. We evaluated performance using a fragile egg task in which the hand must transfer a 43.7 g object over a barrier while keeping compression forces below 6.7 ± 0.1 N (mean ± standard deviation). A healthy intact arm participant completed the task with 100% accuracy (no breaks) in 0.83 ± 0.11 s. The self-aware bionic hand was able to complete the task with 100% accuracy within 3.40 ± 0.17 s. In contrast, a naïve intact participant controlling the bionic arm with a state-of-the-art surface electromyographic (sEMG) control algorithm (Paskett et al., SfN Global Connectome 2021) was unable to complete the task (0% accuracy) and spent a significantly longer time attempting the task (11.67 ± 11.26 s; $p < 0.05$).The embedded sensors provide unique insight into the differences between human sEMG and machine control. There were no significant differences in the time taken to approach the object (human: 0.93 ± 1.43 s, machine: 1.66 ± 0.13 s; $p = 0.12$), but the machine applied significantly less pressure to the object (human:

~15.33 ± 7.36 mbar, machine: ~3.55 ± 0.50 mbar; $p < 0.0001$). For both metrics, the machine was also significantly more precise (smaller variance; $p < 0.0001$). These preliminary results suggest that self-aware bionic hands can operate at near-human speed with greater-than-human sEMG accuracy and precision. Future work involves sharing control between the human and the computer; we anticipate there will be an optimal level of shared control to maximize performance while still maintaining human autonomy.

Disclosures: **T.C. Hansen:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NSF CHS Grant 1901236. **J.L. Segil:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; VA RR&D CDA1 1IK1RX002011-01A2. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PCT/US2019/040724. **R. Patel:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; VA RR&D CDA1 1IK1RX002011-01A2. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PCT/US2019/040724. **R.F.F. Weir:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; VA RR&D CDA1 1IK1RX002011-01A2. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PCT/US2019/040724. **D.J. Warren:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NSF CHS Grant 1901236, NSF Grant 1533649. **J.A. George:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH 1 DP5 OD 029571-01.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.12

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01NS119160
NIH T32 5T32EB007509

Title: Optimizing and decoding limb stiffness for reaching with functional electrical stimulation

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Abstract: Functional Electrical Stimulation (FES) uses electrical stimulation to reanimate muscles that have been otherwise disconnected from the brain through injury or disease. This technology, when utilized in the upper limbs, gives paralyzed individuals the potential to perform reaching tasks which can significantly improve their independence and quality of life. During normal arm movements, people actively modulate the amount of 'cocontraction' or the degree to which opposing muscles are simultaneously active. Increasing cocontraction helps stiffen our limbs and makes them more resistant to external perturbations while decreasing cocontraction minimizes energy use and facilitates rapid movements. Optimizing the degree of cocontraction is particularly important when restoring arm function via FES because excess cocontraction can lead to muscle fatigue and faster battery drain. Conversely, too little cocontraction can lead to poorly controlled movements that are prone to perturbations from the environment. Our goal is to optimize ways to dynamically modulate cocontraction when controlling arm movements via FES. To that end we are using simulations of a virtual arm model where we can easily compare the pros and cons of various methods. Our current work demonstrates how an automated method of adjusting cocontraction based on decoded velocity and acceleration can improve arm control while balancing the need to conserve power and avoid fatigue.

Disclosures: T. Johnson: None. **D.M. Taylor:** None. **S. Moralle:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.13

Topic: E.05. Brain-Machine Interface

Support: NIH AT009263
MH114233
EB021027
NS096761
Bradford and Diane Smith Graduate Fellowship

Title: Control task cognitive load during continuous EEG neuro-robotic use affects cortical fatigue

Authors: *D. SUMA[1], J. MENG[1], B. J. EDELMAN[2], B. HE[1];
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Abstract: During our recent experimentation demonstrating continuous noninvasive electroencephalographic (EEG) brain computer interfacing (BCI) of both the one and two

dimensional control of virtual cursors and neuro-robotic arms, we observed distinct behavioral responses to the introduction of a physical device. To better understand the dynamics of and improve continuous noninvasive control, we investigated the interactions between task complexity, spatial and temporal cursor-target interactions and the indirect effects of the introduction of a physical interface within an extended human subject population using both multi-level ANOVAs and ANCOVAs. Nine healthy human subjects participated in five sessions of one-dimensional (1D) horizontal (LR), vertical (UD) and two-dimensional (2D) continuous neural tracking utilizing EEG. Here, subjects controlled either a robotic arm or a virtual cursor to continuously track a Gaussian random motion target via kinesthetic hand motor imagery (MI) induced sensorimotor rhythm modulation. Control signals were estimated via online source imaging of each subject's cortical activation using high density (128 Channel) EEG and subject specific regions and frequencies of interest. Sessions consisted of 24, one minute continuous tracking tasks, broken down into a variable number of LR, UD, and 2D tasks, split evenly and block-wise randomized across interface (robotic arm or virtual cursor). Within trial fatigue rates were estimated via the slope of the mean-squared error (MSE) corresponding to three 20 second non-overlapping windows. These fatigue rates were found to be lower for control tasks with lower cognitive demand (UD, both hands MI and rest) compared to those with higher cognitive demand (LR, left- and right-hand MI). Spatial error analysis emphasizing target location, cursor location and error direction revealed that a reduction in tracking quality during robotic arm use was contingent upon visual obfuscation of the target by the physical device. In fact, robotic arm performance was significantly superior to virtual cursor control when the users' visual field was unobstructed. These results emphasize the need to take into account the physical footprint of devices, training complexity, and the synergy within control strategies when designing interfaces for practical real-world control.

Disclosures: **D. Suma:** None. **J. Meng:** None. **B.J. Edelman:** None. **B. He:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.14

Topic: E.05. Brain-Machine Interface

Title: Decoding of eye movement trajectory by neuronal ensembles in cerebral cortex

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Abstract: The vast majority of BMI (brain-machine interface) research has focused on decoding arm movement signals in primary motor cortex to control external prosthetic devices. Despite recent progress in BMIs, questions remain about the recorded cortical signals and how best to translate them into movements. The eye has fewer degrees of freedom so, in principle, it should be easier to develop a BMI for dynamic motor control using neural signals from oculomotor or possibly visual cortex. Here, we investigate the plausibility of an oculomotor BMI by analyzing

the relationship between cortical activity and eye movement trajectories in behaving macaques. We recorded ensemble neuronal activity in the frontal eye fields (FEF) in prefrontal cortex and visual area MT via laminar probes while a male monkey tracked moving visual targets. Using multiple decoding algorithms as well as modern neural network methods, we show that eye movements can be accurately decoded with relatively high precision. In an example session with 69 simultaneously recorded neurons (45 FEF; 24 MT), a neural-network decoder accounted for 87% variance of eye position over time (correlation coefficient between decoded trajectories and data is 0.94, $p<0.001$), with an average position error of 0.84 degrees. The reconstructed eye motion trajectories were successfully reproduced on 84% of the trials with an error constraint of 6 degrees (compared to 3 degrees in behavior). Our results demonstrate the feasibility of a gaze BMI, identify an adequate cortical substrate for its implementation, and supply a novel framework for investigating questions in oculomotor control.

Disclosures: B. Liu: None. **J. Mayo:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.15

Topic: E.05. Brain-Machine Interface

Support: SNSF 197271 (MOVEIT)

Title: A Multimodal Neuroprosthesis: combining virtual reality and neurostimulation for treating sensory neuropathies

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Abstract: Because of sensory loss diabetic peripheral neuropathy (DPN) patients are prone to falls, incorrect balance/walking and consequent foot ulcers, which increase the risk of amputations. They also suffer neuropathic pain (pDPN), associable to the aberrant sensory inputs. The most urgent need for people with diabetic neuropathy is the recovery of correct sensations from the leg/foot and its proper brain integration, with consequent gait/balance amelioration and pain suppression. The aim of the study was to develop the first multimodal neuroprosthesis restoring close-to-natural foot sensations, through transcutaneous electrical nerve stimulation (TENS) and combining it with immersive visual digital technology delivered to the brain through the virtual reality (VR). We tested whether coherent multisensory visual-tactile neural stimulation (VTNS) would positively impact on gait, balance and pain of patients with the pDPN. Physiologically plausible stimulation targeting lower-limb nerve holds promise to restore sensory feedback that will improve balance and walking, and decrease periphery-induced pain. VR-based integration of a realistic foot representation will ameliorate the balance/walking and help in CNS-based component of pain. Combined, they should constitute the multimodal neuroprosthesis improving the overall origins of pPDN. It has never been proved

that the electrical stimulation of the lower limb diabetic nerve by arrays of the multi-pad electrodes could induce stable and reliable sensations from a diabetic foot with reduced sensitivity. Portable VR-based approach has been exploited in the pain and walking treatment of pDPN patients. Finally, experiments are performed to prove that this multimodal neurotechnology would trigger beneficial effects in the pDPN population. Virtual reality system was implemented and programmed with modes for the pain suppression and gait/balance training. The system was successfully tested with subjects suffering the pDPN. Our study showed: i) an effective device to achieve therapeutic and quality of life benefits for highly disabled pDPN subjects, who are currently affected by important functional and psychological problems ii) to augment the overall knowledge about the use of electrical stimulation and VR on in pDPN, iii) an assessment tool and rationale for the future extensive clinical study.

Disclosures: G. Valle: None. **G. Preatoni:** None. **L. Chee:** None. **S. Raspopovic:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.16

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Independent research fund, Denmark

Title: Viral targeting of inhibitory interneurons in the spinal cord of wild type rats using the mDlx promotor

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Abstract: So far, genetic targeting of inhibitory interneurons has primarily been viable in transgenic animals. In recent years, viral approaches have been extensively utilized to transport transgenes into the neurons. Moreover, they can infect various cell types in the nervous system and act as a versatile tool to avoid breeding expensive transgenic animals which are available for small number of species. It would be beneficial to have a promotor that targeting inhibitory interneurons in the wild-type animals. In fact, such a promotor, called mDlx, has been developed and tested in the forebrain. Nevertheless, it was not tested in the adult spinal cord, and so far, specificity is unknown for the spinal interneurons in the adult mammalian system. Here, we used the adeno associated virus (AAV) with mDlx promotor with green fluorescent protein (GFP) reporter to target inhibitory neurons in the lumbar spinal region of adult wild- type rats. Laminectomy was performed and the virus was injected with a glass capillary (with a fine tip) at the lumbar spinal region. 3-4 weeks later, the virus expression was investigated by perfusing the rats with paraformaldehyde and sectioning the spinal cords. The sectioned cords were immediately inspected under the fluorescent microscope to verify the expression of GFP. Immunohistochemistry was performed to test the specificity of the virus to inhibitory interneurons. We found that the mDlx enhancer primarily targeted GAD-65 expressing neurons

in the dorsal, central and ventral region of the adult spinal cord whereas very few if any GAD-67 neurons, which are abundant in laminae I-III were infected by mDlx. To contrast the expression of inhibitory neurons using mDlx enhancer, we also used AAV virus with CamKIIa promotor to infect excitatory and hSynapsin to target all the neurons in the adult lumbar spinal cord. We noticed that cells infected with the CamKIIa enhancer has shown colocalization with some GluA1 and VGluT1 neurons, indicating glutamatergic neurons whereas, hSynapsin has shown to target 90% neurons in the dorsal and about 95% neurons in the central and ventral spinal regions. Our targeting of specific inhibitory interneurons in the adult spinal cord of wild-type rats demonstrates the potential of using a viral strategy with mDlx promotor in the mammalian sensory-motor system.

Disclosures: J. Kaur: None. **R. Berg:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.17

Topic: E.07. Rhythmic Motor Pattern Generation

Support: CONACYT
VIEP-BUAP

Title: Internal-switches of a central pattern generator for scratching in the cat

Authors: *J. G. OJEDA;
Benemérita Univ. Autónoma De Puebla.

Abstract: Internal-switches of a central pattern generator for scratching in the cat
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The spinal neurons exhibit an ON-OFF and OFF-ON firing activity related to the central pattern generator (Cuellar et al., 2018, Front Cell Neurosci 12:68). However, this activity was only examined around the "postural stage" of fictive scratching, disregarding the firing behavior during and at the end of the scratching episodes. Here we extended such observations to include the analysis of the whole episodes. The experiments were performed in two decerebrate cats, following the National Institutes of Health Guide for the Care and Use of Laboratory Animals (85–23) and the Mexican regulations (NOM-062-ZOO-1999). We recorded the multiunit neuronal activity from the lumbar dorsal horn and the intermediate nucleus, as well as the electroneurographic activity from the flexor and extensor nerves. A spike sorting software was employed to select the unitary spikes from 743 neurons. The scratching episodes were divided into three stages: "before," "during," and "after." We found the following firing patterns associated with the whole scratching episodes at those stages: 1) ON-OFF-ON, 2) OFF-ON-OFF, 3) ONstart-OFF-ONend, 4) OFFstart-ON-OFFend, 5) ON-OFF-OFF, and 6) OFF-OFF-ON. Because these patterns resemble "switches" or "gates" turning ON or OFF the flow of firing

electrical activity, we suggest that they could be useful to develop new models of neural computation for the central pattern generator.

Disclosures:

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.18

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Local

Title: Dorsal root site activated by nmda triggers limb pattern generators

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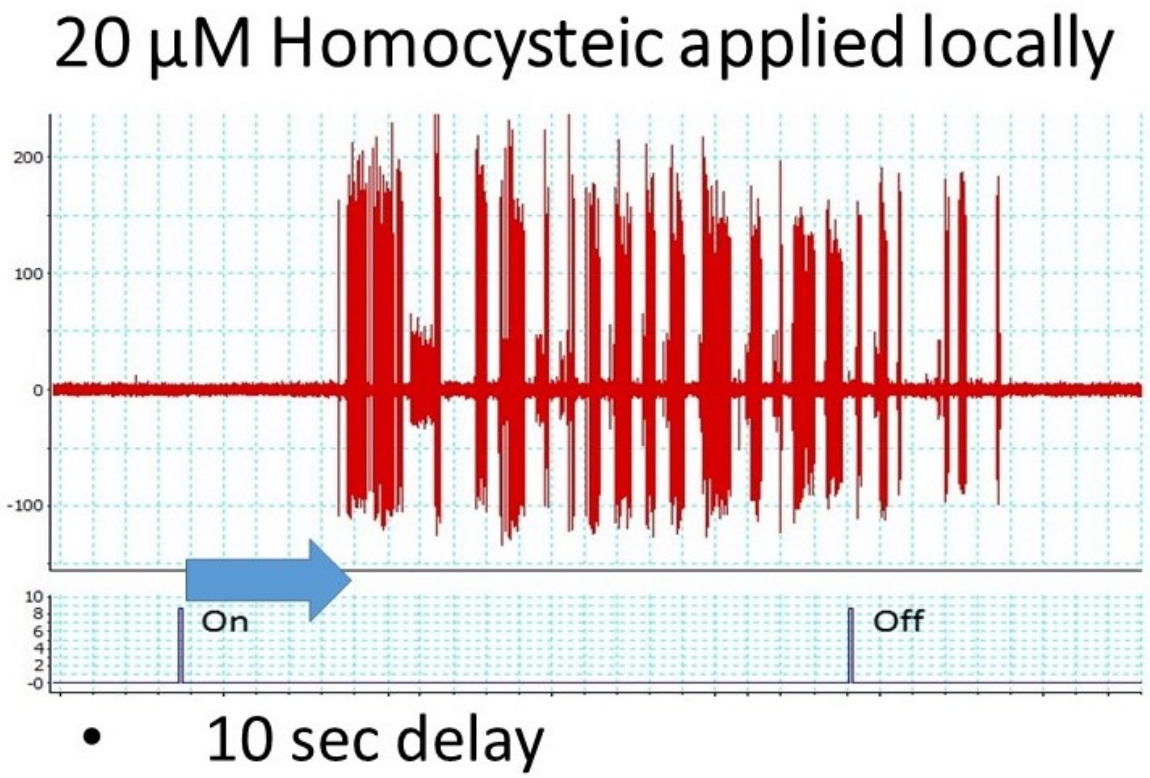
Abstract: It is well established that the spinal cord contains the neuronal circuit for rhythmic locomotion, and application of NMDA can trigger this circuit. The site of action of NMDA remains to be established. In search for this site, we discovered that NMDA acts quickest and at lowest concentration if applied to the spinal dorsal roots. This finding may have clinical significance, because it allows for the application of agents to promote locomotion outside the CNS.



Disclosures: J.T. Hackett: None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.19

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Independent research fund Denmark
Carlsberg foundation

Title: Rhythmic movement by spinal motor networks is governed by rotational population dynamics

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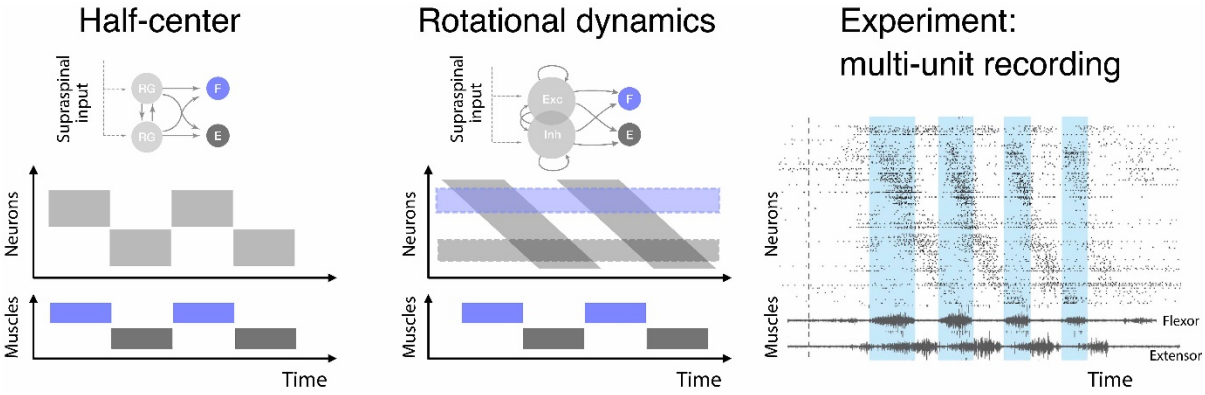
Abstract: While the orchestration of movements is accomplished by motor cortex and the brainstem, networks in the spinal cord are responsible for the detailed muscle commands,

including the generation of rhythmic movements such as locomotion. Although spinal neurons are well characterized at the cellular level, the mechanisms underlying rhythmic generation is largely unknown. It is known that the motor cortex exhibits rotational dynamics associated with movement (Churchland et al, 2012), i.e. the neural populations follow a rotating trajectory through their state space, but it is unknown whether the spinal cord has similar dynamics. To investigate this issue, we analyzed simultaneous multi-electrode recordings from the spinal cord of hundreds of neurons and discovered similar low-dimensional rotational dynamics characterized by a wide phase distribution in relation to the muscle activation. This is in opposition to alternation, which is predicted by the half-center theory (figure). To further appreciate how such slow population dynamics can yield rotational dynamics in the spinal cord, we develop a new theory, briefly involving firing rate dynamics of networks of recurrently connected excitatory and inhibitory spinal interneurons. By analyzing the spectrum of the linearized effective coupling matrix of the rate dynamics we illustrate how a tonic supraspinal drive can induce rhythmic network activity if two conditions are met: 1) the real part of the maximum eigenvalue of the effective coupling matrix is larger than a critical value, and 2) this eigenvalue has a non-zero imaginary part. Using numerical simulations of networks fulfilling these two conditions we show that the resulting population activity is largely determined by the eigenvector corresponding to the largest eigenvalue, displaying rotational dynamics with wide phase distributions consistent with the experimental data. Finally, we demonstrate how such population states can be flexibly modulated via a selective drive to allow multi-functional motor output patterns including multiple gaits.



Disclosures: **R.W. Berg:** None. **P.C. Petersen:** None. **M. Vestergaard:** None. **H. Lindén:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.20

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIHR00DC12536
NIHR01DC016413

McDonnell Center for Cellular and Molecular Neurobiology Postdoc Fellowship 2020

Title: A rostro-caudal map of V1 inhibition in axial spinal circuits

Authors: *M. SENGUPTA, V. DALIPARTHI, M. BAGNALL;
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Abstract: The structure of neuronal connectivity is key to function. In cortex, the structure of inhibitory circuits influences synaptic gain, spike timing, and membrane potential oscillations. Though the spinal cord is responsible for most motor output, the organization of inhibitory networks within it is much less clear. Most spinal interneurons project axons along the rostro-caudal (R-C) axis of the cord, yet connectivity along this axis remains ill explored owing to the inaccessibility in mammalian models. In this study, we use larval zebrafish to map connectivity of a major inhibitory population: V1 (En1+) neurons that send ascending axons rostrally. They directly inhibit both motor and sensory targets, and some V1 neurons function as Renshaw cells, with a role in flexor/extensor alternation. Suppression of V1 neurons results in slower locomotor speeds, in both fish and rodents. To investigate whether V1 neurons are important in R-C coordination, we generated a broad map of connectivity, along the R-C axis, from V1 neurons to both motor and sensory targets, using single cell labelling, optogenetics and electrophysiology *in vivo*. We find that V1 neurons exhibit long axons running approximately 6-7 segments rostrally and exclusively ipsilaterally. To test connectivity, we recorded IPSCs in post-synaptic targets while optogenetically stimulating V1 neurons in different positions in the spinal cord with a DMD. We first looked at a classical V1 target: motor neurons (MNs), both fast and slow. Surprisingly, V1 neurons formed only local connections with motor neurons (up to 2 segments and 3 segments for fast and slow MNs respectively), despite the long reach of V1 axons anatomically. V1 connectivity to V2a neurons, a major excitatory driver of locomotion, extended more distally than MNs (up to 5 segments) but the strength of this synaptic inhibition diminished rapidly beyond 1-2 segments. This pattern of short range connectivity was also observed for other motor targets, including V2b neurons and commissural pre-motor neurons. In contrast, V1 neurons made robust inhibitory contacts throughout the rostral extent of their axonal projections (up to 7-9 segments) onto Commissural Primary Ascending neurons (CoPAs), a dorsal horn sensory population. This connectivity was specific to the CoPAs. Overall, these data demonstrate that V1 neurons systematically connect to different targets along the R-C axis, inhibiting motor targets locally, and shifting to sensory targets, distally. The implications for such a unique pattern of connectivity to functions of V1 neurons is still unknown. Current work involves building a computational model to test these functional implications.

Disclosures: **M. Sengupta:** None. **M. Bagnall:** None. **V. Daliparthi:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.21

Topic: E.07. Rhythmic Motor Pattern Generation

Support: ANR-17-CE16-002

Title: Anatomical and functional investigation of the diversity of reticulospinal neurons controlling movements

Authors: *G. USSEGLIO, E. GATIER, A. HEUZÉ, C. HÉRENT, J. BOUVIER;
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Abstract: Orienting behaviors require multiple motor adjustments, notably of the head to displace the sensory apparatus, and of the limbs to modulate locomotor speed and when needed, to change trajectory. Brainstem reticulospinal (RS) neurons are key candidates for funneling sensory and goal-directed orienting signals to the executive motor circuits of the spinal cord. However, the intermingled organization of the reticular formation has hindered the identification of the RS subtype(s) specifically linked to orientation. Additionally, whether distinct motor actions are controlled synergistically by a unique RS population or instead by dedicated subtypes is still unresolved.

To address this we examined, in mice, the function and diversity of a genetically circumscribed class of glutamatergic RS neurons, the V2a neurons (expressing the transcription factor Chx10). First, using rabies-based transsynaptic tracings, we reveal that V2a neurons receive abundant synaptic inputs from the contralateral superior colliculus, making them a candidate relay of orienting commands. Second, we show that unilateral photo-activations of V2a neurons evoke multiple orienting-like motor responses, including a yaw rotation of the head and the snout and a displacement of the body axis towards the stimulated side. During locomotion, such activations lead to a transient locomotor arrest followed by a striking change in path trajectory. Next, using retrograde tracers and circuit optogenetics we reveal that these multiple motor actions are supported by distinct projection-defined V2a subsets, with at least 1) a lumbar-projecting subset whose activation, even unilaterally, arrests locomotion but neither impacts trajectory nor evokes orienting movements, and 2) a cervical-projecting subset dedicated to head orientation and whose activation suffices to change the animal's trajectory. This argues that the impact of V2a RS neurons on path trajectory owes to their capacity to impose the head orientation. Our ongoing experiments further indicate that cervical projecting V2a neurons might be neck pre-motoneurons and also hints at the existence of yet additional projection-defined V2a subsets that may drive other components of orientation.

Our work hence places medullary V2a neurons as important orchestrators of orienting movements. It also highlights that, while the reticular formation may be organized in functional modules defined by transcription factors (i.e. V2a RS neurons for orienting), a further specialization within modules following a muscle-group connectivity framework may allow the versatile expression of individual components of multi-faceted behaviors.

Disclosures: G. Usseglio: None. **E. Gatier:** None. **A. Heuzé:** None. **C. Hérent:** None. **J. Bouvier:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.22

Topic: E.07. Rhythmic Motor Pattern Generation

Support: CIHR
Will-to-Win/SCRC PhD Studentship
Marie Sklodowska-Curie Grant 665735 (Bio4Med)
Polish Ministry of Science and Higher Education (agreement no 3548/H2020/COFUND/2016/2)

Title: S

Authors: *K. E. ARMSTRONG[1], M. NAZZAL[1], S. BHAVYA[1], X. CHEN[1], U. SLAWINSKA[2], K. STECINA[1], L. M. JORDAN[3];
[1]Univ. of Manitoba, Winnipeg, MB, Canada; [2]Nencki Inst. of Exptl. Biol., Warsaw, Poland; [3]Dept Physiol., Univ. Manitoba, Winnipeg, MB, Canada

Abstract: Serotonergic (5-HT) pathways to the spinal cord have been shown to play an important role in locomotion. Additionally, a shared region of the medulla is a key site providing sympathetic input to support systems for locomotion, such as the cardiovascular system. Our lab has demonstrated that electrical stimulation of 5-HT neurons within the parapyramidal region (PPR) in the brainstem can elicit locomotion in an in vitro, neonatal rat preparation. We have also presented preliminary evidence that chemogenetic stimulation of these neurons can increase ENG activity and blood pressure in adult rats (Armstrong et al., SFN 2019). The aims of this study were to determine the contribution of serotonergic neurons to the initiation and/or the facilitation of locomotion in adult rodents. For the selective excitation of 5-HT neurons, excitatory DREADDs (n=9) were stereotaxically injected into the PPR using AAV viral vectors in adult Tph2-cre female rats. After a recovery period (21-97 days), experiments were performed in decerebrated (un-anesthetized) rats while recording lumbar cord dorsum potentials, hindlimb ENGs and arterial blood pressure. Mesencephalic locomotor region stimulation was used to induce fictive locomotor activity in order to assess changes after applying the clozapine-N-oxide (CNO) via intracerebral (i.c.) injections. Both ENG output and blood pressure increased after i.c. injections of CNO. The i.c. saline injections (n=4) or CNO injections into non-DREADD-infected controls showed no facilitation of ENG or blood pressure. Additionally, optogenetic constructs (ChR2) were targeted to this same region (n=6) and light stimulation-induced facilitation of blood pressure and hindlimb ENG activity was also achieved in these preparations. The injection site was verified post-hoc in frozen-sectioned tissue by mapping the fluorescent reporter protein for DREADDs (mCherry) or for ChR2 (eYFP). The utilization of the fluorescent constructs as tracers for the projections of PPR 5-HT neurons in our animals showed diverse branching of 5-HT terminals both in the thoracic and lumbar segments from the transfected subpopulation of neurons in most animals. These results suggest origin of a shared 5-HT network simultaneously regulating locomotor and cardiac control.

Disclosures: **K.E. Armstrong:** None. **M. Nazzal:** None. **S. Bhavya:** None. **X. Chen:** None. **U. Slawinska:** None. **K. Stecina:** None. **L.M. Jordan:** None.

Digital Abstract Session

P201. Motor Systems

Program #/Poster #: P201.23

Topic: E.08. Respiratory Regulation

Support: NIH HL111215
NS 110169
OT2 TR 001983
Craig H. Nielsen Foundation Pilot Research Grant 56714
The Kentucky Spinal Cord and Head Injury Trust
The Commonwealth of Kentucky Challenge for Excellence

Title: Large-scale optical recording of respiration-modulated neurons in ventrolateral medulla reveals cycle-to-cycle variability consistent with multifunctionality

Authors: B. GOUREVITCH[1], T. PITTS[2], K. E. ICEMAN[3], ***N. M. MELLEN**[3];
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Abstract: Respiration-modulated neurons form a distributed network in ventrolateral medulla. The post-inhibitory complex (PiCo) dorsal to the facial nucleus (VIIn), the parafacial respiratory group (pFRG), ventral to the VIIn, and the pre-Botzinger Complex (pre-BötC) are all endogenously rhythmic, and are hypothesized to mediate respiratory rhythm generation, but their interactions are poorly understood. All three networks can be recorded optically in the sagittally-sectioned rodent hindbrain (SSRH) preparation. In this study, neonate (P3-P5) mice in which the genetically-encoded Ca^{2+} indicator GCaMP6F was expressed in all cells due to spontaneous germline recombination were used, enabling an inclusive sampling of hindbrain respiratory networks. We recorded respiration-modulated networks along the ventral respiratory column at sampling rates fast enough (100 Hz) and recording epochs long enough (600 s) to robustly characterize the intrinsic cycle-to-cycle variability in both motor output and neuronal onset times relative to motor output (lags) and burst duration. The assumption behind these experiments was that constituents of a dedicated respiratory motor pattern generating network would consistently be among the earliest neurons active in each cycle. We found no anatomical parcellation of earliest neurons. Using Kendall's tau to quantify cycle-to-cycle lag variability, we found that although in aggregate pFRG neurons were significantly earlier than neurons in other regions, subsets of neurons from all regions were active earliest, and varied cycle-to-cycle. In part, variability in onset times could be accounted for by system-level variables: significant correlations between preceding expiratory durations and lags was observed for large subsets of neurons along the neuraxis. In addition, in a subset of experiments, non-parametric methods identified consistent "synfire chain" activation patterns associated with the shortest preceding

expiratory durations. The observation that inspiratory bursts arise out of the action of a diffuse network of neurons extending over a millimeter, whose constituents vary on a cycle-to-cycle basis is incompatible with the prevailing view that these are motor pattern generating networks. Rather, we propose that these networks are multifunctional, regulating the coordination of orofacial, ingestive, and respiratory behaviors, as well as blood-gas homeostasis.

Disclosures: **N.M. Mellen:** None. **K.E. Iceman:** None. **T. Pitts:** None. **B. Gourevitch:** None.

Digital Abstract Session

P202. Cerebellum: Cortex and Nuclei

Program #/Poster #: P202.01

Topic: E.02. Cerebellum

Support: NIH Grant MH112168

Title: Microrna-206 acts in cerebellar purkinje cells to regulate sensorimotor gating and fear-related behaviors

Authors: ***M. P. HEYER**[1], M. ISHIKAWA[3], P. J. KENNY[2];
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Abstract: The cerebellum is primarily known for its roles in motor learning and coordination. Emerging evidence suggests that the cerebellum also regulates more complex behaviors related to cognition, affect, and reward, and dysfunction in this brain structure may contribute to schizophrenia and other neurodevelopment disorders. The cerebellum shares extensive reciprocal connectivity with the neocortex, basal ganglia and hindbrain nuclei, and is thought to convey sensory experiences that shape cortical development and function. Nevertheless, the genes, cells, and circuits that govern cerebellar interactions with higher-order brain systems are poorly understood. Using brain-wide in situ hybridization, we find that the schizophrenia-associated microRNA, miR-206, is specifically enriched in cerebellar Purkinje cells. Mice with a targeted deletion of miR-206 exhibit impaired pre-pulse inhibition, an endophenotype of schizophrenia, stress-induced hypolocomotion, impaired extinction of fear memories, and sex-dependent cognitive deficits. Pre-pulse inhibition impairments were recapitulated by conditional deletion of miR-206 in parvalbumin-expressing cells, including Purkinje cells, suggesting that altered cerebellar output may contribute to these behavioral abnormalities. Consistent with this possibility, the spontaneous firing frequency of Purkinje cells was increased in miR-206 null mice. High-throughput sequencing of RNA isolated by crosslinking immunoprecipation after miR-206 deletion in Purkinje cells revealed potential target mRNAs in pathways related to neuronal excitability, glutamate signaling, and dendritic morphology & development. Together, these findings suggest that miR-206 regulates the function of cerebellar Purkinje cells and that this action controls sensorimotor, cognitive, and affective behaviors that are relevant to schizophrenia.

Disclosures: **M.P. Heyer:** None. **M. Ishikawa:** None. **P.J. Kenny:** None.

Digital Abstract Session

P202. Cerebellum: Cortex and Nuclei

Program #/Poster #: P202.02

Topic: E.02. Cerebellum

Support: NIH Grant R01 MH115604 04

Title: Cerebellar modulation of the hypothalamus

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Abstract: Although it constitutes a mere 10% of the total volume of the human central nervous system, the cerebellum contains more than half of our neurons. It is well-known for its involvement in motor control and coordination, but recent observations made on patients suffering from a myriad of mental disorders, or those with cerebellar lesions have led to a growing appreciation for its roles in the regulation of cognitive and somatic functions as well. To delineate the non-motor functions of the cerebellum it is important to have a detailed map of its connectivity with other brain regions, through anatomical neuronal tracings, we studied monosynaptic projections from the deep cerebellar nuclei (DCN). To do so, we used an AAV1-cre virus in the DCN of a reporter mice (called RCE:LoxP), in which neurons require Cre to express GFP. This virus possesses transsynaptic spread properties and allows the labelling of the somas of target-cells from monosynaptic projections. We observed the labelling of DCN target-cells in several hypothalamic regions: in the Medium Pre-Optic Area (MPOA), in the Lateral Hypothalamic Area (LHA), and in the Paraventricular Nucleus of the Hypothalamus (PVH). Immunohistochemistry and *in situ* RNA hybridization also revealed that some of the PVH cells which receive a DCN input also express oxytocin and vasopressin. These results suggest that the cerebellum may have a modulatory role in the hypothalamus. In future experiments, we plan to characterize this novel brain circuit by combining cell-type specific neuroanatomical tracing with in vivo and in vitro electrophysiological assays combined with behavioral tasks paired with live neuronal activity recordings in freely behaving mice.

Disclosures: **N.S. Cayla:** None. **J. Willet:** None. **M. Oñate:** None. **K. Khodakhah:** None.

Digital Abstract Session

P202. Cerebellum: Cortex and Nuclei

Program #/Poster #: P202.03

Topic: E.02. Cerebellum

Support: Wellcome Trust

ERC
Marie Skłodowska-Curie Fellowship

Title: Cerebellar Purkinje cell activity determines the latency and precision of sensory-driven motor initiation

Authors: ***S. TSUTSUMI**[1,2], O. CHADNEY[1], T.-L. YIU[1], E. BÄUMLER[1], L. FARRAGIANA[1], M. BEAU[1], M. HÄUSSER[1];
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Abstract: The cerebellum receives a variety of sensory and motor information to shape precise behavior. However, the cerebellar cortical mechanisms realizing the timed initiation of sensorimotor behavior remain largely unexplored. Here we invented a behavioral paradigm which requires animals to rapidly discriminate multi- vs. uni- sensory information to generate quick motor responses, to probe for the active sensorimotor timing. We investigated the role of Purkinje cell activity in the left folium Crus I, a hemispheric lobule implicated in sensorimotor integration, by combining optogenetic manipulation of simple spikes, high density electrical recordings (Neuropixels) of both simple and complex spikes, and two photon calcium imaging of complex spike signals in head-fixed mice (> P60) during the task. Optogenetic manipulation of simple spikes either abolished (N = 4 mice; 98 ± 1 vs. 55 ± 8% initiation, P = 0.0023) or delayed (N = 4 mice; 210 ± 30 vs. 360 ± 20 ms latency, P = 0.00091) the sensory-driven motor initiation. The magnitude of short latency (~100 ms) simple spike aberration was correlated with the delay and abolition of the initiation. Two photon calcium imaging across the entire Crus I revealed that task-relevant complex spike signals were organized into highly conserved alternating parasagittal stripes (zones). Coherent complex spike signals within these zones represented the salient sensory information. The complex spike coherence was specifically enhanced before the sensory-driven motor initiation, but not before the voluntary motor initiation. Moreover, the presence of the coherent complex spike signals after the sensory stimuli predicted the temporal precision of motor initiation. Finally, the enhancement of sensory-evoked complex spike coherence upon associated motor initiation was acquired together with the temporal precision in the sensory-driven motor initiation. These results reveal the complementary cerebellar cortical systems in controlling the precise sensorimotor timing: simple spikes in Crus I determine whether and when to initiate the sensory-driven motor behavior, whereas the acquired enhancement in sensory-evoked complex spike coherence in Crus I zones contributes to its temporal precision.

Disclosures: **S. Tsutsumi:** None. **O. Chadney:** None. **T. Yiu:** None. **E. Bäumler:** None. **L. Farragiana:** None. **M. Beau:** None. **M. Häusser:** None.

Digital Abstract Session

P202. Cerebellum: Cortex and Nuclei

Program #/Poster #: P202.04

Topic: E.02. Cerebellum

Support: R01DA044761
R01MH115604
R01NS105470

Title: Mechanism of action of ethanol in EA2: the δ-subunit containing $GABA_AR$ hypothesis

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Abstract: Loss of function mutations in the *CACNA1A* gene in humans, which encodes the P/Q-type voltage-gated calcium channel (Cav2.1), cause episodic ataxia type 2 (EA2). EA2 is characterized by attacks of severe ataxia and dystonia that are triggered by stress, caffeine or alcohol. There are several mouse models of EA2, including the tottering mouse which has a mutation in the pore-forming subunit of Cav2.1, resulting in reduced channel current density. These mice exhibit baseline ataxia and episodes of severe dyskinesia and dystonia (motor attacks) that are also triggered by stress, caffeine or alcohol. Our lab reported previously that the baseline ataxia is due to decreased precision in the pacemaking of cerebellar Purkinje cells (PCs). The lab has also shown that stress and caffeine trigger motor attacks by acting on cerebellar α1 adrenergic receptors and A1 adenosine receptors, respectively, resulting in PC burst firing *in vivo* and increased PC irregularity *in vitro*. Although ethanol produces similar firing *in vivo* and *in vitro,* the mechanism by which it triggers attacks is unknown. In fact, the mechanism by which ethanol produces the multitude of reported neurobehavioral, cellular and molecular effects in humans and rodents remains an open question. One of the main hypotheses is that ethanol potentiates the activity of δ-subunit containing γ-aminobutyric acid type A receptors (δ-$GABA_ARs$). We test this by asking whether the δ-$GABA_AR$-specific agonist THIP is sufficient to produce attacks in tottering mice. While ethanol administered via intraperitoneal (IP) injections at 1.5 g/kg triggers attacks in tottering mice, we found that IP injections of THIP up to 4 mg/kg, does not. Ethanol causes ataxia in wild type C57BL6 mice, as assessed by performance on the balance beam, and this effect is exacerbated by THIP at 4 mg/kg. However, THIP alone does not produce ataxia. As a positive control, THIP at 10mg/kg reduces locomotor activity and causes sedation in wildtype mice. Given these results, we conclude that ethanol-triggered motor attacks and ethanol-induced ataxia in wild type mice are not mediated by δ-$GABA_ARs$. The cellular effects of ethanol are known to be species-specific and in C57BL6 mice, ethanol can directly inhibit α6-subunit containing $GABA_ARs$ (α6-$GABA_ARs$) in cerebellar granule cells. We tested this possibility by inhibiting these receptors with IP furosemide at 10m/kg. We found that this was not only insufficient to trigger motor attacks in tottering mice, but also insufficient to cause ataxia in wildtype C57BL6 mice. We therefore conclude that ethanol neither triggers motor attacks in EA2 nor produces ataxia in wildtype C57BL6 mice by acting on δ-$GABA_AR$ or α6-$GABA_AR$.

Disclosures: J.O. Tindi: None. **K. Khodakhah:** None.

Digital Abstract Session

P203. Transmitters and Neuromodulation

Program #/Poster #: P203.01

Topic: E.03. Basal Ganglia

Support: JSPS KAKENHI 26530983
JSPS KAKENHI 16H01622
JSPS KAKENHI 25282247
JSPS KAKENHI 15K12770
JSPS KAKENHI 20H03549
JSPS KAKENHI 20K20671
Grant-in-Aid for Scientific Research on Innovative Areas 26112001

Title: Differential cortical and pallidal inputs to the striosome and matrix compartments in the rat striatum

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Abstract: Differentiation of striatal two compartments, the striosome and matrix, is not yet fully understood. Specific neural inputs to these compartments can drive their local circuits. We explore here compartment-dependent excitatory and inhibitory inputs using adeno-associated virus (AAV) and conventional neural tracers in 8-12 weeks male rats (N>3 rats and N=2-4 sections for all morphological experiments). As shown in earlier reports, the primary motor (M1) and secondary motor (M2) area projected to the striatum (Str). Both motor areas projected to the dorsolateral regions, although M2 projection was frequently found in the more medial Str. M1 preferentially projected to the matrix, whereas M2 equally projected to the striosome and matrix. Since the matrix also receives thalamic inputs more frequently than the striosome, what inhibitory inputs balance with these rich excitatory inputs? Regarding inhibitory inputs to Str, GP is one of the main sources. Thus, we visualized GP projection to Str using anterograde or retrograde AAVs, and discovered that GP densely projected to the matrix, whereas only small amounts of axons are observed in the striosome. Since many axonal fibers pass through GP, some axons from other brain regions could be also labeled. To label GP neurons projecting to Str more specifically, we applied a combination of injection of retrograde AAV encoding Cre (AAV-rg-Cre) to Str and injection of another AAV to GP, which expresses fluorophores in a Cre-dependent manner. Again, GP projection preferred the matrix to the striosome. Recently, it is known that GP projection neurons consist of two types: one type also projects to STN, as well as Str, whereas another type projects only to Str. To reveal whether both types prefer the matrix, the STN-projecting-GP neurons were labeled with injection of AAV-rg-Cre to STN followed by another AAV injection to GP. We found that their axons also preferred the matrix, although axon density in Str was small. It strongly suggests that both GP projection neuron types selectively project to the matrix. Therefore, rich excitatory inputs from M1 and thalamus to the matrix can be balanced with selective inhibitory inputs from GP.In summary, we found that M1 and M2

differentially projected to the striosome/matrix compartments of Str. In addition, GP selectively project to the matrix. Altogether, biased excitatory and inhibitory neural projections from the motor cortical areas to Str, and from GP to Str can affect the matrix-specific local neural circuitry, and take important roles to prepare and conduct appropriate motor behaviors.

Disclosures: F. Karube: None.

Digital Abstract Session

P203. Transmitters and Neuromodulation

Program #/Poster #: P203.02

Topic: E.03. Basal Ganglia

Support: R00NS087098
DP2NS105553

Title: Dopamine modulates the size of striatal projection neuron ensembles

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Abstract: Dopamine (DA) is a critical modulator of brain circuits that control voluntary movements. However, our understanding of its influence on the activity of target neurons in vivo remains limited. Here, we use two-photon Ca2+ imaging in the striatum of behaving mice to simultaneously monitor the activity of direct and indirect pathway spiny projection neurons (SPNs) during acute and prolonged manipulations of DA signaling. We find that DA strongly and differentially regulates the number of direct and indirect pathway SPNs recruited during behavior, and that chronic loss of DA neurons profoundly alters SPN responses to DA. Our results extend existing models of DA modulation and provide novel insights into the pathophysiology of Parkinson's disease.

Disclosures: M. Maltese: None. **N.X. Tritsch:** None. **J.R. March:** None. **A.G. Bashaw:** None.

Digital Abstract Session

P203. Transmitters and Neuromodulation

Program #/Poster #: P203.03

Topic: E.03. Basal Ganglia

Support: NIH/NIAAA DICBR ZIA AA000416

Title: 2-arachidonoylglycerol mobilization following brief synaptic stimulation in the dorsal lateral striatum requires glutamatergic and cholinergic neurotransmission

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Abstract: The endocannabinoid (eCB) system is a major source of synaptic modulation in the dorsal lateral striatum (DLS) and is required for proper motor output and striatal-dependent learning. Several forms of eCB-dependent plasticity have been described in the DLS, however most experimental protocols used to induce plasticity do not recapitulate the firing patterns of striatal-projecting pyramidal neurons in the cortex or firing patterns of striatal medium spiny neurons. Therefore, it is unclear if current models of eCB signaling in the DLS provide a reliable description of mechanisms engaged under physiological conditions. To address this uncertainty, we investigated mechanisms of eCB mobilization following brief synaptic stimulation that mimics *in vivo* patterns of neural activity in the DLS. To monitor eCB mobilization, the novel genetically encoded fluorescent eCB biosensor, GRABeCB2.0, was expressed in corticostriatal afferents of C57BL6J mice and electrically-evoked eCB transients were measured in the DLS using a brain slice photometry technique. We found that brief bouts of synaptic stimulation induce long lasting eCB transients that are modulated by stimulation frequency and duration. Inhibition of monoacylglycerol lipase, but not fatty acid amide hydrolase, increased the amplitude and prolonged the duration of the eCB transient, suggesting that 2-AG is the predominate eCB generated following brief synaptic stimulation. 2-AG transients were robustly inhibited by the muscarinic M1 receptor (M1R) antagonist, VU 0255035, and augmented by a M1R positive allosteric modulator, VU 0486846 (VU'846), indicating that acetylcholine (ACh) release is required for 2-AG production. Additionally, the dopamine D2 receptor (D2R) agonist, quinpiriole, inhibited the 2-AG transient. Combined, these results suggest that cholinergic interneurons (CINs) are required for 2-AG production and D2Rs expressed on these neurons limit production by inhibiting ACh release. To test this hypothesis, we generated transgenic mice lacking D2Rs specifically on CINs. In slices from these mice, quinpirole did not inhibit 2-AG production, confirming that D2Rs on CINs can control the magnitude of 2-AG production. Interestingly, the AMPA receptor (AMPAR) antagonist, DNQX, also robustly inhibited 2-AG production and blocked 2-AG augmentation by VU'846. These results suggest that converging glutamatergic and cholinergic signals are required for efficient 2-AG production following brief synaptic stimulation. Collectively, the present study provides new insights on circuit and cellular mechanisms controlling 2-AG mobilization in the DLS.

Disclosures: D.J. Liput: None. **H. Puhl:** None. **A. Dong:** None. **K. He:** None. **Y. Li:** None. **D. Lovinger:** None.

Digital Abstract Session

P203. Transmitters and Neuromodulation

Program #/Poster #: P203.04

Topic: E.03. Basal Ganglia

Support: ZIA-AA000421

Title: Dopamine depresses lateral inhibition in the nucleus accumbens through a noncanonical mechanism

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Abstract: The nucleus accumbens (NAc) is a brain region that subserves healthy motivated behaviors and is an important site of dysfunction in addiction and depression. Acting as a "limbic-motor interface," projection neurons in the NAc, medium spiny neurons (MSNs), integrate excitatory glutamate signals from cortical and limbic regions with dopamine (DA) signals from midbrain to facilitate motivation and reward learning. However, NAc also contains an extensive network of inhibitory GABA synapses between MSNs, which remains understudied. It is currently unknown how DA interacts with this network of MSN GABA synapses. By modulating synaptic strength within this network of lateral inhibition, DA is situated to influence NAc information processing by shifting the balance of excitation/inhibition at the micro circuitry level. MSNs express two types of dopamine receptors: either G_{olf} coupled D1 receptors or $G_{i/o}$ coupled D2 receptors (D2MSNs). Here we ask: how does DA acutely affect GABA transmission from D2MSNs onto neighboring MSNs? We hypothesize that endogenously released DA from the midbrain can inhibit local GABA transmission from D2MSNs to neighboring MSNs. Using a dual optogenetic approach to test this hypothesis in mouse brain slices, we show that DA neuron stimulation preceding D2MSN GABA release by 500-1000ms acutely depresses GABA transmission from D2MSN synapses. To further probe this depression, we applied exogenous DA and found the IC_{50} for the depression of GABA transmission to be in the micromolar range, an order of magnitude higher than that of previous studies using heterologous systems. We next hypothesized that this DA depression of D2MSN GABA transmission is mediated presynaptically by D2Rs expressed on D2MSNs. Through a series of pharmacological and uncaging studies, we indeed found that this depression is driven presynaptically. However, to our surprise the depression is only partially explained by D2R activation. Genetically deleting D2Rs or pharmacologically blocking D2-like receptors only prevented ~50% of the DA mediated depression. Moreover, D1-like receptor antagonists had no effect on this DA mediated depression, suggesting a novel target of DA. Current experiments are aimed at determining this novel mechanism by which dopamine inhibits synaptic transmission. Taken together, these results shed light on the actions and mechanisms by which DA acutely modulates the lateral inhibitory network in the NAc. This insight is necessary for understanding how DA shapes NAc circuitry and information processing to promote motivated behaviors, both in health and disease.

Disclosures: D.A. Burke: None. **V.A. Alvarez:** None.

Digital Abstract Session

P203. Transmitters and Neuromodulation

Program #/Poster #: P203.05

Topic: E.03. Basal Ganglia

Support: ZIA AA000407

Title: Mechanisms of presynaptic modulation of striatal efferents by $G\alpha_{i/o}$ coupled GPCRs

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Abstract: Dorsal striatal control over basal ganglia (BG) output nuclei is required for appropriate action selection and performance. Presynaptic modulation of SPN terminals plays a role in determining net striatal output and is disrupted in models of BG dysfunction. Potential mediators of presynaptic modulation at SPN terminals are the $G\alpha_{i/o}$ coupled GPCRs, $GABA_B$ and CB1, however the exact mechanisms by which these receptors modulate neurotransmitter release are unclear. Further, which calcium (Ca^{2+}) channel subtypes contribute to presynaptic Ca^{2+} influx at SPN terminals is unknown. Here, we studied Ca^{2+} channel subtype contributions to presynaptic Ca^{2+} influx and GPCR modulation of presynaptic Ca^{2+} transients (preCaTs) and transmission in the direct pathway SPN (dSPN) terminals in the SNr, dSPN collateral terminals in the GPe and indirect pathway SPNs (iSPN) terminals in the GPe. Brain slice photometry was used to measure electrical stimulation-evoked preCaTs in slices from transgenic mice expressing the genetically encoded Ca^{2+} sensor, GCaMP6f, in dSPNs or iSPNs. In parallel experiments, optically-evoked IPSCs (oIPSCs) were recorded in slices from transgenic mice expressing channelrhodopsin in dSPNs or iSPNs. Bath application of N- or P/Q-type Ca^{2+} channel blockers (ω-conotoxin GVIA and ω-agatoxin IVA, respectively), but not a L-type Ca^{2+} channel blocker (Nifedipine), decreased the amplitude of presynaptic Ca^{2+} transients in all three sets of SPN terminals. Bath application of the $GABA_B$ receptor agonist, baclofen, significantly reduced the amplitude of preCaTs and the amplitude of oIPSCs at all three sets of SPN terminals. Interestingly, applying CGP55840 after baclofen reversed the effect of baclofen on preCaTs to baseline amplitudes at iSPN terminals in the GPe, while increasing the amplitude of the preCaT above baseline at dSPN terminals in the GPe and dSPNs in the SNr. Combined, these results suggest that presynaptic $GABA_B$ receptors couple to N- and/or P/Q-type Ca^{2+} channels to inhibit neurotransmitter release at dSPN and iSPN terminals, and $GABA_B$ receptors may tonically inhibit presynaptic calcium channels in dSPN but not iSPN terminals. Surprisingly, bath application of the CB1 receptor agonist WIN55212-2 did not significantly inhibit preCaTs, but strongly reduced the amplitude of oIPSCs in all three sets of SPN terminals, suggesting that CB1 receptors might inhibit neurotransmitter release at dSPN and iSPN terminals through mechanisms independent of presynaptic Ca^{2+} influx. Overall, our results contribute to unravelling the specific mechanisms underlying presynaptic modulation of SPN terminals in the SNr and GPe.

Disclosures: G. Sitzia: None. **D. Liput:** None. **K.P. Abrahao:** None. **G.M. Calandra:** None. **D.M. Lovinger:** None.

Digital Abstract Session

P203. Transmitters and Neuromodulation

Program #/Poster #: P203.06

Topic: E.03. Basal Ganglia

Support: NIA BRAIN Initiative: RF1MH114126
NIH Office of Research Infrastructure Programs (ORIP): P51OD010425
National Center for Advancing Translational Sciences (NCATS): UL1TR000423
Paul G. Allen Family Foundation

Title: Enhancer-based viral tools for targeting and functional analysis of striatal cell types and circuits across mammalian species

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Abstract: Basal ganglia dysfunction is central to diverse neurological disorders including Huntington's disease, Parkinson's disease, Obsessive compulsive disorder, and addiction. Among the basal ganglia structures, the striatum integrates synaptic inputs from diverse brain regions and plays a major role in regulating motor output and reward. Neuron populations in the striatum include medium spiny neurons (MSNs), diverse local GABAergic interneurons, and large cholinergic interneurons. Of particular interest, striatal MSNs consist of two functionally distinct but intermingled populations: those that send axon projections to the substantia nigra (SNr) forming the direct pathway (dMSNs), and those that send axon projections to the globus pallidus externa (GPe) forming the indirect pathway (iMSNs). Understanding the balance between these distinct pathways is critical and may lead to breakthroughs in the treatment of movement disorders.

Transgenic lines for *in vivo* targeting of striatal cell types are well established in mice, and have been invaluable for genetic dissection of intrinsic properties, synaptic connectivity, and circuitry basis of behavior. However, these tools are restricted to the mouse model and can't be leveraged for cross-species studies, including functional studies in non-human primates (NHPs) or potential therapeutic applications in human patients. To address this crucial gap, we developed adeno-associated viral vectors (AAV vectors) with compact cell type-specific enhancer elements to target several important neuron classes and subclasses in the striatum including iMSN, dMSN, pan-MSN, and cholinergic interneuron populations. Putative striatal cell type enhancers were identified from published or open access human and mouse epigenetic datasets and genome browsers and cloned into AAV vectors for functional testing.

We characterized brain-wide expression patterns of our novel AAV vectors in mice and present multiple lines of validation on labeling specificity. Importantly, many of these cell type enhancer AAVs also drive transgene expression in macaque *ex vivo* putamen slice cultures and *in vivo*

following stereotaxic injection. As a final validation of labeling specificity and demonstration of a functional application, we have performed targeted Patch-seq recordings to measure electrophysiology, morphology, and transcriptomes of virus labeled neuron types in mouse and monkey brain slices. These novel viral tools will facilitate additional detailed functional characterization of striatal cell types across mammalian species, with possible applications for human gene therapy to treat disorders of the basal ganglia.

Disclosures: **N. Weed:** None. **S. Owen:** None. **B. Kalmbach:** None. **Y. Kojima:** None. **V. Omstead:** None. **X. Opitz-Araya:** None. **R. Martinez:** None. **R. Martinez:** None. **L. Ng:** None. **B. Gore:** None. **J. Mich:** None. **S. Yao:** None. **B. Lee:** None. **B. Levi:** None. **G.D. Horwitz:** None. **E. Lein:** None. **J. Ting:** None.

Digital Abstract Session

P204. Systems Behavior

Program #/Poster #: P204.01

Topic: E.03. Basal Ganglia

Support: Swiss National Science Foundation Grant P2EZP3_168841
Swiss National Science Foundation Grant P400PB_180841
NIMH R01 Grant MH093672
NIMH R03 Grant NS096494

Title: New players in the basal ganglia circuitry: striatal D1R expressing axonal collaterals to the GPe act as a second direct pathway to support motor control

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Abstract: In the classical model of the basal ganglia two segregated and functionally opposing pathways arise from the striatum to regulate motor control. Direct pathway striatal projection neurons (dSPNs) expressing dopamine D1 receptors (D1R) project monosynaptically to the substantia nigra (SNr) to promote movement while indirect pathway SPNs (iSPNs) expressing D2R project monosynaptically to the globus pallidus externus (GPe) to oppose movement. Recent functional studies, however, reveal that during native movements both pathways are activated. Moreover, anatomical and physiological studies have put into question the organization of the basal ganglia. For instance, we and others have shown that a large fraction of

dSPNs possess axon collaterals within the GPe. These “bridging” collaterals are plastic in the adult animal and regulated by D2Rs and neuronal activity. These findings, though indirect, suggested an important role of dSPN bridging collaterals in regulating the balance of the direct and indirect pathway and motor function. Here, we used a combination of genetic targeting, in vivo calcium imaging, dSPN terminal-specific manipulations (chemogenetics, optogenetics) and behavioral tracking (DeepLabCut) to determine the role of dSPN bridging collaterals in motor function. We found that dSPN bridging collaterals were activated during specific movements in the rotarod. dSPN terminal inhibition also decreased locomotion and impaired rotarod performance. Recent physiology work shows that dSPNs preferentially target the non-canonical, striatal-backprojecting, arkypallidal Npas1 neurons in the GPe. We here found that stimulation of dSPNs inhibited native Npas1 signals in awake behaving mice. Finally, we found that Npas1 stimulation recapitulated the effects of dSPN inhibition by decreasing locomotion and rotarod function. We propose a model by which dSPN bridging collaterals support movement via inhibition of Npas1 neurons in the GPe. Thus, direct pathway GPe terminals act in concert with the canonical terminals in the SNr by inhibiting a potential stop signal going back to the striatum. Future work on bridging collaterals is expected to inform applications to disease states characterized by basal ganglia imbalances, such as Parkinson’s disease or Huntington’s.

Disclosures: **M.A. Labouesse:** None. **J.M. Villarin:** None. **A. Torres-Herraez:** None. **X. Sun:** None. **S. Lam:** None. **J. Bonaventura:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Application 62/627,527 with the U.S. Patent Office. **M. Michaelides:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Application 62/627,527 with the U.S. Patent Office.. **F. Carvalho Poyraz:** None. **J. Greenwald:** None. **A. Tang:** None. **S. Chan:** None. **O. Yizhar:** None. **C. Kellendonk:** None.

Digital Abstract Session

P204. Systems Behavior

Program #/Poster #: P204.02

Topic: E.03. Basal Ganglia

Support: NIH grant R01-NS099323-01
NIH grant R01-NS105349
EMBO Postdoctoral Fellowship
HFSP Postdoctoral Fellowship

Title: Motor skill learning and execution in a distributed brain network

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Abstract: The remarkable capacity of the brain to acquire and execute motor skills depends on a distributed motor network. While many components have been identified, less is known about their specific roles and interactions during skill learning and execution. Here we probe this network through the lens of complex, spatiotemporally precise motor sequences we train in rats. We focus on the basal ganglia and the contributions made by their main inputs, from motor cortex and thalamus, respectively. Using electrophysiological recordings, we find that the dorsolateral striatum (DLS), the main motor-related input nucleus of the basal ganglia, encodes the detailed kinematic structure of the learned motor sequences. We further show that a loss of the DLS renders animals unable to execute the learned idiosyncratic motor patterns, causing them to revert to simple species-typical behaviors. In addition, we find that not only the DLS, but also its motor cortical inputs are necessary for learning the skills we train. This very same pathway, however, becomes dispensable after the behaviors are acquired. In line with this, the loss of motor cortical inputs leaves the DLS activity encoding the kinematic structure of the behavior largely unaffected. In contrast, thalamic inputs to the DLS remain crucial for the generation of the learned skills and loss of these inputs disrupts performance akin to DLS lesions, causing a reversion to the same species-typical behavior. Together, our results suggest that the basal ganglia can play a role in the control of complex learned behaviors which goes beyond traditional models of basal ganglia function. They further suggest that motor cortex 'tutors' sub-cortical motor circuits during learning, potentially by guiding plasticity at thalamostriatal synapses. Such adaptive reprogramming of lower-level motor circuits may broaden their flexibility and allow them to store and generate complex learned motor skills.

Disclosures: S.B. Wolff: None. **R. Ko:** None. **A. Dhawale:** None. **B. Ölveczky:** None.

Digital Abstract Session

P204. Systems Behavior

Program #/Poster #: P204.03

Topic: E.03. Basal Ganglia

Support: Marion Bowen Neurobiology Postdoctoral Fellowship
EY019663
EY024153

Title: Optogenetic activation of the inhibitory nigro-collicular circuit evokes orienting movements in mice

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Abstract: In contrast to predictions from the current model of basal ganglia (BG) function, we report here that increasing inhibition from the BG to the superior colliculus (SC) through the substantia nigra (nigra) using *in vivo* optogenetic activation of GABAergic terminals produces contralateral orienting movements in freely moving mice. Orienting movements resulting from

activation of inhibitory nigral terminals are unexpected because decreases and not increases in nigral activity are generally associated with orienting movements. To determine how orienting movements may result from activation of inhibitory terminals, we performed a series of slice experiments and found that the same optogenetic stimulation of nigral terminals used *in vivo*, evoked post-inhibitory rebound depolarization and spiking in SC output neurons *in vitro*. In addition, only high frequency (100Hz) stimulation evoked contralateral movements *in vivo* and triggered rebound spiking *in vitro*. Furthermore, the latency of orienting movements relative to the stimulation *in vivo* was similar to the latency of rebound spiking *in vitro*. Taken together, our results point toward a novel hypothesis proposing that inhibition from the BG into the SC may play an active rather than passive role in the generation of orienting movements in mice.

Disclosures: C.A. Villalobos: None. **M.A. Basso:** None.

Digital Abstract Session

P204. Systems Behavior

Program #/Poster #: P204.04

Topic: E.03. Basal Ganglia

Support: UF-NS108177
U19-NS113201
EY-12196
Harvard Lefler Predoctoral Fellowship
Stuart H.Q. and Victoria Quan Predoctoral Fellowship

Title: Dynamic dopaminergic activity controls the moment-to-moment decision of when to move.

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Abstract: Parkinson's disease has long suggested a vital role for dopamine in movement initiation, but there is surprisingly little evidence connecting the activity of the endogenous dopaminergic system to movement onset. Although "standard" stimulus-response paradigms have not revealed a clear role for dopaminergic activity in movement initiation, most natural movements are not short-latency reactions to abrupt stimuli. Intriguingly, Parkinson's patients struggle to *self-initiate* movement more than to react to sensory cues (a phenomenon known as "paradoxical kinesia"), which suggests a role for dopamine might be revealed by instead examining movements that rely on an internal generative process to decide when to move. One type of self-generated movement is self-timed movement, in which subjects must decide *when* to move in the absence of overt cues, and pharmacological studies have suggested that exogenous dopamine can affect the timing of these movements. We hypothesized that dopaminergic activity may influence movement initiation by guiding its moment-to-moment timing. We recorded

dopaminergic system activity with fiber photometry in gender-balanced cohorts of mice as they prepared to initiate self-timed movements (GCaMP6f in genetically-defined dopamine neurons (n=12); the novel dopamine indicator dLight1.1 (n=5) or DA2m (n=4) in striatal neurons). We controlled for optical/movement artefacts with co-expressed tdTomato fiber photometry, neck EMG, back mounted accelerometer, and high-speed video. We found that even when accounting for movement, task variables, and trial history, dopaminergic signals were highly predictive of single-trial movement timing via a slow "ramp-up" that unfolded over seconds between the start-timing cue and the self-timed movement, reminiscent of a ramp-to-threshold process. Moreover, optogenetic activation of dopamine neurons systematically early-shifted movement timing (n=12 mice), whereas inhibition caused late-shifting (n=4 mice), and no-opsin stimulation caused no consistent behavioral effect (n=5 mice). Optogenetic stimuli were subthreshold for generating/preventing movement outside the task, suggesting that dopaminergic activity influenced movement onset by adjusting the moment-to-moment probability of a planned movement. Applying a reward-prediction error model for dopaminergic activity recapitulated the dopaminergic signal in our task and explained puzzling findings from a recent perceptual timing task, suggesting a unifying interpretation for the role of dopaminergic activity across timing paradigms.

Disclosures: **A.E. Hamilos:** None. **G. Spedicato:** None. **Y. Hong:** None. **F. Sun:** None. **Y. Li:** None. **J.A. Assad:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); J.A.A. is a co-founder of OptogeniX, which produces the tapered optical fibers used in some experiments..

Digital Abstract Session

P204. Systems Behavior

Program #/Poster #: P204.05

Topic: E.03. Basal Ganglia

Support: NSF 1940957
NIH NS098744

Title: Developmentally regulated pathways for motor skill learning in songbirds

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Abstract: During motor skill learning, cortico-basal ganglia circuits mediate comparisons between self-generated motor actions and a goal behavior that the animal is trying to learn; the results of these comparisons guide refinement of the animal's own behavior towards the goal motor pattern. Information stemming from copies of motor commands - efference copy - may play an essential role in guiding this learning process. Vocal learning in songbirds serves as a powerful model for investigating mechanisms of motor skill learning, because, like humans, juvenile songbirds learn complex vocal motor patterns by copying the song of an adult tutor (the

goal behavior). Juvenile song production is mediated by a projection from the cortical nucleus $LMAN_{core}$ to RA, a region of the motor cortex that directly drives vocal motor output. $LMAN_{core}$→RA neurons send a collateral projection to AId, a region adjacent to RA, in juvenile birds during early vocal development. Both RA and AId neurons project to the thalamic nucleus DLM, which forms a feedback pathway to cortico-basal ganglia circuitry. These projections from motor-command neurons in $LMAN_{core}$ to AId and RA and thence to the thalamus provide pathways by which information about vocal motor output could be reintegrated into cortico-basal ganglia circuitry, potentially aiding in the refinement of juvenile vocalizations. However, no studies have examined whether the strength of these efferent projections changes during the early stages of sensorimotor learning. We investigated developmental changes in these axonal pathways by conducting tract-tracing experiments to label the projections of $LMAN_{core}$ to AId and of RA to DLM in juvenile songbirds (35-50 dph). The volume and density of terminal label in AId stemming from injections into $LMAN_{core}$ (n=7) declines substantially between 35 and 45 dph, during the earliest stages of sensorimotor learning. In contrast, the projection from RA to DLM is consistently sparse during the early stages of sensorimotor learning and does not change in volume or density (n=15). The developmental decrease in connectivity between $LMAN_{core}$ and AId indicates a loss of efference copy in this pathway and suggests that substantial changes in the neural substrate are intertwined with changes in learning: projections that are present only during early stages of sensorimotor learning may mediate unique, temporally restricted processes of goal-directed learning.

Disclosures: A. Chung: None. **S.W. Bottjer:** None.

Digital Abstract Session

P204. Systems Behavior

Program #/Poster #: P204.06

Topic: E.03. Basal Ganglia

Support: Natural Sciences and Engineering Research Council of Canada (Grant #2017-06411)

Title: The mTOR pathway impacts motor learning skills in mice

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Abstract: The mammalian target of rapamycin (mTOR) is known to play a central role in cognitive memory function. For instance, the underlying changes in biological processes that are associated with Alzheimer's disease, a condition with progressive decline in cognitive function as a primary clinical symptom, are associated with mTOR dysfunctions. (Hoeffer and Klann, 2010) However, whether mTOR play a role in motor memory processes is still under investigation. Recent study have shown that mTOR is using molecular partners like ribosomal protein S6 kinase beta-1 (p70-S6K) to initiate formation of new neuronal connections. (Graber et

al., 2013) Our study investigates the role of mTOR and p70-S6K during the learning of a complex motor skill by using selective pharmacological inhibitors. The effect of systemic treatments with rapamycin and PF4708671, inhibitors of mTOR and p70 S6K respectively, were investigated during the accelerating rotarod test on day 1, 2 and 3, using three cohort of mice (N=84). The first cohort (n=36) was injected 1 hour or 15 minutes before trainings to study intra-sessions effect. The second cohort (n=30) was injected after trainings to examine inter-sessions effect. The third cohort (n=18) was injected before day 4, with no injections on day 1, 2 and 3. This last cohort was also tested for motor strength and coordination. Preliminary results with first cohort demonstrated significant differences between mice treated before training with vehicle and those treated with PF4708671 on day two and three, but no significant effect with rapamycin. The experiments using the second and third cohorts revealed that injections of rapamycin or PF4706871 had no effect on motor learning when injected after trainings, and that both treatments were not affecting general motor capacity such as muscular strength. In conclusion, our data suggest that p70-S6K function is important during motor learning sessions (intra-sessions effect). However, we didn't observe significant impact of mTOR inhibition during motor learning. Our results also indicate that activation of those two proteins might not be crucial after trainings (inter-sessions effect). The way mTOR pathway impacts motor learning skills is important in order to better understand the basis of neurophysiology and neurodegenerative diseases. GRABER, T. E., MCCAMPHILL, P. K. & SOSSIN, W. S. 2013. A recollection of mTOR signaling in learning and memory. *Learn Mem,* 20**,** 518-30. HOEFFER, C. A. & KLANN, E. 2010. mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends Neurosci,* 33**,** 67-75.

Disclosures: A. Allain: None. **M. Cyr:** None.

Digital Abstract Session

P205. Finger and Grasp Control: Normal Human Behavior

Program #/Poster #: P205.01

Topic: E.04. Voluntary Movements

Title: What you see vs. what you need: How reliance on visual information changes in response to sequence motor learning

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Abstract: Vision supplies vital feedback information for motor execution and feedforward information for motor planning. As individuals learn a motor task, how the nervous system uses visual information to control movements will change. However, little research has examined how the role of feedback and feedforward information changes in relation to motor learning. In the present study, participants completed an online "video game" with a sequence-learning task during which small objects dropped downward on the monitor and traveled through 1 of 4 vertical channels at a constant velocity. As the object(s) crossed a target located at the bottom of

the vertical channel, the participant was instructed to press a keyboard key(s) corresponding to the correct target(s). The dropping objects appeared in a 10-object repeating sequence (i.e. a fixed spatiotemporal relationship between the 10 individual objects) and participants received auditory feedback following accurate responses to help accelerate motor learning. Participants completed 8 trial blocks (practice, baseline, 5 training blocks, and post testing). The repeating sequence was included in the 5 training blocks and in post testing where the 10-object sequence was repeated 15 times per block. Participant reliance on feedback and feedforward visual information was probed by either blacking out object trajectories in the upper (feedforward) or lower (feedback) half of the game screen with the same expectation for participants to press the correct keys as the object crossed the target locations. We hypothesized that at baseline, before practicing the task, accuracy would decrease more when visual feedback information in the lower half was removed as compared to feedforward information at the top half, signifying a greater reliance on feedback. We also hypothesized that following motor learning, performance would decrease more when feedforward visual information at the upper half was removed, signifying a greater reliance on feedforward information. The results support our hypothesis with performance decreasing by 73% without feedback compared to 7% without feedforward information at baseline. Following the motor learning paradigm, performance dropped 30% without feedback and 33% without feedforward visual information. These results suggest that participants shift reliance from feedback to feedforward visual information following sequence learning.

Disclosures: A. Cates: None. **K. Gordon:** None.

Digital Abstract Session

P205. Finger and Grasp Control: Normal Human Behavior

Program #/Poster #: P205.02

Topic: E.04. Voluntary Movements

Title: Effect of local vibration on finger force production during isometric pressing tasks

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Abstract: Vibration stimulation has been studied to analyze the neural and mechanical connections that lie underneath the limbs control. It has been evidenced that long-time vibration exposure produced nerve and muscle injuries (Krajnak et al., 2013; Barcenilla et al., 2011). However, more recent investigations using vibration applied to either upper or lower limbs revealed an enhancement of human performances such as jumping height, extension strength, speed, grip force etc (Arpinar-Avsar et al., 2013). Direct stimulation has been proved to activate the muscles at both primary and secondary endings leading to the tonic vibration reflex effect

(TVR) (Luo et al., 2005). TVR showed an enhancement of the muscle's force on the flexor side indicating activation on the agonist muscle (Eklund 1978). However, it is unknown if the muscle response the same during voluntary (maximal and low force tasks) and involuntary contractions. It appears that vibration provides both temporal and spatial aspects that need to be further evaluated. In this study, we investigated the effect of vibration stimulation on finger forces during voluntary contraction and resting tests. We hypothesize that the vibration stimulation effect is dependent on muscle voluntary activation status. In addition, the effect of vibration on muscle responses is interacted with the stimulation site, as well as the enrolled finger combination. 12 healthy adult subjects participated and performed isometric pressing tasks by right four fingers in 2 separate sessions: vibration and control sessions. In vibration session, 5 vibrators (MicroPrecision, 100Hz frequency) were attached at 4 distal sites for fingers and 1 proximal site for the wrist, to stimulate both agonist and antagonist muscle tendons (flexor and extensor respectively). Individual finger forces have been recorded by force/torque sensors (ATI nano17) under 3 force conditions: voluntary maximal force (MVC), voluntary sub-maximal force (sub-MVC), and resting. Each force condition was performed by each individual finger (1D: I, M, R or L) or the four fingers together (4D), resulting in 76 pseudo-randomized trials. Our results showed that MVC is significantly decreased by vibration stimulation on flexor side, while the involuntary forces during resting condition from same side presented significant higher values with vibration compared to control session. Our findings suggest that the additional sensory stimulation of muscle vibration pose opposite effect toward to muscle activation, that is, an inhibition effect upon agonist muscle tendon during voluntary contractions, yet an activation effect on muscle tendon during resting period.

Disclosures: W. Zhang: None. **F. Bruyer-Montéléone:** None. **E. Shi:** None. **V. Apadaylo:** None. **L. Soloveychik:** None. **S. Imoke:** None. **A. Zou:** None. **Z. Ahmed:** None.

Digital Abstract Session

P205. Finger and Grasp Control: Normal Human Behavior

Program #/Poster #: P205.03

Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS085122
NIH Grant 2R01HD058301
NSF Grants CBET-991804550

Title: Involvement of Dorsal and Ventral Premotor cortices in online updating to reach and grasp perturbations

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Abstract: Dexterous and robust control of reach-to-grasp involves rapid sensorimotor updating for online adjustments to changing external goals (e.g., perturbations of objects we interact with) in order to interact with objects in the surrounding environment seamlessly. It is now well-established that nodes within the dorsolateral pathway involving the ventral premotor cortex (PMv) are involved in sensorimotor processing of hand preshaping, and nodes within the dorsomedial pathway involving dorsal premotor cortex (PMd) processing the reach component during reach-to-grasping actions. Recent evidence, however, that neural populations in both pathways respond to both reach- and grasp-related aspects of the movement suggest that neural control of reach-to-grasp may not be as parcellated as previously thought. In this investigation, we test the degree to which PMv and PMd mediate online updating to transport and grasp perturbations during reach-to-grasp action. Seven healthy right-handed subjects (26.2 ±7.7 years old), after providing informed consent, performed reach-to-grasp movements in a virtual environment. Movements were performed in unperturbed conditions toward a 3.6 cm (small) virtual object located 24 cm (near) from the starting hand position. In a minority of the trials (25%), either the object size (small-to-large, 3.6-7.2 cm) or distance (near-to-far, 24-36 cm) was unexpectedly perturbed (early-100 ms or late-300 ms after movement onset), requiring subjects to make online compensatory responses to grasp (aperture) and reach (transport). Transcranial magnetic stimulation (TMS) was used to perturb processing in PMv and PMd (50% of trials, 120% of RMT) at the time of object perturbation. Movement kinematics of grasp aperture and wrist transport were analyzed using standard parametric statistical tests. Our preliminary data revealed two main findings: (i) TMS to PMv delayed the latency of corrective response in aperture (50 ms, $p = .001$) for late transport perturbation condition (despite there being no perturbation of grasp); (ii) TMS to both PMv (20 ms, $p = .013$), and PMd (30 ms, $p = .003$) delayed the latency of corrective responses of aperture to perturbations of object size when the perturbation was applied early into the movement, and in the case of PMv, the effect held for late perturbations as well (40 ms, $p = .003$). These preliminary findings provide evidence that PMv and PMd may both integrate grasp and transport information at different stages of the reach-to-movement. If true, the data warrant a reevaluation of the classical two-streams model of information processing for reach-to-grasp. We are currently investigating this in a larger sample size.

Disclosures: **M.P. Furmanek:** None. **M. Yarossi:** None. **M. Mangalam:** None. **K. Lockwood:** None. **E. Tunik:** None.

Digital Abstract Session

P206. Finger and Grasp Control: Age, Pathology, and Physiology

Program #/Poster #: P206.01

Topic: E.04. Voluntary Movements

Title: The dose-response effectiveness of active music therapy for upper extremity stroke rehabilitation: a systematic review and meta-analysis study

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Abstract: Background. Previous studies have found that active music therapy (AMT) can improve upper extremity (UE) function in people with stroke. Actively playing a musical instrument is highly motivating compared to rote exercises due to the engaging music features. When playing instruments, such as a piano, the affected UE can be naturally involved in practice, which leads to functional improvements. To investigate the effects and the optimal dosage of AMT in improving UE function in stroke, we conducted a systematic review and meta-analysis.
Methods: Our systematic review included searches in PubMed, Cochrane, MEDLINE, Embase, CINAHL, and ProQuest, with three key categories terms: stroke, music-based therapy, and upper extremity. The review process were conducted by two individuals separately with a third person involved if agreement was not achieved. For meta-analysis, data were extracted from the commonly-used assessments for UE function and finger dexterity in the field, including Action Research Arm Test and Nine Hole Pegboard Test. The intervention dosage in total minutes was also extracted from each article.
Results: Twenty-one articles were selected from the systematic review. Thirteen studies remained in the meta-analysis after excluding duplicate or missing data set (n=2), studies without the common assessments (n=4), and single subject studies (n=2). Two meta-analyses were conducted: one included studies with a stroke control group (n=5) and one without (n=8). While the effect size of most selected studies were not significant, both meta-analyses showed a significant overall effect of AMT on UE function post-intervention (p=0.021 and $p < 0.000$). This indicated the potential benefit of AMT in stroke. There was no heterogeneity in either meta-analysis, suggesting a low variability among studies. Further meta-regression also demonstrated no dosage-response effect (p=0.663 and p=0.231). Greater treatment time did not associate with better motor outcomes.**Conclusion/Discussion:**Our findings suggest that AMT has the potential to UE motor function in stroke survivors, compared to control treatments and pre-intervention performance. However, with the small number of articles and mostly non-randomized controlled trails (n=10), more high quality of research is needed to conclude a solid suggestion. In addition, the majority of the selected articles included AMT for a total of 5-10 hours (n=10). The low variability did not provide enough information for understanding optimal dosage of AMT. Music-based therapy is a developing but promising intervention. Future research should continue focusing in this area to help recovery for stroke survivors.

Disclosures: S. Archer: None. **A. Tomlin:** None. **G. Warren:** None. **Y. Chen:** None.

Digital Abstract Session

P206. Finger and Grasp Control: Age, Pathology, and Physiology

Program #/Poster #: P206.02

Topic: E.04. Voluntary Movements

Title: Effects of aging on rapid grip force during bimanual manipulation of an active object

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Abstract: Reports of an age-related deterioration in rapid grip force responses, which may be attributed to declined somatosensory and cognitive functions, are limited to a one-hand condition despite the higher frequency of using two hands together in daily living of older adults and the higher risk of dropping objects. Unexpected perturbations during bimanual movements elicit goal-oriented and cortically-meditated bilateral rapid motor responses. As aging is associated with declined somatosensory and cognitive functions, bilateral rapid motor responses may differ between young and older adults, such that older adults exert stronger grip forces following perturbation and the unperturbed hand is more involved in stabilizing the object in older adults. We tested this hypothesis by comparing the rapid grip force responses of both hands in young and older adults. A total of 13 right-handed young adults (24.2 ± 4.0 years old, 5 men) and 13 right-handed older adults (68.7 ± 7.1 years old, 5 men) performed a bimanual grip-lift movement, in which subjects held and leveled an object using thumbs and index fingers of both hands. Two different magnitudes of unpredictable pulling loads were applied to the right side of the object while subjects were leveling the object. Measures of rapid motor response included: latency, maximal grip force (MGF), ΔGF (difference between MGF and stabled grip force at pre-perturbation), peak grip force rate (PGF rate), and maximal grip-to-slip forces ratio (G/S ratio) of both hands. Additionally, the equivalence between left and right hands from kinetic data was quantified (L/R ratio). Regardless of age, the latency of the left hand was slower than the right hand, while the hand difference depended on perturbation magnitudes ($p < 0.05$). Older adults had higher values of MGF and PGF rate compared to young adults ($p < 0.05$). The analyses of MGF, ΔGF, and PGF rate demonstrated hand difference regardless of age; the magnitude of hand difference was dependent on the perturbation magnitude only in young adults but not in older adults ($p < 0.01$). The analysis of G/S ratio demonstrated that older adults exerted grip force three times higher than the slip force in both hands, while such relation between grip and slip forces was seen only in the right hand in young adults ($p < 0.005$). The analyses for L/R ratio suggested that the indirectly perturbed (left) hand was more involved in stabilizing the object in older adults compared to young adults ($p < 0.05$). The study extended the understanding of age-related changes in rapid grip force responses to unexpected perturbations. The age differences may be due to both declined peripheral and central nervous systems with aging.

Disclosures: H. Hibino: None. **N. Watson:** None. **S.L. Gorniak:** None.

Digital Abstract Session

P207. Reaching Control: Action and Sensation

Program #/Poster #: P207.01

Topic: E.04. Voluntary Movements

Support: grants-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (18020030, 18047027, 26120003, 19H05724, 19H01092)
Japan Science Technology Agency Precursory Research for Embryonic Science and Technology program (all to K.S.)
D.K. was a Japan Society for the Promotion of Science summer fellow supported by National Science Foundation Grant OISE-1108063.

Title: Are primates just big frogs? Forelimb force direction and amplitude are independently controlled by spinal motor modules

Authors: *A. YARON[1], D. KOWALSKI[2], H. YAGUCHI[1], T. TAKEI[3], K. SEKI[1];
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Abstract: Modular organization of the spinal motor system is thought to reduce the cognitive complexity of simultaneously controlling the large number of muscles and joints in the human body. Although modular organization has been confirmed in the hindlimb control system of several animal species, it has yet to be established in the forelimb motor system or in primates. Expanding upon experiments originally performed in the frog lumbar spinal cord, we examined whether co-stimulation of two sites in the macaque monkey cervical spinal cord results in motor activity that is a simple linear sum of the responses evoked by stimulating each site individually. Similar to previous observations in the frog and rodent hindlimb, our analysis revealed that in most cases (77% of all pairs), the directions of the force fields elicited by co-stimulation were highly similar to those predicted by the simple linear sum of those elicited by stimulating each site individually. A comparable simple summation of EMG output, especially in the proximal muscles, suggested that this linear summation of force field direction was produced by a spinal neural mechanism whereby the forelimb motor output recruited by co-stimulation was also summed linearly. We further found that the force field magnitudes exhibited supra-linear (amplified) summation, which was also observed in the EMG output of distal forelimb muscles, implying a novel feature of primate forelimb control. Overall, our observations support the idea that complex movements in the primate forelimb control system are made possible by flexibly combined spinal motor modules.

Disclosures: A. Yaron: None. **K. Seki:** None. **D. Kowalski:** None. **T. Takei:** None. **H. Yaguchi:** None.

Digital Abstract Session

P207. Reaching Control: Action and Sensation

Program #/Poster #: P207.02

Topic: E.04. Voluntary Movements

Support: NSF Grant 1753915

Title: Visuo-proprioceptive realignment: Rate, retention, and conscious awareness

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Abstract: We estimate hand position via proprioception and vision, which may not agree. E.g., viewing the hand underwater: water refracts light, displacing the visual estimate of the hand from the proprioceptive estimate. The brain compensates by realigning one or both. We have previously studied this phenomenon by gradually displacing a visual indicator of fingertip position from the true, unseen fingertip position, with no performance feedback. The mismatch can reach 70 mm without most subjects noticing. Subjects realign both visual and proprioceptive estimates, but usually this compensation totals only about 45 of the 70 mm perturbation. In Experiment 1 we asked whether the rate of visuo-proprioceptive misalignment affects the magnitude of realignment. The Slow, Medium, and Fast groups experienced misalignment at a rate of 0.84, 1.67, or 3.34 mm every two trials, respectively. Once misalignment reached 70 mm, this mismatch was maintained for the remaining trials. Results suggest that realignment in the Fast and Medium groups was similar during the gradual misalignment phase: 61 and 56 mm in total. No further realignment was evident during the maintenance phase. The Slow group realigned only 43 mm in total, suggesting that a slower misalignment rate may be less effective at eliciting realignment. Experiment 2 asked whether direct vision of the hand and/or active movement of the hand could disrupt retention of realignment. Four groups of subjects experienced misalignment at the medium rate to a maximum of 70 mm. After the misalignment phase, the Active/Vision group was given direct vision of the misaligned hand and asked to trace circles with their index fingers. The Active/No Vision group traced circles, but with no visual information. The Rest/Vision group was given direct vision of the misaligned hand while resting the hand on their lap. The Rest/No Vision group rested their hand without vision. After 5 minutes, subjects performed more trials to assess how much visual and proprioceptive realignment was retained from the end of the misalignment phase. Preliminary data shows evidence of retention in all four groups. Experiment 3 asked how much misalignment must occur before subjects begin to notice. Subjects experienced misalignment at the medium rate for 8 blocks of trials, reaching a max of 140 mm mismatch. After each block, subjects were questioned about their perception of the mismatch. Once they noticed the mismatch, subjects reported awareness of only about half the true mismatch magnitude. This suggests that visuo-proprioceptive perception of the hand is flexible enough to accommodate considerable spatial perturbation.

Disclosures: H.J. Block: None. **M. Wali:** None. **R. Babu:** None. **A. Hsiao:** None.

Digital Abstract Session

P207. Reaching Control: Action and Sensation

Program #/Poster #: P207.03

Topic: E.04. Voluntary Movements

Support: 1K01HD092558-01

Title: Evaluation of sensory integration and its impact on sensory-motor function post-stroke

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Abstract: Introduction: Post-stroke motor recovery can be frustratingly intractable. Despite rehabilitative efforts that focus on motor recovery, functional deficits remain even when strength is restored. Normal motor control requires real-time feedback from the sensory system to operate effectively. However, current standard of care rehabilitation approaches and testing metrics simply ignore the sensory side of the sensory-motor equation. Recent work from our lab provides evidence that amplified proprioceptive sensory feedback improves post-stroke motor control in an upper limb reaching task. The aim of this study was to examine the use of new functional metrics for upper limb sensory-motor function to provide insight into the effects of somatosensory impairment on upper limb motor control. Methods: A cohort study was conducted in which participants (N=2) with chronic stroke and residual upper limb dysfunction completed the Fugl-Meyer Assessment (FMA) evaluating motor function and two recently developed tests evaluating sensory-motor function. 1) During the block sorting test, subjects are asked to identify, manipulate, and transfer polyurethane rubber blocks of varying stiffness (soft, medium, or hard). Outcomes include accuracy, alpha and beta error rates, foraging times, overall efficiency, and discrimination efficiency. 2) The grasping precision test requires participants to quickly and accurately achieve a desired grasp force. Fitts'-based outcomes of interest include speed and peak precision, and the relationship between speed and precision (throughput). Results: The participants performed comparably on the FMA; however, they demonstrated variable performance on sensory-motor testing. One participant was within normative ranges of speed and efficiency on the block test, demonstrating mild difficulty with discrimination accuracy (75% correct) while the other was unable to discriminate firmness (accuracy = 38%) and had deficits with speed (discrimination efficiency of 1.2 %correct/sec). Maximal grip was similar for both patients at 166 N and 185 N. Throughput during the grip test was below normative values for both at 0.87 bits/s and 0.67 bits/s. Conclusion: Although the FMA did not resolve differences between subjects, the grip and block tests revealed distinct differences in how participants integrated sensory information into function, indicating that data from both systems are necessary to provide comprehensive upper limb function assessments. Functional tests with high resolution that evaluate the integration of the sensory system can guide clinical interventions and measure the effects of training on sensory-motor function.

Disclosures: S.M. Linder: None. **Z.C. Thumser:** None. **D.T. Beckler:** None. **P.D. Marasco:** None.

Digital Abstract Session

P207. Reaching Control: Action and Sensation

Program #/Poster #: P207.04

Topic: E.04. Voluntary Movements

Support: NSF Grant 1849067

Title: Timing estimates are biased by viscous movement environments

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Abstract: Time perception is a phenomenon that is highly influenced by sensory and cognitive processes. However, there are surprisingly few investigations into the involvement of the motor system on timing. This is particularly important, as organisms rely on continuous sensorimotor feedback to navigate a world with rich temporal dynamics. Recent research suggests that movement parameters (e.g., speed and direction) can bias timing estimates, and more generally, that movement can improve timing sensitivity. While this work characterizes the role of volitionally modulated movement parameters, the influence of external modulation (e.g., environmental perturbations) is relatively unexplored. In this experiment, 18 right-handed participants (7 female, 11 male, M age = 21.5(4.1)) completed a temporal reproduction task in which intervals were timed during hand movement in viscous movement environments. We hypothesized that viscosity would restrict movement to shorter lengths, and likewise bias timing estimates to be shorter. Participants performed this task using a robotic arm manipulandum. During the encoding phase, they were instructed to continuously move their right arm through the two-dimensional workspace (i.e., “try not to stay still”) while listening to an auditory tone ranging from 1-4s. In each trial, one of four viscosity values (0, 12, 24, or 36 Ns/m²) was randomly applied to the robotic handle to increase resistance, and thus shorten movement. Next, they reproduced the duration in a central location (handle locked in place) by holding and releasing a button attached to the handle. Notably, including movement during encoding only allowed us to isolate observed movement effects to perceptual timing. Participants performed 280 trials, and trials were excluded if reproduction times were outside of 3 standard deviations from the mean. Our analyses confirmed that viscosity shortened movement in a graded way [$F(3,51)=149.82$, $p < 0.001$, $\eta 2p =0.898$]. By utilizing a Bayesian observer model, we isolated the individual components of the timing process (e.g., bias, estimation noise, and production noise), which revealed an effect of viscosity on perceptual bias (offset parameter) [$F(3,51)=3.72$, $p = 0.017$, $\eta 2p =0.18$]. This suggests that viscosity impacted perceptual, but not cognitive processes during timing. These results strongly indicate that further investigations into external movement influences are needed in timing research, and can help move research towards understanding time-keeping in real world settings where motor plans undergo continuous updating and encounter various environmental perturbations.

Disclosures: **W. Zhou:** None. **W. Joiner:** None. **R. De Kock:** None. **M. Wiener:** None.

Digital Abstract Session

P207. Reaching Control: Action and Sensation

Program #/Poster #: P207.05

Topic: E.04. Voluntary Movements

Support: NIH Grant 5R00NS097620
Cedars-Sinai

Title: Coordinated movement-related activity emerges across motor cortex and cerebellum during skill learning

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Abstract: The control of forelimb movement and learning new motor skills is distributed across several regions of the brain. This allows for precise limb coordination that is mediated by appropriate descending commands as well as sensory feedback while executing a consolidated task. Cerebellum and the motor cortex (M1) are two densely interconnected regions that are linked to motor learning. Yet, little is known about how the network dynamics in these two regions change when learning a dexterous skilled motor behavior. We have performed simultaneous recordings in M1 and cerebellum in awake behaving rodents (n=3) and profiled the properties of cortico-cerebellar communication that develops while learning a skilled reach-to-grasp task.Single-unit and local field-potential (LFP) recordings were collected from the M1 and cerebellum's Crus II and paramedian lobules while the rats were trained in a skilled forelimb reaching task. We analyzed how the behavior changes from early days of training to late days and the associated power-spectral changes in M1 and cerebellum. We also analyzed movement-related neural coherence across the M1 and cerebellum and specifically, the changes in phase relationships between the spiking activity and the LFP frequency bands both within and between the two regions. Finally, we also explored the representation of neuron activity in M1 and cerebellum through Gaussian-process factor analysis (GPFA). Once the rats had become proficient we saw coordinated low-frequency (< 4 Hz) activity across the M1 and cerebellum that was closely related to the sub-movements in the reach-to-grasp task. Movement-related LFP coherence between M1 and cerebellar LFP also increased in the low frequency range. Furthermore, we also found that this low-frequency oscillatory activity in LFPs modulated the phasic spiking activity of the neurons in each region. GPFA analysis found more correlation within the low-dimensional factors representing population spiking activity in the M1 and cerebellum in individual trials as the skill consolidates. Our work suggests that coordinated low-frequency activity is present in cortico-cerebellar networks and that such rhythmic activity may allow for the coordination of neuronal activity across regions that sub-serves skill learning.

Disclosures: **P. Fleischer:** None. **A. Abbasi:** None. **N. Danielsen:** None. **T. Gulati:** None.

Digital Abstract Session

P207. Reaching Control: Action and Sensation

Program #/Poster #: P207.06

Topic: H.10. Human Learning and Cognition

Support: NIH NIGMS GM103642
NIMHD 2U54MD007587
NIH R25 NS08068

Title: The Effects of Musical Education in Rhythmic Perception and Production in Puerto Rican Adults with or without Musical Training

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Abstract: Our remarkable musical abilities seem to be rooted in biological principles and the inherent capacities of the human brain. In this study, we seek to understand the intrinsic rhythmic capabilities in Puerto Rican adults, as well as consider how musical education influences these abilities. To account for these skills, Puerto Rican adults (over 21 years of age), with or without musical training, participated in a series of rhythmic perception and production tests. The tests were the following: Metronome Synchronization, Free Tapping and the Montreal Battery of Evaluation of Musical Abilities (MBEMA). The rhythmic outputs were quantified by measuring the Inter Beat Intervals (IBI). We expect that musicians will display less variability and more accuracy in both rhythmic perception and production. Although all participants reflected a consistent rhythmic output, preliminary data reflects less variability in musicians during the rhythmic production tests when compared to non-musicians. In addition, the participants obtained an overall score of 85% or higher in the rhythmic perception test. This supports that although there seems to be inherent capabilities for rhythmic abilities, musical training may enhance these skills. By characterizing rhythmic perception and production in Puerto Rican adults and children, we hope to shed light on the maturation of rhythmic abilities during brain development and adulthood.

Disclosures: A.F. Edwards-Cintrón: None. **M. Rodríguez:** None. **D. Pérez:** None. **A. Miquelajáuregui:** None. **A. Ravignani:** None.

Digital Abstract Session

P207. Reaching Control: Action and Sensation

Program #/Poster #: P207.07

Topic: D.08. Visual Sensory-motor Processing

Support: CIHR
VISTA

Title: Visuomotor transformations in ventral premotor cortex during head-unrestrained reaches.

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Abstract: Reaching involves a coordinated sequence of gaze, head, and arm movements toward a visual stimulus. Several studies have examined eye-head-hand coordination in the human, but the underlying neural mechanisms, especially those controlling head motion, have not been studied. We addressed this problem by recording single neurons from ventral premotor cortex (PMv) while two trained monkeys performed a reaching paradigm, that allowed unencumbered head motion and reaching in depth. Animals touched one of three central LEDs (different initial hand locations) at waist level while maintaining gaze on a central fixation dot (with a jitter of 7-10° from trial to trial) and were rewarded if they touched a target appearing at one of 15 locations in a 40° x 20° (visual angle) array. Preliminary analysis of 271 neurons in both monkeys showed an assortment of target/stimulus, gaze, pre-reach and reach related responses in PMv. Most neurons could be described as falling into three main groups. 'Early" neurons increased firing rate during the target presentation and gaze onset. "Early-late" neurons responded in a sustained way from target presentation until reach. Finally, "Late" neurons increased firing rate during the pre-reach and peak when the monkey reaches. We first tested for gaze, head and hand gain fields during the different neuronal responses and found in both animals that 38% of target, gaze, pre-reach and reach-aligned responses were gain modulated by initial hand position. A small fraction of neurons showed gain fields for initial eye position (4%), and for both initial eye and hand position (6%). After removing the gain field effects, we fitted the residual data against various spatial models and found that "Early" neurons best coded the target (T) during target presentation and, during gaze shifts, preferentially coded displacement of the arm (dA). This T-dA transformation occurred 150-200 ms after target onset. "Early-late" neurons coded dA from target presentation until reach and "Late" neurons best coded T during pre-reach and arm position in future space (Afs) during reach. A more complete analysis will aim to describe the complete coding and distribution of gaze, head and reach signals in this region.

Disclosures: V. Nacher: None. **P. Abedi-Khoozani:** None. **V. Bharmauria:** None. **H. Arora:** None. **X. Yan:** None. **S. Sun:** None. **H. Wang:** None. **J.D. Crawford:** None.

Digital Abstract Session

P208. Reaching Control: Movement Selection and Strategy

Program #/Poster #: P208.01

Topic: E.04. Voluntary Movements

Support: PRIN 2017 to A Battaglia Mayer prot. N. 201794KEER_002

Title: Acting alone or together? Measuring the cost of inter-individual motor coordination in non-human primates

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Abstract: Motor coordination between individuals is costly but can also be advantageous when making a goal accomplishable. We previously showed (Visco-Comandini et al, Cortex, 2015) that monkeys engaged in a joint action task pay their cost to achieve a common goal, by adjusting the motor behavior to coordinate with their mates. We now study monkeys' attitude to act together, when allowed to freely choose whether to achieve a goal individually or jointly, on the basis of the payoff of each option. Two rhesus monkeys, using an isometric joystick, guided each a cursor shown on a screen from its center to one of two alternative targets (left/right) to gain the offered amount of reward, which was cued by a level bar inside each target. The targets' color instead cued the type of action (SOLO or TOGETHER) required to gain the offer. In the SOLO option, monkeys obtained the expected reward by moving their cursor alone, while in the TOGETHER one, the animals had to reciprocally coordinate their forces to bring jointly their cursors into the selected target and gain their treat. The rewards associated to the two options differed by 5 possible values (Δ_i, i=0, ⋯ ,4) equally spaced from 0 to 0.60 ml. As a control, monkeys were required to choose between two SOLO or two TOGETHER options, with different payoff.When choosing between targets associated to the same action type both monkeys aimed at the higher offer with similar probability. When facing the SOLO vs TOGETHER choice both animals showed an utter preference for the SOLO action regardless of the offer. This preference decreased without being reverted just for a reward difference higher than 0.15 ml in favor of the TOGETHER option, increasing the choice rate from 0% to about 40%, in both animals. We hypothesized that the offered payoff difference initially adopted was always insufficient to motivate monkeys to choose unequivocally the costly TOGETHER condition over the SOLO one, even when maximally rewarded (Δ_4). However, by doubling the Δ_i - thus ranging from 0 to 1.20 ml - the action condition preference drastically changed: the SOLO action was not always preferred, and 100% of choice rate in favor of the TOGETHER option was observed for a difference in the two options' values above 0.90 ml. The indifference point between the TOGETHER and SOLO offers was found at $\Delta = 0.30$ ml, indicating the perceived cost of acting together. In conclusion, when monkeys chose between acting alone or together, their choice was not merely dictated by the payoff value, but their economic evaluation was subdued to the high cost of motor interindividual coordination. Thus, monkeys can accurately estimate this cost and use it to decide whether to act individually or jointly with others.

Disclosures: I. Lacal: None. **A. Schito:** None. **L. Caratelli:** None. **A. Battaglia-Mayer:** None.

Digital Abstract Session

P208. Reaching Control: Movement Selection and Strategy

Program #/Poster #: P208.02

Topic: E.04. Voluntary Movements

Title: Prolonged eye-hand decoupling deficits in young adults with concussion history from adolescence: issues with task novelty or ongoing task demand?

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Abstract: Previous work reported prolonged spatial planning deficits during eye-hand decoupling tasks in young adult non-athlete college students with a sport-related concussion history (CH) from adolescence. The individuals with CH displayed a larger initial direction error and a trend to fail more often to decouple the movement direction of the eyes and hand at response onset than controls with no concussion history. The previous work focused on performance across the whole series of trials. We now expand this work and investigate potential time-related changes of these deficits along this series of trials, to examine whether these eye-hand decoupling deficits are rather due to problems with adapting to new task constraints or with meeting ongoing task demand. We re-analyzed data from 21 CH participants (M=48 months post-concussion) and 20 controls with no-history of concussion (NoH) (M=21 yrs. in both groups). Participants performed two eye-hand coordination tasks, a direct task with vision and motor action in alignment, and an eye-hand decoupling (EHD) task where vision and motor action were spatially decoupled. Twenty trials were performed per task condition, with five movements from a central target to each of four target directions (up, down, left, right), thus, movement trials were split into five blocks of four trials (i.e., trial 1-4, 5-8, 9-12, 13-16, and 17-20). We analyzed movement planning (direction reversal error [DRE] at response onset, reaction time, initial direction error [IDE] 100 ms after response onset) and execution related variables (movement time, path length, endpoint error) across blocks (1-5) and groups (CH, NoH) in condition EHD.ANOVA revealed CH trended to perform more DRE than NoH in the EHD condition ($p=0.08$), independent of blocks. For IDE, ANOVA revealed a main effect of group ($p<0.001$), block ($p<0.01$), and a significant block x group interaction ($p<0.05$). Post hoc test revealed IDE was larger in CH than NoH in block 1 and 2 (both $p<0.01$) but not in block 3-5 (all $p>0.05$). All other variables did not show significant group or group x block effects in condition EHD (all $p>0.05$).The present results suggest that the previously reported spatial movement planning deficits of young adults with a concussion history from adolescence while performing a series of eye-hand decoupling trials relate to issues with adapting to new task constraints. Our findings also suggest a trend for ongoing inhibition control issues (i.e., a larger DRE) along a series of twenty trials in the CH group. The current work enhances our understanding of performance issues during eye-hand decoupling tasks in young adults with concussion history about four years post-injury.

Disclosures: **M. O'Neil:** None. **B. Jones:** None. **M. Dalecki:** None. **B. Phillips:** None.

Digital Abstract Session

P208. Reaching Control: Movement Selection and Strategy

Program #/Poster #: P208.03

Topic: E.04. Voluntary Movements

Support: NSF CAREER 1943276
NIH R01NS110866

Title: The importance of inhibition in grooming action choice

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Abstract: Complex behaviors such as courtship or catching food require series of coordinated actions. How nervous systems solve the problem of assembling flexible behavioral sequences is an important unresolved question. We study grooming behavior in the fruit fly, *Drosophila* as a model to investigate circuits that regulate sequential behaviors. Flies covered with dust select different motor programs among many competing choices. They clean only one body part at a time and prioritize anterior body cleaning over posterior (Seeds et al., 2014; Hampel et al., 2015, 2017). Here, we investigate the role of specific inhibitory interneurons in regulating the choice of grooming actions. Inhibition controls movement of limb joints (Grillner, 2006; Kiehn, 2011; Goulding et al., 2014) and regulates selection among different behaviors (Gaudry and Kristan, 2009; Stephenson-Jones et al., 2011; Mann et al., 2013). Humans and flies share Gamma-aminobutyric acid (GABA) as a major inhibitory neurotransmitter (Hosie et al., 1997; Ennel et al., 2007; Fei et al., 2010). Although inhibition is key for motor control, common design principles that enable execution of flexible motor sequences are not yet obvious.
We performed a targeted behavioral screen to identify specific inhibitory neurons that disrupt normal grooming. We use sensory cues to initiate grooming and test behavioral consequences of optogenetic activation of interneurons. We identified subsets of GABAergic lineage 13 neurons that are involved in both inter- and intra-segmental coordination. Lineage 13 consists of two hemilineages, 13A and 13B that project to the ipsilateral and contralateral regions respectively in a given segment of ventral nerve cord (VNC) (Harris et al., 2015). Some disrupt essential left-right limb coordination. Activation of other inhibitory neurons disrupts the grooming hierarchy, causing flies to prioritize posterior body cleaning over anterior. Inhibition could act directly at the sensory level, since RNAi knockdown of Rdl receptors in mechanosensory neurons results in a similar phenotype. Preliminary connectivity analysis using an electron-microscopy dataset (Maniates-Selvin et al., 2020) revealed interesting presynaptic connections. These include commissural interneurons, possibly involved in left-right coordination; descending neurons that project from brain to the VNC; and intersegmental neurons, possibly involved in sequential action selection. The neural circuit motifs identified in this study will provide insights into how a relatively simple nervous system coordinates the order and execution of sequential actions.

Disclosures: D.S. Syed: None. **J.H. Simpson:** None.

Digital Abstract Session

P208. Reaching Control: Movement Selection and Strategy

Program #/Poster #: P208.04

Topic: E.04. Voluntary Movements

Support: Marquette University Strategic Innovation Fund
Marquette University Athletic and Human Performance Research Center
Marquette University Opus Dean's Endowment

Title: Independent Adaptations of Movement Direction and Extent During Goal-directed Reaching

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Abstract: Existing concussion assessments focus on symptoms, signs, and changes in overt behavior and measurement of cognitive performance that engage explicit memory systems but do not assess implicit motor memories. Recent findings (Brown JA et al, 2015) show that people with a history of concussion exhibit pronounced deficits in movement timing and accuracy relative to healthy controls when the brain has to resolve more than one level of decoupling between vision and action. Here we sought to develop a robust concussion assessment by establishing normative performance on a challenging reach task requiring simultaneous adaptations to distinct perturbations of movement direction and extent. Seven subjects performed 600 ballistic, 10 cm, out-and-back reaches to 5 targets at -60°, -30°, 0°, 30°, 60° from the participant's sagittal plane. Subjects moved a 2-joint robot handle outward in the horizontal plane against spring-like loads that changed unpredictably from trial to trial. Direct view of the arm was blocked. Subjects received feedback of hand location on a monitor in the vertical plane. Visual feedback was faithful during the first 150 trials (Baseline), a 30° visuomotor rotation was applied for 300 trials (Rotation) and then removed for 150 trials (Washout). Kinematic performance was quantified using reach direction error and extent error at the point of maximum extent. All subjects reached with minimal direction and extent errors during Baseline. At the start of the Rotation Block, both direction and extent errors increased, but the time course of subsequent error reduction differed markedly between the two error measures. Whereas direction errors were corrected within 10-15 trials, extent errors took more than 50 trials to return to baseline. On removing the rotation (Washout), direction errors showed clear aftereffects whereas reach extent was unaffected by removal of the imposed rotation. Because the rotation impacted movement direction but not extent, and the spring loads impacted movement extent but not direction, the results show that the sensorimotor compensations used to adapt movement direction are distinct from those mediating adaptation of movement extent. Thus, our findings support separate planning and control of reach direction and extent (Bhat RB & Sanes JN, 1998).

Future studies are planned to use this composite task to study the impact of concussion on these two aspects of sensorimotor control.

Disclosures: D. Peters: None. **L.A. Mrotek:** None. **D. Lantagne:** None. **R. Scheidt:** None. **J. Hoelzle:** None. **D. Thomas:** None.

Digital Abstract Session

P208. Reaching Control: Movement Selection and Strategy

Program #/Poster #: P208.05

Topic: E.04. Voluntary Movements

Support: NSF M3X-1825942
NIH-R01-HD087089
NSF M3X-1826097

Title: Variability of Arm Kinematics in Hitting a Target with a Bull Whip

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Abstract: Over evolution, humans have become exquisitely adept at manipulating complex objects, ranging from chopping onions to spreading a tablecloth. However, to date motor control studies have focused on simple highly controlled motor tasks typically excluding contact or interaction to assure experimental control. As an overture to study the variety of skills that humans display in their daily actions, this study chose one of the most challenging motor skills: manipulation of a flexible underactuated object—a bull whip. We studied whole-body movements when swinging a 1.6m long bull whip to hit a target at ~2.2m distance with the tip of the whip. A first question was how different manipulation styles achieved hitting accuracy. Sixteen subjects were asked to throw a whip in two variants: hitting the target in a single-shot discrete movement and in a rhythmic action that kept the whip in the air. All subjects performed both styles for 5 blocks, each consisting of ~30 trials. Three-dimensional body and whip kinematics were tracked using a marker-based motion capture system. Seven joint angles of the arm were calculated.Performance was quantified by target hits and mean and variability of error. The 16 participants showed a wide range of performance success. However, all participants showed better hitting performance in the discrete style, although improvements across blocks only occurred in the rhythmic style. Based on this wide range of performance levels, the next focus was on how inter-joint coordination of the arm prepared an accurate whip throw. Given the redundancy of the multi-joint arm, principal components analysis assessed the dimensionality of the throwing movement evolving across the duration of the throw. We hypothesized that more

successful subjects reduce their variability as they approached the critical throw onset. We further hypothesized that, as subject improve, they learn to exploit the redundancy in the task and reduce joint angle variability in the latent dimensions that affect task performance. First analyses showed results consistent with these hypotheses.This analysis of a highly complex motor task aims to shed light onto how humans learn to adjust their joint coordination when interacting with a complex object. This study presents the first step in a new experimental paradigm that challenges current motor control notions and sets the stage for more hypothesis-driven analysis.

Disclosures: **M. Edraki:** None. **R. Sharif Razavian:** None. **A.D. Krotov:** None. **M. Russo:** None. **M.C. Nah:** None. **N. Hogan:** None. **D. Sternad:** None.

Digital Abstract Session

P208. Reaching Control: Movement Selection and Strategy

Program #/Poster #: P208.06

Topic: D.07. Vision

Support: Mitacs Accelerate International Award
NSERC Discovery Grant RGPIN-2014-05248

Title: Going through the motions: Evaluating the consistency of mouse-tracking derived measures of decision difficulty across three reach-decision tasks

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Abstract: As decisions require actions in order to have an effect on the world (Cisek & Kalaska, 2010), measures derived from movements can be used to provide a powerful index of decision-making processes (e.g. Gallivan & Chapman, 2014). Measures of trajectory curvature (interpreted as a competitive pull from the non-chosen choice; Spivey, Grosjean, & Knoblich, 2005), reaction time, and movement time obtained during mouse-tracked, reach-decision tasks thus provide a metric of the relative difficulty of decisions (McKinstry, Dale, & Spivey, 2008). While these measures of decision difficulty have been demonstrated across a variety of decision domains, they are reported in different studies with different groups of participants. To our knowledge, no study has determined whether the same measures of decision difficulty are consistent for an individual across different decision tasks. To that end, the current study aims to 1) replicate task-specific metrics of competition between choice options in three independent decision domains differing in choice stimuli, objectivity and processing requirements and 2) assess whether within-participant measures of decision difficulty remain consistent across the decision domains. Deploying a classic mouse-tracking, reach-decision paradigm, participants (n = 46) were asked to complete a numeric-size congruency (SC) task requiring objective perceptual judgements of which of two digits with different physical sizes was numerically larger (Faulkenberry *et al.*, 2016), a sentence verification (SV) task requiring semi-subjective

conceptual judgements about the truth value of statements varying in truth value and negation (Maldonado *et al.*, 2019), and a photo preference (PP) task requiring subjective judgements of preference between two images varying in pleasantness (Koop & Johnson, 2013). Broadly, task-specific results replicated previous work: SC: We found an increase in decision difficulty when digit choice options were incongruent in physical and numeric size; SV: Measures of decision difficulty increased when participants were asked to affirm negated sentences compared to non-negated sentences, with greater negation-driven difficulty effects for true statements than false statements; PP: Images matched in pleasantness showed increased decision difficulty compared to image options that differed in pleasantness. Ongoing analyses are examining 1) across-participant correlations to determine if participants exhibiting high degrees of decision difficulty are consistent across tasks and 2) within-participant correlations to examine how measures of mouse-derived decision difficulty are related.

Disclosures: A.A. Ouellette Zuk: None. **C.S. Chapman:** None.

Digital Abstract Session

P209. Reaching Control: Motor Learning ? Human Psychophysics

Program #/Poster #: P209.01

Topic: E.04. Voluntary Movements

Title: Computational limits on the speed of learning internal models for arm reaching

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Abstract: An important aspect of motor learning is the formation of internal models, which encode the physics of a motor task in the form of mappings between motor commands and actions (forward models), and vice versa (inverse models). Different learning algorithms such as feedback error learning, reinforcement learning and distal learning have been proposed to explain the formation of inverse models. These algorithms attempt to minimize errors by adjusting parameters of the inverse model. Here we used a distal learning-based model to investigate the aspects of isometric and kinematic arm reaching tasks that influence the speed of learning an inverse model of the task. Using convex optimization techniques, we analyzed the shape of the error surface in the space of the inverse model parameters. The eigenvalues of the Hessian of the error surface around a minimum describe the shape of the surface, which for gradient-following algorithms, also determines the maximum speed of learning for a given task. We show that for reaching tasks, the shape of the error surface and the speed of learning are determined by the shape of the manipulability ellipsoid of the arm, and by the distribution of targets in the task. In particular, the rounder the manipulability ellipsoid and the target distribution, the faster learning can take place. We tested these results experimentally in a virtual quasi-isometric reaching task where the force manipulability ellipse of the arm is controlled through a virtual squeezing mapping between arm forces and cursor movement. The results (5 subjects, 3 male) showed that rounder virtual force manipulability ellipses allow faster learning

of a 30° visuomotor rotation. This is consistent with our theoretical predictions. Furthermore, our analytical framework was able to predict the speed of learning in several experiments published in the literature. For instance, differences in the speed of learning incompatible and compatible virtual surgeries, and differences in the speed of learning a visuomotor rotation with different numbers of targets are well accounted for by our analysis. By identifying factors that influence the speed of learning, our results provide theoretical principles for the design of motor tasks that allow for faster learning.

Disclosures: V.R. Barradas: None. **N. Schweighofer:** None. **Y. Koike:** None.

Digital Abstract Session

P209. Reaching Control: Motor Learning ? Human Psychophysics

Program #/Poster #: P209.02

Topic: E.04. Voluntary Movements

Support: NSERC

Title: The effect of instructions on de novo learning and the mechanisms that distinguish it from motor adaptation

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Abstract: The environments that people move in are constantly changing. Such changes may lead to movement errors, which we must process in order to produce correct movements. This error processing occurs regardless of whether people are acquiring a new motor skill (de novo learning) or adapting a well-known movement (motor adaptation). However, these two types of motor learning should develop differently. Furthermore, while reach aftereffects or the persistent deviation in reaches after perturbation removal are typically observed following adaptation, switching between response mappings in de novo learning should not lead to aftereffects. Here, we conducted two experiments that differentiate de novo learning from adaptation and explore the mechanisms of de novo learning further. In experiment 1 (N = 16), we distinguished the two motor learning types by having each participant train with two perturbations in counterbalanced order: a 30-degree visuomotor rotation and a reversal of cursor feedback in the opposite direction of a mirror axis. Importantly, we matched the movements required to reach the targets in both tasks. We found no order effects, suggesting that learning for one perturbation does not affect the other. Although participants countered for both perturbations by the end of only 90 training trials, learning for the rotation task was more gradual while variability in learning was greater for the mirror task. Participants generally took longer to initiate and execute reaching movements in the mirror task. Moreover, participants only exhibited reach aftereffects after completing the rotation task. In experiment 2, we developed an online version of the mirror task, with target locations placed farther from the mirror axis than in experiment 1. Here, we compared how learning progressed when either providing participants with explicit instructions about the nature

of the mirror reversal (N = 115) or not providing such instructions (N = 112). Surprisingly, learning occurred quickly, even for the non-instructed participants. Regardless of instructions, however, asymptotic learning of participants differed depending on target location and reach aftereffects were not observed. These results show how the development of de novo learning is distinct from adaptation, and future work should investigate the behavioural and neural processes underlying these two types of motor learning.

Disclosures: R.Q. Gastrock: None. **B.M. 't Hart:** None. **D.Y.P. Henriques:** None.

Digital Abstract Session

P210. Reaching Control: Motor Learning: Human Neurophysiology

Program #/Poster #: P210.01

Topic: E.04. Voluntary Movements

Support: Travis Roy Foundation

Title: Mapping of cervical spinal cord in humans shows stronger muscle responses to lateral than midline stimulation

Authors: *J. R. MCINTOSH[1,4], S. C. KARCESKI[5], O. MODIK[5], E. SHELKOV[5], N. PATEL[6], S. OH[1,2], J. L. GOLDBERG[4], N. Y. HAREL[7,8], M. VIRK[4], J. B. CARMEL[1,3];
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Abstract: When individuals who have had spinal cord injury are treated with continuous lumbar epidural spinal cord stimulation they are able to stand and walk, demonstrating the effectiveness of this technique for restoration of movement. However, the question of how best to deliver electrical stimulation for sustained arm and hand function recovery remains open. In particular, how epidural stimulation activates human cervical spinal cord circuits is poorly understood. Work in rats has indicated that stimulation delivered over the dorsal root entry zone recruits afferent axons more strongly than stimulation directly over the midline. Based on this, we hypothesized that in humans, stimulation applied more laterally, where afferents enter the spinal cord at the dorsal root entry zone, would produce stronger muscle responses compared to stimulation delivered over the midline. We mapped cervical spinal cord responses to epidural stimulation in 10 participants undergoing clinically indicated cervical laminectomy. EMG was recorded in multiple arm and leg muscles, and a hand-held bipolar electrode was used to stimulate the exposed dorsal spinal cord. We measured the threshold stimulation intensity required to evoke activation of a targeted muscle (triceps or ADM) at the midline of the most caudal surgically exposed segment (usually C7). Multiple rostrocaudal and midline-lateral sites were then stimulated at 120% of this threshold. Analysis was conducted on the area under the curve (AUC), calculated by integrating the evoked responses. Stimulating laterally produced

larger muscle activation than stimulating at midline across subjects (median midline AUC = 0.93μVs, lateral AUC = 2.30μVs, Wilcoxon signed-rank test, p = 0.014, n = 10), corresponding to a 131% increase. By demonstrating greater responses when stimulation was delivered directly over the dorsal root entry zone, our results are consistent with a growing body of work indicating that epidural stimulation of the spinal cord primarily activates afferents. More generally, this study paves the way for further work investigating associative plasticity that can be induced in the cervical spinal cord by combining brain stimulation and spinal epidural electrical stimulation to aid recovery in spinal cord injury.

Disclosures: J.R. McIntosh: None. **S.C. Karceski:** None. **O. Modik:** None. **E. Shelkov:** None. **N. Patel:** None. **S. Oh:** None. **J.L. Goldberg:** None. **N.Y. Harel:** None. **M. Virk:** None. **J.B. Carmel:** None.

Digital Abstract Session

P210. Reaching Control: Motor Learning: Human Neurophysiology

Program #/Poster #: P210.02

Topic: E.04. Voluntary Movements

Support: French National Research Agency in the framework of the " Investissements d'avenir" program (ANR-15-IDEX-02)

Title: Decoding motor error correction and adaptation through phase and amplitude components of the EEG signal.

Authors: *L. STRUBER, M. BAUMONT, P.-A. BARRAUD, V. NOUGIER, F. CIGNETTI; TIMC-IMAG, Univ. Grenoble Alpes, CNRS, Grenoble INP*, Grenoble, France

Abstract: In order to achieve goal-directed movement, our brain simulates movement dynamics with an internal model. Error between actual and expected consequences of the movement drives update of this model, a.k.a. motor adaptation. Only a few studies focused on the oscillatory brain activities associated with recalibration of an internal model. We proposed here a sensitive data-driven multivariate approach to explore EEG signal characteristics and come up with an extended understanding of motor adaptation. To this end, 19 healthy subjects performed aiming movements with a joystick, while a visuomotor distortion (constant rotation) was applied between the joystick and the visual feedback. Two other experimental conditions were also considered, one without distortion, including neither error nor model update, and one with a random rotational perturbation in which errors were present but without model update. Oscillatory power and phase locking signals were estimated from 128-channel EEG through Hilbert transform. Then, a multiple kernel learning (MKL) approach with a high number of features was considered to decode early from late stages of visuomotor adaptation. Secondarily, adaptation condition was also classified against the other conditions to tease apart internal model update and error correction processes. MKL discriminated between early and late adaptation (accuracy: 87%; p = 0.002) from modulations of EEG power during post-movement.

Specifically, weights of the kernels revealed three frequency bands contributing to the discrimination: a theta band contribution in frontal and premotor regions, a beta band contribution in the supplementary motor area, and a gamma band contribution in motor regions, explaining respectively 29%, 18% and 30% of the classification. For all these band/region couples, adaptation process led to an increase of post-movement event-related synchronization. Further investigation showed a strong contribution of high frequency bands (beta and gamma, 46% and 41%) in the classification of the no perturbation vs. random perturbation conditions (accuracy: 74%; $p = 0.016$), between which only error size varied. Besides, classification of no perturbation vs. adaptation (accuracy: 82%; $p = 0.002$), in which both error and model update differed, showed a strong contribution of theta band (66%), and to a lesser extent of beta and gamma bands (11 and 15%, respectively). Altogether, our results indicate that post-movement frontal theta synchronization is most related to model update, whereas post-movement motor high-frequency beta and gamma synchronization is most associated to error processing.

Disclosures: L. Struber: None. **M. Baumont:** None. **P. Barraud:** None. **V. Nougier:** None. **F. Cignetti:** None.

Digital Abstract Session

P210. Reaching Control: Motor Learning: Human Neurophysiology

Program #/Poster #: P210.03

Topic: E.04. Voluntary Movements

Title: Neural correlates of visuomotor adaptation with workspace manipulation

Authors: *R. N. ADDISON, F. STEINBERG, A. W. VAN GEMMERT;
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Abstract: Background: Visuomotor adaptation tasks provide insights into how humans integrate sensory information to interact with our environment. Successful updates of motor plans ensues in visuomotor adaptation. Effective visuomotor adaptation might be workspace specific and associated neural activity, in particular the central and parietal areas, may differ due to adaptation within different workspaces. **Objective:** This study examines neural activity changes due to visuomotor adaptation to workspace location manipulation. **Methods:** Twenty right-handed healthy young adults were instructed to move a stylus from a home position to a target on a digitizer tablet using a stylus while visual feedback of the drawing trace was rotated 45 degrees on the monitor. Three different workspace location conditions were employed: 1) Digitizer located central to the body's midline (control condition; CON), 2) lateral to the body requiring the hand holding the stylus not to cross the participant's midline (NMC), and 3) lateral to the body requiring the hand holding the stylus to cross the participant's midline of the body (CML). Participants started and ended the experimental session with the CON condition, while NMC and CML conditions were counterbalanced across participants. During performance, neural activity through electroencephalography (EEG) with 32 electrodes was recorded and for each condition the power spectra of alpha and beta waves were computed. A repeated measures

ANOVA with workspace location as within factor was performed for five behavioral variables (Pathlength, movement time, resultant velocity, normalized jerk, and endpoint error), as well as for neural activity measures (alpha and beta waves). **Results:** All behavioral measures, except normalized jerk, and mainly the neural activity measures of the central and parietal areas were significantly affected by the workspace conditions. Tukey's test for post hoc analysis revealed differences in behavioral measures and neural correlates of the different workspace manipulation. Workspace condition differences of alpha waves were mostly observed unilaterally in the right hemisphere of the central and parietal areas (C4, CP2, CP6, P4, and P8), whereas beta waves showed bilaterally differences between workspace conditions (C3, CP1, CP2, P3, and P4). **Conclusions:** Workspace location affects alpha and beta neural activity of central and parietal areas together with behavioral performance measures when performing a visuomotor task. Therefore, this study supports the view that these brain areas are pivotal to process spatial information during the adaption process when novel tasks are performed.

Disclosures: R.N. Addison: None. **F. Steinberg:** None. **A.W. Van Gemmert:** None.

Digital Abstract Session

P210. Reaching Control: Motor Learning: Human Neurophysiology

Program #/Poster #: P210.04

Topic: E.04. Voluntary Movements

Title: Beta Band Decreases in Hippocampus During Motor Execution in a Delayed Reach Task

Authors: A. M. TANG, R. MARTIN DEL CAMPO-VERA, K.-H. CHEN, A. S. GOGIA, R. SEBASTIAN, G. NUNE, C. Y. LIU, S. KELLIS, *B. LEE;
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Abstract: Introduction:
The role of the hippocampus in motor planning and execution is a documented area of ongoing investigation. Much of previous research has focused on the hippocampal beta frequency band (13-30 Hz), particularly focusing on beta band increases and decreases during phases of movement planning and execution. During simple reaching tasks, the beta band has been shown to decrease during movement. Conversely, beta band increases have been observed during Go/No-Go tasks requiring movement inhibition. However, despite the increasingly clear relationship between movement and beta band modulation, it remains unclear if modulation is a reflection of planning or execution itself. In this study, a delayed reach task is utilized to elucidate the temporal properties of movement-related beta band modulation.

Methods:
Twelve participants with epilepsy underwent hippocampal stereo electroencephalographic (SEEG) implantation. Local field potentials (LFPs) were sampled throughout a delayed reach task, in which patients were cued on the movement destination, but were required to remain stationary for a 1-4 second delay prior to motor response. For each phase of the task, beta band

neural modulation was compared to baseline power levels during the fixation phase. Differences were assessed by observation of 95% confidence intervals for trial-averaged spectral power.
Results:
After artifact removal, eight patients were available for analysis. Five out of eight patients had decreases in beta power during the response phase. When compared to baseline, cue phase beta power increased in three out of eight patients. During the delay phase, three out of eight patients demonstrated beta power increases.
Conclusion:
This study found decreases in beta power during movement execution in a task that introduced a delay between cue and execution. This finding supports previous studies noting decreases in the beta band during movement execution itself and adds that the beta band has similar modulation when the planning component is forced to delay. Furthermore, during the delay requiring movement inhibition, increases in beta band were not generally observed. This finding suggests that the mechanism behind beta band increases in previous Go/No-Go studies requires continued investigation.

Disclosures: **A.M. Tang:** None. **R. Martin Del Campo-Vera:** None. **K. Chen:** None. **A.S. Gogia:** None. **R. Sebastian:** None. **G. Nune:** None. **C.Y. Liu:** None. **S. Kellis:** None. **B. Lee:** None.

Digital Abstract Session

P210. Reaching Control: Motor Learning: Human Neurophysiology

Program #/Poster #: P210.05

Topic: E.04. Voluntary Movements

Title: An investigation of individual differences to anodal tDCS during practice with complex, whole-arm, implicit sequence task

Authors: *B. GREELEY;
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Abstract: While transcranial direct current stimulation (tDCS), a form of non-invasive brain stimulation, over the primary motor cortex (M1) has been demonstrated to improve motor learning, there is considerable individual variability in response to tDCS. For example, it is estimated that approximately 40-70% of individuals respond to anodal (excitatory) tDCS in an expected manner (e.g., tDCS enhances motor learning). Therefore, there is a need to identify sources of variability to tDCS in order to understand and predict tDCS efficacy. Here we investigate two potential sources of variability to tDCS efficacy: resting motor threshold (RMT) and the duration and quality of sleep following a single session of anodal (excitatory) tDCS. Using a within-subject design (3 day washout period), eleven (age range 20-35 years old, 3 males) healthy young adults received either sham or anodal tDCS (5x7cm electrodes, 2mA, 10 minutes) over the dominant M1 while practicing 4 blocks of the serial targeting task, a whole-arm implicit motor sequence task, on a KINARM robot. In the task, participants reached to a

series of targets that were arranged in a circular array. Targets were presented one at a time. Unknown to participants, a reoccurring 6-element sequence was embedded in the target presentations, flanked by the presentation of pseudo-random targets. To quantify learning the reaction time from the sequence targets was subtracted from the random targets. RMT was obtained via transcranial magnetic stimulation over the extensor carpi radialis motor "hotspot." Twenty-four hours following practice, participants returned and practiced 1 block of the serial targeting task without the application of tDCS to assess retention and completed a survey to assess sleep duration and quality. While a repeated measures ANOVA revealed that there was no difference in learning between the anodal and sham tDCS groups, cluster analysis indicated 54% of participants benefited from the application of anodal tDCS. Additionally, neither RMT or sleep duration or quality predicted motor retention. These preliminary results suggest that other approaches such as individualized current modeling or structural imaging methods may be more effective in accounting for sources of variability to tDCS.

Disclosures: B. Greeley: None.

Digital Abstract Session

P211. Cortical Planning and Execution: Behavior

Program #/Poster #: P211.01

Topic: E.04. Voluntary Movements

Support: NIH DP2 NS106663
NYS DoH SCIRB C33610GG
NYS DoH SCIRB C32633GG

Title: Temporal cortical regulation of motor behavior on a modified forelimb dexterity test

Authors: *H. MOHAMMED, Y. LI, P. DI GRAZIA, A. BERNSTEIN, S. AGGER, E. HOLLIS, II;
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Abstract: Hand and arm manual dexterity is a hallmark of humans and non-human primates. While rodents are less dexterous than primates, they provide powerful models for testing neural circuit function in behavioral output, including dexterous behaviors. In rodents, the single pellet reach task has been used extensively to study both dexterous forelimb motor learning as well as recovery from injury; however, mice exhibit high variability in task acquisition in comparison to rats and a significant percentage fail to learn the task. We have created a recessed version of the task that requires greater dexterity. This subtle modification increases both task difficulty as well as the proportion of mice that show an improvement with training. Furthermore, motor cortex inactivation shows a greater effect on the execution of the recessed forelimb reach task, with distinct effects on reach targeting vs grasping components depending on the timing of inhibitory activation. Kinematic analysis revealed differences in reach targeting upon transient cortical inhibition prior to reach onset. In summary, the recessed single pellet reach task provides a

robust assessment of forelimb dexterity in mice and a tool for studying skilled motor acquisition and execution.

Disclosures: H. Mohammed: None. **Y. Li:** None. **P. Di Grazia:** None. **A. Bernstein:** None. **S. Agger:** None. **E. Hollis:** None.

Digital Abstract Session

P211. Cortical Planning and Execution: Behavior

Program #/Poster #: P211.02

Topic: E.04. Voluntary Movements

Support: PRIN 2017 Grant. n. 201794KEER_002 to ABM

Title: Pre-instruction about future action improves joint action performance in monkeys

Authors: I. LACAL, A. SCHITO, L. BABICOLA, L. NALBANT, R. GUPTA, ***A. BATTAGLIA MAYER**;
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Abstract: The ability to optimize collective behavior to achieve common goals is not a prerogative of our species, although humans exhibit exceptional skills in acting together, due to their capacity to predict a coagent's behavior and to flexibly adjust to it. We have shown (Visco-Comandini et al, Cortex, 2015) that monkeys engaged in a joint action task use different strategies to coordinate with each other. We now investigate under which conditions monkeys' dyadic performance can be facilitated. Three pairs of rhesus monkeys were trained to perform an isometric center-out task requiring a force application to guide visual cursors in 8 directions, either individually (SOLO) or jointly (TOGETHER) with a partner, in an intermingled fashion. Two different task structures were used: i) in the 'no Pre-Instruction' (no-PI) task the direction of cursor's movement and the action type (SOLO or TOGETHER), required to gain the reward, were cued simultaneously, by presenting a peripheral target in one location, its color signalling the action requested; ii) in the Pre-Instruction (PI) task the action type was prompted in advance by the color of the central target, during an ad hoc delay time, followed by the appearance of the peripheral target. The TOGETHER performance was always poorer than the SOLO one, reflecting the higher task demand due to the necessity of a reciprocal motor coordination. However, the presence of the pre-instruction about the action type favored the joint behavior, significantly increasing the dyadic success rate. Even though, as previously shown, reaction-times (RTs) were generally reduced in the TOGETHER condition respect to SOLO one, few exceptions confirmed that this was not always the adopted strategy. We hypothesized that the way each monkey copes with interacting contexts critically depends on the idiosyncratic motor behavior of the respective partner. We found that inter-individual differences in monkeys' action kinematics, as observed in SOLO condition, predict the quality of joint performance. Interestingly, this correlation was observed only for PI trials, i.e. when the pre-instruction about the future action type was provided. The presence of a PI period was associated to RTs'

decrease, that resulted in reducing the RTs differences between the interacting subjects and therefore in their synchronization. We conclude that pre-cueing the future action type facilitates inter-individual motor coordination during joint action, particularly in the temporal domain. It might provide an optimal 'kinematic setting' that ultimately induces the reduction of inter-individual differences, maximizing the chance of success between interacting partners.

Disclosures: I. Lacal: None. **A. Schito:** None. **L. Babicola:** None. **L. Nalbant:** None. **R. Gupta:** None. **A. Battaglia Mayer:** None.

Digital Abstract Session

P212. Cortical Planning and Execution: Neurophysiology ? Human

Program #/Poster #: P212.01

Topic: E.04. Voluntary Movements

Title: Estimating maximal muscle electromyographic activity from the relationship between muscle activity and voluntary activation

Authors: *J. DIONG[1], K. KISHIMOTO[1], S. C. GANDEVIA[2], J. E. BUTLER[3], M. E. HEROUX[4];
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Abstract: Maximal muscle activity recorded using surface electromyography (EMG) is an important neurophysiological measure. It is often used to normalize EMG activity recorded during passive movement or active contractions. However, maximal muscle activity cannot be accurately measured in people with an impaired ability to voluntarily activate their muscles. In this study, we determined whether maximal muscle activity can be estimated from muscle activity produced during submaximal voluntary activation. Twenty-five able-bodied adults (18 males, mean age 29 yrs, range 19-64 yrs) participated in the study. Participants were seated with the knee flexed 90° and the ankle in 5° of dorsiflexion from neutral. Participants performed isometric voluntary ankle plantarflexion contractions to produce the following target torques, in random order: 1, 5, 10, 15, 25, 50, 75, 90, 95, 100% of maximal voluntary torque. Ankle torque, muscle activity in soleus, medial and lateral gastrocnemius muscles, and voluntary muscle activation measured using twitch interpolation were recorded. In all three muscles tested, there was a strong $\log_e$-linear relationship between measures of muscle activation and muscle activity. Linear mixed models were fitted to muscle activation and $\log_e$-transformed EMG data. Each 1% increase in muscle activation was associated with a mean increase in muscle activity of 0.027 ln(mV) [95% CI 0.025 to 0.029 ln(mV)] in the soleus muscle, 0.025 ln(mV) [0.022 to 0.028 ln(mV)] in the medial gastrocnemius muscle, and 0.028 ln(mV) [0.026 to 0.030 ln(mV)] in the lateral gastrocnemius muscle. The relationship between voluntary muscle activation and muscle activity can be described using simple mathematical functions, which could be later used to estimate maximal muscle activity to normalize recorded muscle activity.

Disclosures: **J. Diong:** None. **K. Kishimoto:** None. **S.C. Gandevia:** None. **J.E. Butler:** None. **M.E. Heroux:** None.

Digital Abstract Session

P212. Cortical Planning and Execution: Neurophysiology ? Human

Program #/Poster #: P212.02

Topic: E.04. Voluntary Movements

Support: NSERC RGPIN-2020-05263 to JLN

Title: The neurophysiological effects of an acute exercise bout in young healthy individuals: a systematic review and meta-analysis

Authors: ***S. PHAN**[1], J. COLLINS[2], B. FRANCISCO[4], L. JOHNSON[5], A. ROMAIN[2], K. HAYWARD[6], J. L. NEVA[3,7];
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Abstract: Acute cycling exercise can modulate primary motor cortex (M1) excitability of the non-exercised upper-limb in young healthy individuals. Evidence shows that measures of intracortical excitability, via transcranial magnetic stimulation (TMS), are altered after an acute bout of exercise [1-4], with some studies showing enhanced corticospinal excitability [4-5]. However, it is unclear which M1 excitability measures are consistently altered after acute exercise and which exercise intensity elicits the greatest modulation [1-5]. We conducted a systematic review and meta-analysis to synthesize the current state of the literature to further understand the impact of acute exercise on M1 excitability (PROSPERO CRD42017065673). This work has three aims: (1) characterize the effects of acute exercise on M1 excitability, (2) determine which TMS measure shows consistent and robust effects and (3) examine which exercise intensity (i.e., low, moderate, high) elicits the greatest M1 excitability response. A literature search was conducted up to April 2020 using Medline Ovid, Embase, and PsychINFO. Eligible studies included healthy participants (aged 18-40), an acute bout of lower-limb cycling exercise and measures of M1 excitability of the non-exercised upper-limb using TMS. Screening of titles and abstracts was performed (3047 studies), followed by full text screening (61 studies), resulting in 22 included studies (n=457 participants). Data extraction was performed using a prespecified form. Descriptive statistics (mean, frequency) were used to characterize the impact of acute exercise on M1 excitability. Preliminary results demonstrated most studies included a measure of intracortical excitability (49%), with several including measures of corticospinal excitability (39%). Moderate-(43%) and/or high-intensity (39%) were the most common exercise

intensities included in the studies. Descriptive results suggest that acute exercise more commonly impacted intracortical excitability compared to corticospinal excitability, and moderate-to-high intensity exercise more frequently elicited these effects compared to low-intensity exercise. A meta-analysis of continuous data will be performed on the extracted data (mean, SD) to determine which TMS measure shows the greatest response to acute exercise and sensitivity analyses to control for exercise intensity. [1] Singh et al. (2014) *BMC Sports Sci Med Rehabil*, 6: 23. [2] Mooney et al., (2016) *Exp Brain Res*, 234: 3669-3676. [3] Neva et al. (2017) *Eur J Neurosci*, 45(10): 1343-1355 [4] Lulic et al. (2017) *PLoS One*, 12 (3): e0173672. [5] Ostadan et al., (2016) *Neurobiol Learn Mem*, 136 :196-203

Disclosures: S. Phan: None. **J. Collins:** None. **B. Francisco:** None. **L. Johnson:** None. **A. Romain:** None. **K. Hayward:** None. **J.L. Neva:** None.

Digital Abstract Session

P212. Cortical Planning and Execution: Neurophysiology ? Human

Program #/Poster #: P212.03

Topic: E.04. Voluntary Movements

Support: CIHR Grant PJT-14853

Title: The impact of acute high intensity exercise on transcallosal inhibition in older adults

Authors: *B. CHAU, B. GREELEY, C. B. JONES, L. A. BOYD;
Physical Therapy, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Background: While studies have investigated the effect of exercise on corticospinal, intracortical, and interhemispheric processes in young adults, few studies have focused on older adults. Current evidence supports the hypothesis that there is a shift from predominantly inhibitory to excitatory interhemispheric interactions as we age. Other work suggests that changes observed in transcallosal inhibition (TCI) with age (i.e., reduced ipsilateral silent period (iSP) duration and area), may be mitigated by physical activity. Therefore, the main purpose of this experiment was to advance understanding of how high intensity interval exercise training (HIIT) alters patterns of interhemispheric excitability in the healthy older adult population. **Methods**: 41 healthy older adults participated in this study. 21 were pseudo-randomized into the exercise group (8 males, mean age = 66.8 ± 8.9) and 20 in the rest group (5 males, mean age = 65.8 ± 6.3). Participants in the exercise group completed an acute bout of HIIT on a recumbent bike. HIIT exercise began with a 5 min warm up (10 W), followed by three repetitions of 3-minute high intensity bouts of exercise (75% of their max power output as determined from their cardiac stress test) which were interspersed with 3-minute bouts of active recovery (at 10 W) for a total 23 minutes of exercise. Participants in the rest group sat for the same duration of time while their attention was controlled by watching a nature documentary. TCI of the upper limbs, indexed by iSP area, was assessed via transcranial magnetic stimulation while participants squeezed a dynamometer at 50% of their maximum voluntary contraction before (baseline),

immediately (Post 1), and 30 minutes (Post 2) following HITT or rest. **Results**: A mixed design ANOVA was performed with between group factor GROUP (exercise, rest), and within group factors: TIMEPOINT (baseline, post 1, post 2) and HEMISPHERE (dominant, non-dominant) to evaluate changes in iSP area. There was an interaction effect between timepoint and hemisphere ($F(2, 78)= 3.211$, $p= .046$). Post-hoc analyses using Bonferroni correction revealed that this interaction effect was driven by hemispheric differences within the Exercise group at timepoint Post 1; iSP area was significantly greater in the non-dominant compared to the dominant hemisphere ($p= .008$). **Conclusion**: The current study showed following HIIT, that there was a hemispheric difference in TCI in older adults. The present research provides insight on how exercise could be used to mitigate age related changes in the brain and informs how exercise therapies could be employed in association with rehabilitation in clinical populations.

Disclosures: B. Chau: None. **B. Greeley:** None. **C.B. Jones:** None. **L.A. Boyd:** None.

Digital Abstract Session

P212. Cortical Planning and Execution: Neurophysiology ? Human

Program #/Poster #: P212.04

Topic: E.04. Voluntary Movements

Title: Characterizing changes in PMd-M1 interhemispheric inhibition across execution of simple unimanual and bimanual actions.

Authors: *R. DENYER, B. GREELEY, L. A. BOYD;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Understanding the influence of frontal cortical regions on primary motor cortex (M1) output during movement preparation and execution will inform hierarchical models of motor control and improve treatment of movement disorders. In the current study, single and dual stimulation transcranial magnetic stimulation (TMS) were used to measure the inhibitory influence of non-dominant dorsal premotor cortex (PMd) on dominant M1 at rest and in the pre-movement period of unimanual and bimanual simple reaction time tasks in heathy volunteers. Motor evoked potentials (MEPs) generated in the flexor dorsal interossei by single stimulation TMS over M1 (% maximum stimulator output that generated ~1 mV MEPs at rest) were used to track corticospinal excitability as participants prepared to execute button presses in response to a visual GO stimulus. The inhibitory influence of PMd on M1 was tracked by assessing the effect of a conditioning TMS stimulus over non-dominant PMd on MEPs generated by a test stimulus over dominant M1 (conditioning stimulus intensity = 110% of resting motor threshold, interstimulus interval = 9ms). MEPs were generated at rest, 50ms and 100ms after the presentation of the GO stimulus. In line with previous work, preliminary results showed that corticospinal excitability linearly increased as reaction time approached, for both unimanual and bimanual movements. In contrast, a release in inhibition from PMd to M1 was found at 50ms, but not at 100ms post GO stimulus in the unimanual task. PMd-M1 inhibition was not modulated in the bimanual task. These results tentatively support the notion that PMd is central to the early

shaping of motor programs generated in response to external cues, and that PMd modulates inhibitory output depending on whether unimanual or bimanual responses are required.

Disclosures: R. Denyer: None. **B. Greeley:** None. **L.A. Boyd:** None.

Digital Abstract Session

P213. Cortical Planning and Execution: Premotor and Motor Cortex ? Neurophysiology ? Animal

Program #/Poster #: P213.01

Topic: E.04. Voluntary Movements

Support: NIH Grant R01 NS-058659
NSF Grant 1656882

Title: Neuronal activity reorganization in motor cortex for successful locomotion after a lesion in the ventrolateral thalamus

Authors: *I. N. BELOOZEROVA;
Georgia Inst. of Technol..

Abstract: Thalamic stroke is a common disease that leads to ataxia if the cerebellum-receiving ventrolateral thalamus (VL) is affected. The compensation mechanism for this deficit and the contribution of different brain regions to the compensation are unclear. This slows development of rehabilitation approaches. The goal of this study was to clarify neuronal mechanisms of motor cortex that are involved in compensation for ataxia during locomotion when a part of the VL is inactivated or lesioned. In freely ambulating cats we recorded the activity of neurons in layer V of motor cortex as cats walked on the flat surface and horizontal ladder. Walking on the ladder is an accuracy demanding task that suffers if the VL is damaged. We first reversibly inactivated approximately 10% of the VL unilaterally using an AMPA/kaniate glutamatergic transmission antagonist CNQX and analyzed how neuronal activity in motor cortex reorganized for this 2-4 hour long small inactivation. We focused on pyramidal tract projecting neurons (PTNs) and on subpopulations of neurons with somatosensory receptive fields on different segments of the forelimb. We next lesioned 50-75% of the VL bilaterally with kainic acid and analyzed the activity of motor cortex neurons during the following month. We found that when a small part of the VL was inactivated, discharge rates of motor cortex neurons decreased but otherwise the activity was near normal, and cats walked fairly well. Individual neurons retained their ability to respond to the demand for accuracy during ladder locomotion, however, the great majority changed their response. In different neurons the changes were often opposite, so that only few net population changes were observed. When the VL was lesioned, the cat walked normally on the flat surface, but was ataxic on the ladder during several days post-lesion often missing crosspieces. When ladder locomotion normalized, neuronal discharge rates on the ladder were normal and the shoulder-related group was preferentially active during the swing phase of the stride rather than at the end of the stance phase. We concluded that motor cortex compensates for

a VL leason and contributes to maintenance of successful locomotion on a complex terrain by reorganizing responses of individual neurons and neuronal subpopulations to the accuracy demands during locomotion. These data may be useful for the development of new rehabilitation approaches for patients with thalamic stroke or lesion, approaches that assist the function of motor cortex at the level of single neurons and specific subpopulation of neurons.

Disclosures:

Digital Abstract Session

P213. Cortical Planning and Execution: Premotor and Motor Cortex ? Neurophysiology ? Animal

Program #/Poster #: P213.02

Topic: E.04. Voluntary Movements

Support: NIH Grant R01DE027236
NIH Grant R01AG069227

Title: Decoding lingual-palatal contacts from population responses in primate orofacial sensorimotor cortex

Authors: *D. TANG[1], A. SIMONOFF[1], J. LAURENCE-CHASEN[1], B. J. SESSLE[2], C. ROSS[1], N. HATSOPOULOS[1], F. I. ARCE-MCSHANE[1];
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Abstract: Complex orofacial sensorimotor behaviors, such as feeding and speech, rely on precise control from the orofacial sensorimotor cortex (oSM). The ways in which tactile and proprioceptive feedback contribute to this process have yet to be characterized. We evaluated the presence of information on lingual-palatal contacts in oSM and how this was affected by absent tactile inputs while monkeys engaged in natural feeding. Neuronal activity from four chronically implanted microelectrode arrays in the primary motor (rostral M1 and caudal M1) and somatosensory (areas 3a/3b and areas 1/2 of S1) regions of oSM was recorded simultaneously with 3D tracking of tongue kinematics using bilateral videoradiography with the X-Ray Reconstruction of Moving Morphology (XROMM) workflow. To determine palate locations, we used a CT-scan based reconstruction of the cranium. Local anesthetic block of trigeminal nerve sensory branches knocked out tactile inputs from the palate, teeth, and tongue, while preserving proprioceptive inputs. We implemented two K-Nearest Neighbor (KNN) classifiers to evaluate the ability of spiking activity of M1 and S1 neurons to predict 1) contact of any part of the tongue to six palatal regions encoded by a sequence of six bits (total of 64 possible combinations) and 2) contact of tongue-tip to two anterior palatal regions. Classifier performance was assessed using classification loss for 10-folds of cross- validated data. For decoding multiple lingual-palatal contacts, we found that classifier performance was better than chance for all cortical regions, with M1 being the most reliable predictor of contact with (control) and without

tactile feedback. The removal of tactile inputs degraded performance when assessing spiking activity from areas 1/2. In contrast, performance improved for areas 3a/3b. For single lingual-palatal contact, the classifier correctly identified palatal regions above chance with and without nerve block. The weak effect of absent tactile inputs on decoding lingual-palatal contact suggests alternative sources of sensory information, possibly from proprioceptive inputs from the tongue. The robust representations of lingual-palatal contacts in oSM suggest that their cortical control plays an important role in vital and critical functions such as feeding. The results may have important implications for the evaluation and treatment of sensorimotor disorders affecting the orofacial system.

Disclosures: **D. Tang:** None. **A. Simonoff:** None. **J. Laurence-Chasen:** None. **B.J. Sessle:** None. **C. Ross:** None. **N. Hatsopoulos:** None. **F.I. Arce-McShane:** None.

Digital Abstract Session

P213. Cortical Planning and Execution: Premotor and Motor Cortex ? Neurophysiology ? Animal

Program #/Poster #: P213.03

Topic: E.04. Voluntary Movements

Support: NIH 1R01NS092894-01
NSF IIS-1527747

Title: Sensorimotor beta oscillations contribute to reward processing

Authors: ***T. YADAV**[1], D. FRANCIS[2], J. T. FRANCIS[1];
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Abstract: The role of beta oscillations in motor processing has been widely studied in both the animal and human sensorimotor cortices. However, whether these oscillations contribute to the processing of non-movement aspects of a motor task such as reward magnitude is not fully understood. In our previous work (An et al., 2019), we established that local field potentials (LFP) in the primary motor cortex (M1) are modulated by reward expectation. As a next step, the aim of present research is to study the influence of reward magnitude on LFPs specifically beta oscillations recorded from M1 and somatosensory (S1) cortices. For this, we trained a non-human primate (Female, Macaca mulatta) to perform planar center-out reaching movements to targets in 4 possible directions (0, 90, 180 and 270 degrees relative to horizontal line). For every successful trial, the monkey received a cued amount of juice reward (1/2/3 drops). Prior to the analysis, data collected from 17 sessions were pooled and matched for significant differences in reaction time, peak movement speed and time to reward (time from cue presentation to the start of reward delivery). A linear fixed effects model with two predictors namely reward level and time bin was used to fit beta amplitude during the hold period (0.7 sec), pre-movement period (0.5 sec) and movement period (0.8 sec) of each successful trial. A significant interaction between reward level and time bin was seen for both M1 and S1 (F-test, $p<0.05$). Post-hoc

analysis (Wilcoxon rank sum with false discovery rate, $p<0.001$) was used to identify significantly different reward pairs in each time bin. Reward-related distinction in beta amplitude began to appear 300ms and 450ms after the presentation of reward predicting cue in M1 and S1 regions respectively. Further, these differences continued to remain significant until the beginning of movement in M1 and end of movement in S1. The results of this study indicate that beta amplitude during hold on cue and pre-movement periods decreases with increase in reward magnitude in both regions. From these preliminary results, we have shown that sensorimotor beta oscillations indeed reflect processing of reward information.

Disclosures: T. Yadav: None. **J.T. Francis:** None. **D. Francis:** None.

Digital Abstract Session

P213. Cortical Planning and Execution: Premotor and Motor Cortex ? Neurophysiology ? Animal

Program #/Poster #: P213.04

Topic: E.04. Voluntary Movements

Support: Canadian Institute of Health Research PJT 153445

Title: Convergence of proprioceptive and visual feedback on neurons in primary motor cortex

Authors: *K. P. CROSS, D. J. COOK, S. H. SCOTT;
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Abstract: Sensory feedback plays a critical role in correcting motor actions such as when our arm is bumped while moving, or when the behavioural goal unexpectedly moves such as a glass tipping over. Vision plays a dominant role for identifying behavioural goals, while vision and proprioception provide feedback about the limb. Primary motor cortex (M1) plays an important role in generating corrections to sensory feedback, however, we know little about how these different sources of feedback are organized in M1 for online control. Here we examined two extreme hypotheses, the convergence and independent-input hypotheses. The convergence hypothesis assumes that 1) proprioceptive and visual feedback of the limb are redundant sources of information and should be optimally combined into a common limb estimate 2) a difference vector is computed between the visual location of the goal and the common limb estimate which is then converted to motor commands by M1. The prediction is that a common group of neurons in M1 should rapidly respond to proprioceptive feedback and visual feedback of the limb and goal. The independent-input hypothesis assumes that each feedback source is not integrated due to the computational complexities of combining multiple feedback sources. The prediction is that each feedback source will influence a random distribution of neurons in M1. We investigated these hypotheses by training monkeys to make goal-directed reaches to a target. On random trials a perturbation was applied that was either a jump to the visual feedback of the goal or limb or a mechanical load that displaced the limb off its intended trajectory (i.e. proprioceptive feedback). Monkeys were able to quickly counter the perturbations and recordings from M1 demonstrated

that each type of perturbation generated a robust change in activity in a subpopulation of neurons with 55%, 52% and 57% of neurons responding to the goal jump, cursor jump and mechanical loads, respectively. Across the subpopulations, there was significant overlap between the different perturbation types with many neurons responding to two or more different perturbations (Monkey M/A: χ^2 =113.9/68.1, df=4, p<0.001/<0.001). The magnitudes of perturbation-related activity were also highly correlated between the different perturbation types (goal with cursor r=0.90/0.97, mechanical with goal r=0.85/0.86, mechanical with cursor r=0.75/0.86) and the low-dimensional subspaces that perturbation-related activity resided in was highly conserved across the different perturbation types. Collectively, these results indicate M1 receives potent and highly convergent sensory feedback about the limb and goal during online control.

Disclosures: K.P. Cross: None. **D.J. Cook:** None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder and CSO of KINARM which commercializes the robotic technology used in the study.

Digital Abstract Session

P214. Cortical Planning and Execution: Neuroimaging

Program #/Poster #: P214.01

Topic: E.04. Voluntary Movements

Support: Tianqiao and Chrissy Chen Institute for Neuroscience at Caltech
NIGMS T32 GM008042

Title: Functional ultrasound neuroimaging reveals heterogenous organization of posterior parietal cortex in non-human primates

Authors: *W. S. GRIGGS[1], S. NORMAN[1], C. RABUT[2], C. DEMENE[3], T. DEFFIEUX[3], V. CHRISTOPOULOS[4], M. TANTER[3], M. SHAPIRO[2], R. A. ANDERSEN[1];
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Abstract: The posterior parietal cortex (PPC) is an important area for the transformation of spatial information into accurate motor movements. PPC sub-regions such as the lateral intraparietal area (LIP) and the parietal reach region (PRR) are especially important for saccades and reaches, respectively. However, our understanding of these areas' functional organization is incomplete, in part due to technical challenges. While electrophysiology recordings can only record from small regions of the PPC at a time, fMRI and other 'whole-brain' techniques have insufficient spatiotemporal resolution. Now, functional ultrasound (fUS) is available as an innovative neuroimaging technique that measures cerebral hemodynamics with exceptional spatiotemporal resolution (<100 μm; ~100 ms) and a large field of view (several cm) – specifications ideally suited to recording detailed activity of entire cortical regions in parallel. In this study, we used fUS to record changes in cerebral blood volume (CBV) in PPC of two awake

behaving monkeys. Each monkey performed intermingled memory-guided saccades or reaches to targets throughout their visual field. We then analyzed the distribution of response fields within each coronal plane of PPC. We found that many subregions within the PPC, including within LIP, PRR, ventral intraparietal area (VIP), Area 5, and medial parietal cortex (MP), had effector-specific tuning curves. Specifically, activity in these areas was modulated by certain effectors and movement directions. These results address a fundamental gap in our understanding of PPC's functional organization by developing mesoscopic maps of effector and direction specificity previously unattainable with fMRI or electrophysiology methods. These mesoscopic maps reveal a highly heterogenous organization within each PPC subregion with many small patches of cortex encoding different combinations of effectors and directions.

Disclosures: W.S. Griggs: None. **S. Norman:** None. **R.A. Andersen:** None. **M. Shapiro:** None. **C. Rabut:** None. **C. Demene:** None. **T. Deffieux:** None. **M. Tanter:** Other; Iconeus. **V. Christopoulos:** None.

Digital Abstract Session

P214. Cortical Planning and Execution: Neuroimaging

Program #/Poster #: P214.02

Topic: E.04. Voluntary Movements

Support: NSERC discovery Grant-in-aid

Title: A comparison of cognitive-motor integration performance and resting state functional brain network connectivity in female athletes with and without concussion history

Authors: *A. PIERIAS[1], D. E. GORBET[1], M. WOJTOWICZ[1], A. PRIEUR[1], L. E. SERGIO[2]; [1]York Univ., North York, ON, Canada; [2]Sch. Kinesiol & Hlth. Sci., York Univ., Toronto, ON, Canada

Abstract: Structural neural changes following concussion are often not captured by standard imaging techniques. However, there is growing evidence that damage to white matter tracts and change in functional network connectivity may be observed following concussive injury. We investigated behavioural performance on a cognitive-motor integration (CMI) task in conjunction with alterations in resting state functional connectivity (rs-FC) in brain networks in a population of 30 female varsity athletes, with 16 having a previous history of concussion. Behavioural performance on accuracy, timing, and trajectory measures of a CMI task were assessed between the concussion history group and the control group. Rs-FC within the nodes of the Default Mode Network (DMN), Dorsal Attention Network (DAN), the Frontoparietal Network, and the Anterior Cerebellar Lobule Network, was assessed against performance scores on accuracy, timing, and trajectory measures. Main findings indicate no difference in behavioural performance ($p>0.05$) between those with concussion history and those without, in contrast to previous findings in a group of primarily male varsity athletes. In addition, no difference in rs-FC was noted in correlation with behavioural performance scores on either accuracy, timing, or

trajectory. These findings may suggest sex-related differences in performance on a CMI task, and a resiliency in both functional network connectivity and visuomotor skilled performance in varsity female athletes.

Disclosures: A. Pierias: None. **D.E. Gorbet:** None. **M. Wojtowicz:** None. **A. Prieur:** None. **L.E. Sergio:** None.

Digital Abstract Session

P214. Cortical Planning and Execution: Neuroimaging

Program #/Poster #: P214.03

Topic: E.04. Voluntary Movements

Title: Predicting self-initiated behaviour in mice several seconds prior to movement using widefield calcium imaging

Authors: C. MITELUT[1], Y. ZHANG[2], Y. SEKINO[3], G. SILASI[5], F. BOLANOS[6], J. BOYD[3], N. SWINDALE[4], ***S. SAXENA**[2], T. H. MURPHY[3];
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Abstract: Modern experimental neuroscience has sought to identify the neural correlates of free, or voluntary, actions largely focusing on instructed tasks in humans while recording neural activity via electroencephalography (EEG) or functional magnetic resonance imaging (fMRI). Multiple findings from human studies suggest that significant increases in neural activity (e.g. “readiness potential”-RP) precede both action initiation and awareness, and that behavior could be predicted well before action. These findings have been countered by more recent studies suggesting that only low-value actions (e.g. random pressing of a button) can be predicted and that voluntary action studies should focus more on high-value freely chosen actions (e.g. those involving reward).
Here we study voluntary behavior in mice engaged in a high-value action, i.e. a self-initiated water seeking task in water deprived animals, while recording large-scale neural activity from the dorsal cortex using widefield calcium imaging. Mice were trained to pull a lever to receive a water reward following a minimum of 3 sec of non-lever movement. We identified high-value free behaviors such as lever pulls, as well as low-value behaviors such as spontaneous limb movement or grooming, using DeepLabCut for behavioral tracking. We show that self-initiated lever presses are preceded by increases in widefield calcium activity in multiple cortical areas. Water-seeking behaviors as well as spontaneous movements can be decoded using linear and nonlinear methods by up to several seconds prior to movement (Fig. 1). We finally show that preparation of voluntary behavior is widely distributed across the dorsal cortex, with the hubs of activity being in the motor, retrosplenial and somatosensory cortex. Our findings support the pre-movement determination of behavior, with a detailed quantitative study showing that self-

initiated voluntary high-value actions may be prepared and can be decoded several seconds in advance of movement. Our work paves the way to future studies in humans on behavior prediction from neural activity.

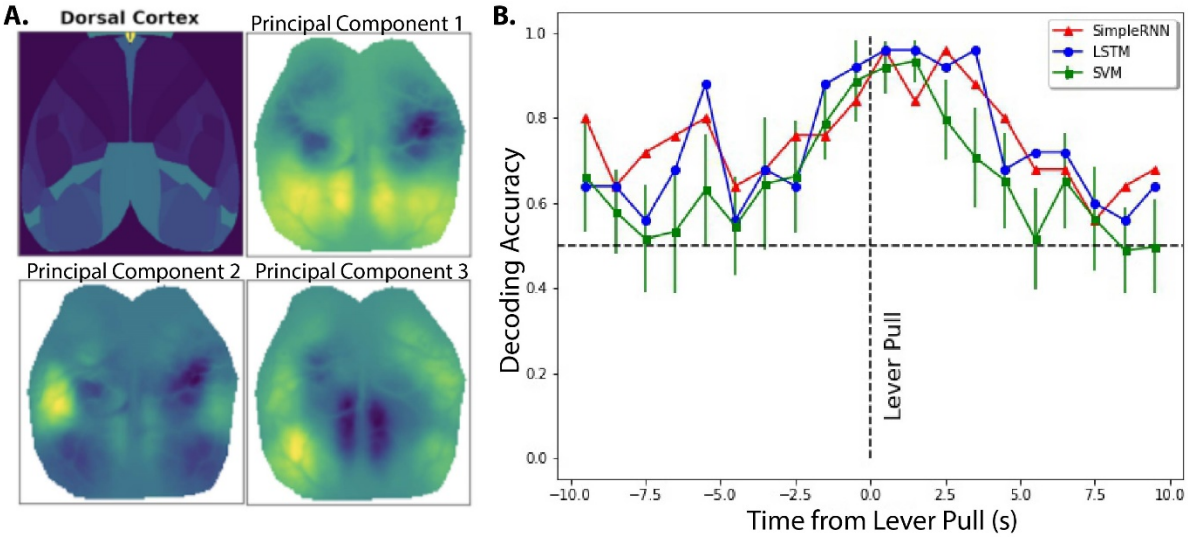

Figure 1. (A) Map of all mouse dorsal cortex areas visible during widefield imaging, and PCA loadings for the first three components activated during self-initiated behaviours. (B) Decoding accuracy of lever pull trials from random trials at various times before / after lever pull initiation, using Support Vector Machines (SVMs), and two different kinds of Recurrent Neural Networks (RNNs): Simple RNNs and LSTMs. Error bars show standard deviation across 10 folds.

Disclosures: **C. Mitelut:** None. **S. Saxena:** None. **Y. Sekino:** None. **J. Boyd:** None. **F. Bolanos:** None. **T.H. Murphy:** None. **Y. Zhang:** None. **G. Silasi:** None. **N. Swindale:** None.

Digital Abstract Session

P215. Oral Motor and Speech

Program #/Poster #: P215.01

Topic: E.04. Voluntary Movements

Support: MOST 105–2628-B-010-008-MY4

Title: The association between masticatory performance and the parahippocampal volume varies as age increases in non-demented older people

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Abstract: Cumulating evidence has suggested that in older people, oral sensorimotor performance is associated with not only the peripheral factors (e.g., tooth loss) but also brain signatures. The cross-sectional findings, however, do not clarify the dynamic association

between the brain and oral sensorimotor performance, which may vary as age increases. Atrophy of the volume of the medial temporal lobe, including the entorhinal and parahippocampal regions, is associated with normal aging. It has remained unclear if, in older people, the association between MP and the brain volume would vary as age increases. Here, we present novel evidence of the association between structural brain signatures and MP in a cohort followed up for at least one year. 31 non-demented older subjects (male:female=12:19, aged 50 to 78 years) were recruited and received T1-weighted magnetic resonance imaging (MRI) and oral examination, including assessment of the NMT and MP. MP was assessed using a color-changeable chewing gum test, with a higher score indicating a better oral mixing ability. The structural MRI data were processed using Freesurfer for extracting the regional volume of cortical and subcortical brain regions. To adjusted for individual variations in brain size, the regional volume was normalized by the intracranial volume on an individual basis. To quantify the association between MP and regional volume, Spearman's rho was used due to the non-parametric distribution of the NMT and MP. We find that (1) individual age is not significantly correlated with the NMT or MP. The NMT, MP, and brain volume of bilateral entorhinal or parahippocampal regions do not significantly differ between the initial and the follow-up stages. (2) Notably, at the initial stage, MP is significantly negatively correlated with NMT (rho=-0.45, p=0.015) and but not significantly correlated with the brain volume of bilateral entorhinal or parahippocampal regions. (3) In contrast, at the follow-up stage (1-1.5 year after), MP is not significantly correlated with the NMT (rho=-0.31, p=0.1) but significantly positively correlated with the brain volume of the right parahippocampal region (rho=0.41, p=0.023). The preliminary findings suggest that in non-demented older subjects, as age increases, the association between MP and the NMT may decrease, while the association between MP and the right parahippocampal region may increase. Such a dynamic association highlights the role of the CNS in an age-related change in oral functions, which would require further investigation.

Disclosures: **C. Lin:** None. **J. Fuh:** None.

Digital Abstract Session

P215. Oral Motor and Speech

Program #/Poster #: P215.02

Topic: E.04. Voluntary Movements

Support: NSF-GRFP
HHMI

Title: Sex, Song, and Estrogen: Vocal Learning Sufficient Gene Expression Specialization Following Estradiol Treatment in Female Zebra Finch

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Abstract: Zebra finches learn to sing by imitating heard vocalizations, a rare ability known as vocal learning. This vocal learning behavior demonstrates striking convergence with human speech in terms of developmental trajectory, neural circuit structure, and specialized gene expression in vocal motor control circuits. Unlike humans, the behavior is sexually restricted in zebra finch, with females limited to producing innate calls. The vocal learning brain regions and circuits form in both sexes during early post hatch life, but components of the circuit later atrophy in females. However, if female zebra finch are chronically treated with estrogen during development, they will develop a complete song system with mature connectivity, enabling them to vocally imitate male conspecifics. To better understand the genetic basis of zebra finch vocal learning and its sexual dimorphism, we chronically treated male and female birds with estradiol or saline from hatch until sacrifice at 30 days. Following sacrifice, we performed laser capture microdissection to isolate song nuclei and surrounding control regions, then short read sequencing on transcriptome libraries prepared from these samples. These RNAseq reads were mapped to a new zebra finch genome assembly, including the female restricted W sex chromosome. Using weighted gene co-expression network analysis, we found gene modules whose differential expression marks the song nuclei in vocal learning males and then examined module expression in females. While LMAN and RA song nuclei were similarly specialized in the females at post hatch day 30, HVC and Area X were unspecialized and absent respectively. Following estradiol treatment, HVC acquired a subset of the gene module specializations present in male HVC and Area X also appears as specialized. Intestingingly, the gene module specialized in female-estradiol HVC did not include the core vocal learning genes previously identified by convergent regulation in human vocal-motor cortex or involvement in human speech disorders. Our data instead indicate that vocal learning sufficient, estradiol induced specialization of female HVC is driven by the increased expression of certain Z chromosome genes, most strikingly the Growth Hormone Receptor, which are depleted in the untreated females.

Disclosures: **M. Davenport:** None. **H. Choe:** None. **H. Matsunami:** None. **E. Jarvis:** None.

Digital Abstract Session

P215. Oral Motor and Speech

Program #/Poster #: P215.03

Topic: E.04. Voluntary Movements

Support: NIH Grant U01NS098969
NIH Grant U01NS117836

Title: Identification and mitigation of technical challenges in experiments probing the cortical-basal ganglia speech network during awake Deep Brain Stimulation surgeries.

Authors: ***A. BUSH**[1], A. CHRABASZCZ[2], V. PETERSON[3], C. DASTOLFO-HROMACK[2], V. SARAVANAN[1], W. LIPSKI[2], M. RICHARDSON[1], M. RICHARDSON[1];
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Abstract: Deep brain stimulation (DBS) surgery allows direct recordings from human basal ganglia and thalamus in awake patients, with adequate temporal and spatial resolution to explore the functional roles of these structures in speech production. However, neural signal contaminations in this setting have not been characterized. Here we report technical challenges encountered and applied solutions, focusing on the characterization of a speech induced vibration artifact in LFP recordings. We developed an intraoperative experimental approach which allows depth recordings from DBS targets, while simultaneously recording from selected cortical regions by means of high-density electrocorticography (ECoG) strips temporarily placed through the burr hole. A syllable repetition task using auditory stimuli was performed by 55 patients (36 males, mean age 66.8y, range 43-79) undergoing awake DBS surgery (# Target/Diagnosis: 28 STN/PD, 21 VIM/ET, 5 GPi/PD, 1 Gpi/Dystonia). We developed a well-documented data processing pipeline in Matlab for offline data synchronization, re-referencing, artifact characterization and rejection. We identified artifactual crosstalk between intra-cranial recording channels at the head-stage connector which could be corrected by custom re-referencing schemes. In addition to the physiological increase in high gamma (70-150 Hz) activity during speech production, time-frequency analysis revealed the presence of a narrowband component in the same frequency band, remarkably similar to the pattern observed in the spectrogram of produced speech. This component was present to different degrees in all types of LFP recordings: DBS leads, micro and macro mapping electrodes, and ECoG strips. We show that this component tracks the fundamental frequency of the participant's voice and speech intensity, correlates with the power spectrum of speech and has coherence with the produced speech audio. However, no corresponding component was identified in any neural channel during the listening epoch, ruling out the possibility of this component representing an auditory response. Attaching a vibration sensor to the stereotactic frame shows the presence of speech induced vibrations with the same pattern as observed in the LFPs. Taken together these observations suggest that speech-induced vibrations can affect the recorded neural signal, in line with observations from other groups. Speech production research in the context of DBS implantation surgery is a new and growing field. Identifying and accounting for potential sources and types of artifacts is crucial to achieve valid, reliable and reproducible results.

Disclosures: **A. Bush:** None. **A. Chrabaszcz:** None. **V. Peterson:** None. **C. Dastolfo-Hromack:** None. **V. Saravanan:** None. **W. Lipski:** None. **M. Richardson:** None. **M. Richardson:** None.

Digital Abstract Session

P215. Oral Motor and Speech

Program #/Poster #: P215.04

Topic: E.04. Voluntary Movements

Support: NIH Grant R01DE027236
NIH Grant 1UL1TR002389-01
NIH Grant R01AG069227

NSF GRFP

Title: Decoding tongue position and shape from population responses in primate orofacial sensorimotor cortex

Authors: *J. LAURENCE-CHASEN[1], C. F. ROSS[1], N. G. HATSOPOULOS[1], F. I. ARCE-MCSHANE[2];
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Abstract: During feeding, drinking, and speech, the tongue both moves and deforms to assume a range of different postures. The extent to which the orofacial sensorimotor cortex encodes the different components of this behavior is not well understood. Here, we used XROMM (X-ray reconstruction of moving morphology) to quantify intraoral tongue movement and deformation while rhesus macaques (*Macaca mulatta,* n=2) fed on grapes and gummy bears. We employed an adaptive Kalman filter to decode either tongue position or tongue shape from cortical neuronal activity recorded from chronically-implanted microelectrode arrays in the orofacial region of the primary motor and somatosensory cortices. Specifically, the position variables comprised the X-, Y-, and Z- positions of four markers in the anterior region of the tongue, and the shape variables comprised the tongue's length, width, roll, and yaw. Each shape variable was computed from 4-6 dimensions of the 3D marker positions, and thus represented a multi-dimensional feature. To compare decoder performance across the range of positions and shapes, we compared the error expressed as a percentage of a given variable's total range. We found that the decoder predicted shape variables with similar accuracy as it did for position variables, as mean performance errors of approximately 20% remained consistent across all variable types. Such robust representations of both tongue position and tongue shape in the orofacial sensorimotor cortex suggest that their cortical control plays a role in critically important functions such as feeding and speech. The results may have important implications for the evaluation and treatment of sensorimotor disorders affecting the orofacial system.

Disclosures: J. Laurence-Chasen: None. **C.F. Ross:** None. **N.G. Hatsopoulos:** None. **F.I. Arce-McShane:** None.

Digital Abstract Session

P216. Plasticity

Program #/Poster #: P216.01

Topic: E.04. Voluntary Movements

Title: Modulation of task-related alpha power after motor learning is dependent on age and task difficulty

Authors: *M. BOOTSMA[1], S. R. CALJOUW[1], M. P. VELDMAN[3], N. M. MAURITS[2], J. C. ROTHWELL[4], T. HORTOBÁGYI[1];
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Abstract: Although a general age-related decline in neural plasticity is evident, the effects of age on neural plasticity after a period of motor practice are inconclusive. Inconsistencies in the literature may be related to the nature of the experimental tasks, which differ in task-related variables such as task difficulty and the type of skill practiced. Therefore, the current study aimed to determine the effects of age and task difficulty on task-related brain activity before, immediately after, and 24-h after motor skill acquisition of a mirror star tracing task. Healthy younger (N=36, 19-24 yr) and older (N=36, 65-86 yr) adults practiced the task at one of three difficulty levels defined by the width of the wall of the star, while electroencephalography (EEG) was acquired. Task performance was assessed in terms of both performance speed and accuracy. On the behavioral level, task difficulty only affected motor skill acquisition in younger but not older adults. While young participants practicing at the low and high difficulty levels moved respectively 32% and 28% faster after practice, young participants practicing at the medium difficulty level predominantly improved tracing accuracy (43% improvement in accuracy, 12% improvement in speed). Furthermore, younger adults only consolidated improvements in speed (29% improvement pre-retention), whereas consolidation in older adults was limited to accuracy (18% improvement pre-retention). On the neural level, a significant decrease of task-related power in the alpha frequency band (8-12 Hz) after practice, indicative of early processes of neural plasticity, was present in practice groups that predominantly improved accuracy (35-85% decrease) but not in the groups that predominantly improved speed (7-18% decrease). Together, these data suggest that both age and task difficulty affect neural plasticity as well as the strategy to prioritize improvements in either speed or accuracy during the mirror star tracing task.

Disclosures: M. Bootsma: None. **S.R. Caljouw:** None. **M.P. Veldman:** None. **N.M. Maurits:** None. **J.C. Rothwell:** None. **T. Hortobágyi:** None.

Digital Abstract Session

P216. Plasticity

Program #/Poster #: P216.02

Topic: E.04. Voluntary Movements

Title: No effect of anodal tDCS on motor cortical excitability and no evidence for responders in a large, double-blind, placebo-controlled trial

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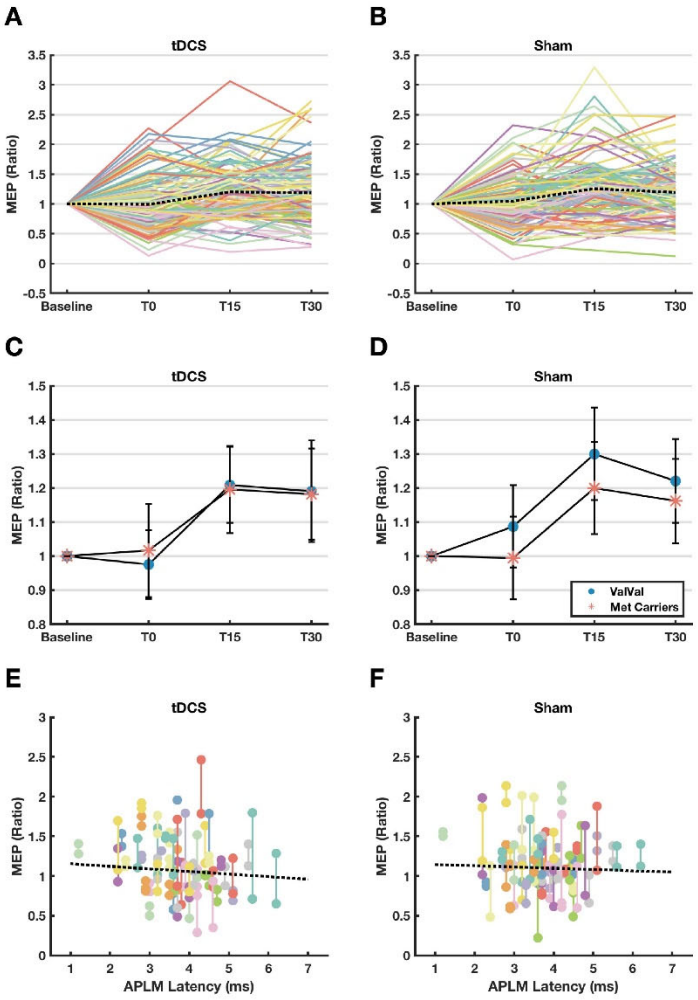
Abstract: Rationale: *Transcranial direct current stimulation* (tDCS) has emerged as a non-invasive brain stimulation technique with potential applications in a variety of clinical fields.

However, it is not yet widely used in clinical settings as conflicting effects of tDCS have been reported. Some researchers have proposed that the variability in the literature is caused by a combination of underpowered studies and individual differences in sensitivity to tDCS. **Objective:** To test the effect of anodal tDCS on cortical excitability and the interaction effect of two participant-specific factors that may explain individual differences in sensitivity to anodal tDCS: the Brain Derived Neurotrophic Factor Val66Met polymorphism (BDNF genotype) and the latency difference between anterior-posterior and lateromedial TMS pulses (APLM latency). **Methods:** In 62 healthy participants, cortical excitability was measured before and after 20 minutes of anodal tDCS stimulation (2mA) in a pre-registered, double-blind, randomized, placebo-controlled trial with repeated measures. The main effect of tDCS and interaction effects of participant-specific predictors were assessed using a linear mixed model. Each participant was tested four times, with the first two and last two sessions constituting measurement pairs. In each measurement pair, a participant received one anodal and one sham tDCS stimulation, resulting in a total of two anodal and two sham sessions per participant. **Results:** We did not find a main effect of anodal tDCS, nor an interaction effect of the participant-specific predictors. Moreover, further analyses did not provide evidence for the existence of responders and non-responders. **Conclusion:** This study indicates that anodal tDCS at 2mA for 20 minutes may not reliably affect cortical excitability. Before tDCS can be used in a clinical setting, future research should focus on improving the tDCS technique, rather than continue the search for participant-specific predictors for the effect of tDCS in its current form.



Effects of tDCS on cortical excitability (N = 59).

A-B: Individual (colored lines) and mean (black dotted line) responses to anodal tDCS and sham stimulation over time (T1: 0 minutes after stimulation, T15: 15 minutes after stimulation, and T30: 30 minutes after stimulation). The evolution of cortical excitability is similar between tDCS and sham stimulation over different time points. ***C-D***: Responses of carriers (red asterisks) and non-carriers (blue dot) of the BDNF polymorphism to anodal tDCS or sham stimulation. Error bars indicate the 95% CI of the mean. The evolution is similar between carriers and non-carriers. ***E-F***: Relation between APLM latency and mean MEP ratio after anodal tDCS or sham stimulation. Connected dots illustrate responses of the same individual in two anodal tDCS and two sham conditions respectively. The black dotted line represents the regression line.

Disclosures: C. Gaiser: None. **Z.D. Jonker:** None. **J.H.M. Tulen:** None. **G.M. Ribbers:** None. **M.A. Frens:** None. **R.W. Selles:** None.

Digital Abstract Session

P216. Plasticity

Program #/Poster #: P216.03

Topic: E.04. Voluntary Movements

Support: NSERC RGPIN 2020-0675

Title: Investigating the influence of sensory input on motor cortex organization

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Abstract: <Evidence indicates that prolonged periods of sensory input through peripheral nerve stimulation can induce changes in the organization of the primary motor cortex (M1). However, the mechanisms of such changes and the influence of cutaneous versus proprioceptive input are unclear. In this study, it is proposed that a Transcranial magnetic stimulation (TMS)-evoked phenomenon known as afferent inhibition may contribute to changes M1 organization induced by sensory enrichment. Short- and long-latency afferent inhibition (SAI, LAI) are TMS measures that reflect the excitability of neural connections between the primary somatosensory cortex (S1) and M1. Changes in SAI or LAI following a period of sensory enrichment may underly the neural mechanisms by which sensory input influences M1 organization. Therefore, we investigated the influence of sensory enrichment on afferent inhibition and on human M1 organization as assessed via maps created via TMS. Four right-handed, healthy humans (18-35 years) were recruited to participate in a pre- and post-intervention assessment. The following 45 min interventions were delivered on three separate sessions to create sensory enrichment: rest, digital nerve electrical stimulation or ulnar nerve electrical stimulation. Sensory enrichment was delivered at three times the perceptual threshold through a “burst” pattern of stimulation, where 20 Hz trains of electrical pulses were delivered for 1 s, with a 5 s rest between trains. Using fast-mapping TMS, the cortical representation of the right first dorsal interosseous (FDI) muscle was mapped pre- and post-interventions. 100 TMS pulses were delivered to pseudo-random locations within a 6x6 cm area centered over the FDI motor hotspot, at the intensity corresponding to 120% of the resting motor threshold with an inter-stimulus interval of 2 s. A MATLAB script was then used to construct a map of the FDI muscle representation from the peak-peak amplitude of the motor-evoked potentials (MEPs) and the location of stimulation registered with Neuronavigation. Measures of SAI and LAI were also acquired before and immediately following each intervention. To do so, digital nerve stimulation was delivered prior to the TMS pulse at an interstimulus interval of N20 + 2 ms for SAI and 200 ms for LAI. The preliminary results show no consistent pattern of intervention-induced changes in motor maps, SAI or LAI following any of the interventions.>

Disclosures: S. Toepp: None. **S. Folgia:** None. **P. Dans:** None. **A.J. Nelson:** None.

Digital Abstract Session

P217. Neuroprosthetics For Motor Systems

Program #/Poster #: P217.01

Topic: E.05. Brain-Machine Interface

Support: NIH 5R00NS097620
Cedars-Sinai

Title: Cortico-cerebellar adaptations associated with learning a cortically-controlled neuroprosthetic skill

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Abstract: Introduction: Brain-machine interfaces (BMIs) or neuroprosthetics have the potential to seamlessly merge the computational power of the motor system with that of artificial electronic systems. Previous studies have identified that learning to control a BMI involves an adaptive process in several cortical and subcortical regions of the brain. However, the cerebellar adaptations associated with neuroprosthetic learning remain incompletely understood. We have performed cerebellar recordings while rodents were trained on a cortically-controlled BMI, and analyzed the cortico-cerebellar adaptations associated with neuroprosthetic learning. **Methods:** We have recorded single units and local-field potentials (LFPs) by implanting microwire arrays in the primary motor cortex (M1) and silicon tetrodes in the cerebellum, while adult Long-Evans rats (n=4) performed a neuroprosthetic task. During the task, activity of experimenter-defined M1 neurons was transformed into the angular velocity of a feeding tube using a simple linear decoder. Rats have to modulate activity in these neurons, hereafter defined as 'direct' neurons, to obtain a reward. We analyzed how the activity in these direct neurons, as well as all other recorded (indirect) neurons in M1 and cerebellum changed while learning the neuroprosthetic task. We also analyzed band-limited oscillatory activity in both regions. **Results:** We found that learning a cortically-controlled BMI task led to indirect neurons showing a robust task-related modulation in the cerebellum and M1. Furthermore, we observed the emergence of task-related 3-6 Hz oscillatory activity in cortico-cerebellar LFPs, after neuroprosthetic learning. Also, task-related direct and indirect units' spike-field coherence (SFCs) were elevated in this frequency-band with learning. These changes were specific to robust BMI learning sessions and were not observed in poor learning sessions. **Conclusion:** Our work has identified cortico-cerebellar adaptations associated with learning a cortically-controlled neuroprosthetic task. This underscores the importance of optimal engagement of neural learning mechanisms in offsite motor regions while the neuroprosthetic task calls for modulation of only M1 activity.

Disclosures: A. Abbasi: None. **N. Danielsen:** None. **T. Gulati:** None.

Digital Abstract Session

P217. Neuroprosthetics For Motor Systems

Program #/Poster #: P217.02

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS K99/R00-NS101127
NIH NINDS R01-NS092626

Title: Common Patterns in Neural Learning and Optimization Across BCI Tasks

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Abstract: The study of brain-computer interfaces (BCIs) provides a unique opportunity to study the collective and individual learning properties of neurons. In this study we investigate, across three distinct task paradigms, the mechanism and patterns by which users modulate their neural activity and selectively optimize use of BCI control dimensions in order to improve task performance.

We analyzed neuronal data from 5 non-human primates who collectively performed three different BCI tasks. One task involved control of a virtual hand posture, a second task involved control of a cursor's position on a screen, and a third task involved control of a cursor's velocity on a screen. Between two and sixteen neurons, and between one and four BCI-control dimensions were used in each task. For each individual assignment, we analyzed the target independent, target dependent, and noise components of the neuronal firing rates. We then analyzed the magnitude and signal-to-noise ratio (SNR) of the firing rates over time. Magnitude was defined as the sum of target dependent variance, and SNR was defined as the ratio of the combined target independent and dependent variances to the total variance. Finally, we looked at optimization of neural modulation for each assignment, as measured by the difference between total firing rate magnitudes in BCI-output and non-BCI neural dimensions for each assignment. For each subject, we observed a pattern of increasing SNR and optimization over time across all assignments. A significant difference in their relative time courses was observed such that subjects reached peak SNR before peak optimization. Moreover, we observed that high SNR and optimization at the beginning of an assignment were associated with high SNR and optimization, respectively, at the end of an assignment, although low SNR and optimization were associated with greater increases in each over the course of an assignment.

Our results suggest a common pattern of BCI learning across distinct task types by which increases in signal-to-noise ratio of firing rates precedes increases in selective modulation of BCI-specific dimensions. Moreover, our results suggest that individual neurons differ in their capacity for adaptive BCI learning such that assignments with high performance early achieve higher end performance. Assignments with lower performance at the start had greater improvement in performance but did not reach the same peak performance as those with better starting performance.

Disclosures: A.M. Miller: None. **F.B. McAfee:** None. **Z. Liu:** None. **A.J. Law:** None. **M.H. Schieber:** None. **A.G. Rouse:** None.

Digital Abstract Session

P217. Neuroprosthetics For Motor Systems

Program #/Poster #: P217.03

Topic: E.05. Brain-Machine Interface

Title: Structural and functional changes of pyramidal neurons In primary motor cortex at the site of an implanted microelectrode array

Authors: *B. GREGORY[1], C. THOMPSON[2], M. RAILING[1], K. WILLIAMS[2], J. BEATTY[1], C. COX[1], E. PURCELL[2];
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Abstract: Implantable microelectrode arrays (MEAs) have created unprecedented opportunities to study brain function and treat neurological diseases. However, the rapid growth in use of these technologies has outpaced clear understanding of variable device longevity and performance caused by interactions with surrounding brain tissue. While gliosis and neuron cell death are well-known to occur following device implantation, the link between the tissue response and cell-specific changes in excitability are unknown. Our studies explore potential mechanisms of signal loss and instability by characterizing dendritic morphology and intrinsic excitability of pyramidal neurons in close proximity to an MEA. We employed fluorescence microscopy and patch-clamp electrophysiology in brain slice preparations containing a silicon or polyimide MEA device, harvested 1 week or 6 weeks after implantation into the primary motor cortex (M1) of adult Sprague-Dawley rats. Deep layer pyramidal neurons with somas <100 μm (Near-Device) and ≥500 μm (Distant-Device) from the implanted device were compared, with stab-injury and non-implanted controls also investigated. Morphology analyses show that Near-Device neurons at both time points have reduced dendritic arborization and reduced spine density. At 1 week, Near-Device neurons show a reduced density of filipodia (proto-spines), however that density is significantly increased after 6 weeks. Whole-cell intracellular recordings show that Near-Device neurons at 1 week show minimal differences in intrinsic properties or frequency of spontaneous excitatory postsynaptic currents (sEPSCs). At 6 weeks, Near-Device neurons show reduced sag amplitude (putative I_h), increased action potential half-width, and reduced sEPSC frequency. Our data suggest that after 6 weeks of implantation, Near-device neurons show a persistent reduction of dendrites, dendritic spine density, and excitatory activity. Additionally, these changes occur with both traditional, silicon-based devices and more flexible, polyimide-based devices. The results propose that a chronic, hypoexcitable network surrounding MEA devices could contribute significantly to MEA signal loss and instability.

Disclosures: B. Gregory: None. **C. Cox:** None. **J. Beatty:** None. **E. Purcell:** None. **K. Williams:** None. **C. Thompson:** None. **M. Railing:** None.

Digital Abstract Session

P217. Neuroprosthetics For Motor Systems

Program #/Poster #: P217.04

Topic: E.05. Brain-Machine Interface

Support: NSF CBET Grant 1724345
NSF Grant 1724263

Title: An Adaptive Model Predictive Control System for Functional Neuromuscular Stimulation Torque Control

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Abstract: Functional neuromuscular stimulation (FNS) is a well-established technique for reanimation of paralyzed limbs through electrical stimulation of muscles or nerves. Both open loop and feedback control techniques have been investigated in FNS systems to modulate stimulation to achieve a desired movement or torque production. We present here an adaptive model predictive control (aMPC) system for FNS and compare its performance to a proportional feedback controller (PFC). This aMPC system is designed around a dynamical model of the relationship between stimuli and neuromusculoskeletal system responses. Model predictive control systems have several advantages over feedback control techniques that include reduced response delays and complexity of gain tuning. Adaption mitigates the need for frequent recalibration of the model. We performed experiments with the aMPC system and a PFC to control isometric torque production, about the ankle in an animal model of paralysis, an anesthetized feline. A Utah Slanted Electrode Array (USEA) chronically implanted in the sciatic nerve was used to excite the lower limb muscles that produce torque. The aMPC system utilizes a linear twitch torque summation model based on single twitch recruitment curves (RC's) for each of the possible 96 electrodes. The RC relate the maximum twitch torque produced to the pulse width of a biphasic current pulse. Each sigmoidal-shaped RC was parameterized by the maximum torque, mid-point pulse width, and slope at the mid-point. The aMPC algorithm is composed of two parts: (1) a sequential determination of optimal stimuli based upon the twitch model and RC's and (2) online model recalibration by changing the parameters of each RC. For each experiment, the foot of the anesthetized feline was secured to the locked pedal of a custom torque monitoring device. The knee was also secured to ensure all torque measured came from the ankle. The evoked torque by the aMPC system for a 10 Ncm tall trapezoidal trajectory with a rise interval of 100 ms had a rise delay of 52 ± 34 ms (measured from the setpoint onset until the actual torque reached the setpoint) with a steady-state ripple of 1.4 ± 0.39 Ncm peak-to-peak, a steady-state error of 1.6 ± 1.1% and a overshoot of 41 ± 22% (n = 5). For the same trajectory, the PFC evoked a torque profile with a rise delay of 390 ± 130 ms, steady-state ripple of 0.93 ± 0.37 Ncm peak-to-peak, steady-state error of 1.3 ± 0.39 % and a overshoot of 27 ± 14 % (n = 5).The results currently demonstrate the reduction in response delay in the aMPC FNS system, with some minor compromises in steady state performance compared to the PFC. Overshoot remained high for both and is being addressed in future experiments.

Disclosures: W.M. Thomas: None. **L. Cavalcanti:** None. **V. Mathews:** None. **D.J. Warren:** None.

Digital Abstract Session

P217. Neuroprosthetics For Motor Systems

Program #/Poster #: P217.05

Topic: E.05. Brain-Machine Interface

Support: Stanford University Wu Tsai Neurosciences Institute
NSF GRFP DGE – 1656518
Stanford School of Medicine's Dean's Postdoctoral Fellowship

Title: A novel method for creating electrolytic lesions using a microelectrode array

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[1]Electrical Engin., [2]Bioengineering, [3]Neurosurg., [4]Wu Tsai Neurosciences Inst., [5]Bio-X, Stanford Univ., Stanford, CA

Abstract: Using multi-electrode arrays to record large populations of neurons has transformed motor systems neuroscience. Lesioning is another powerful method, as it offers strong causal insights. However, these two areas have not yet been combined; the effects of lesions have primarily been studied behaviorally. Combining chronic multi-electrode neuronal recording with lesional studies would enable causal investigation of the recorded population's role in generating skilled motor behavior over long timescales, while simultaneously providing unprecedented insight into neuronal reorganization after injury. In order to combine these methods, we need stable electrophysiology before and after the lesion and the ability to plan the lesion location and titrate its effect size. Although many lesioning methods exist, most are either invasive, potentially disturbing chronic electrodes, or the location and extent of the lesion is hard to control. We present a novel method for creating electrolytic lesions through chronically implanted multi-electrode arrays. We apply current through two electrodes of the array, using a custom-built current source to provide stable current in the face of changes in electrode impedance. Unlike conventional lesioning techniques, we avoid disruptive surgical procedures and can continue recording from the same population immediately after the lesion is performed. Electrode choice controls location, and by changing the duration and amount of current delivered, we can precisely control the extent of the injury. Calibration studies in ex-vivo and in-vivo porcine preparations allowed us to sweep a range of parameters, titrating to values that created damage without fully ablating the tissue. Our first nonhuman primate lesion in motor cortex resulted in a total estimated volume of 1.77 mm^3. Histology from this lesion was characterized by three qualitatively distinct zones: a lesion core of focal ablation, surrounding necrotic tissue from heat induced coagulation, and a periphery of rarefied tissue. The rarefied tissue at the periphery of the injury resembles the peri-infarct tissue after general hypoxic/ischemic trauma in humans, and it contains viable neurons whose survival and functional reorganization is hypothesized to play an important role in motor recovery. By comparing electrophysiology before and after the lesion, we have confirmed that the multi-electrode array continues to produce viable recordings and captures substantial changes in

neuronal population activity. We believe our new lesioning platform enables causal investigation of the relationship between activity of neuronal populations and behavior.

Disclosures: I.E. Bray: None. **S.E. Clarke:** None. **P. Nuyujukian:** None.

Digital Abstract Session

P217. Neuroprosthetics For Motor Systems

Program #/Poster #: P217.06

Topic: E.05. Brain-Machine Interface

Support: Stanford University Wu Tsai Neurosciences Institute
NSF GRFP DGE – 1656518
Stanford School of Medicine's Dean's Postdoctoral Fellowship

Title: Electrolytic lesion through multielectrode array alters population activity and impairs reach

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Abstract: It is widely accepted that the coordinated activity of neuron populations in motor cortex generates voluntary movement, yet much remains unknown about the aspects causally necessary in the planning, production, and coordination of motor system output. Tremendous progress in understanding motor cortex has been made by simultaneously recording the action potentials of hundreds of neurons using multi-electrode arrays in awake-behaving non-human primates and applying multidimensional estimation techniques that reduce the complexity of the data. The resulting low-dimensional models of population activity capture both classic features of the neuronal code (e.g., directional tuning), as well as activity that deviates from traditional theories, but which features are necessary for the control of behavior? Here, we present the results from a novel electrolytic lesion technique that allows us to probe causality through permanent perturbations of a recorded population, while observing how the neuronal activity and task behavior subsequently evolve. The technique is performed painlessly in rhesus monkeys through chronically implanted arrays in motor cortex, allowing for months of undisturbed population recordings right before, and immediately after spatially precise lesions that target the neuronal correlates of the behavior. A data set of ten successive lesions over five months has been gathered from our first animal, demonstrating substantial changes in single neuron activity and population structure that accompanies slowed reaches. The behavior recovers in under a week, during which local neuronal reorganization is captured by the multi-electrode array. Changes in population activity are affected at distances beyond the physical boundaries of the lesion (one sixth of array surface area), indicative of decreased activity that may arise due to loss of co-activation in the local network, or increased inhibition from local and remote sources. By seamlessly integrating recording, electrical stimulation, and lesion capabilities on a single

chronic implant, we are in a unique position to causally assess invariance of neuron mappings into low dimensional state space, as well as how the state space evolves over time.

Disclosures: **S. Clarke:** None. **I.E. Bray:** None. **P. Nuyujukian:** None.

Digital Abstract Session

P218. Advances in Decoding of Neuronal Activity

Program #/Poster #: P218.01

Topic: E.05. Brain-Machine Interface

Support: Dmitrii was supported by EU-funded Marie Skłodowska-Curie Actions "OSCBAGDIS" IF

Title: Distinguishing Parkinson's Limb Tremor from Voluntary Movements in Neural Data

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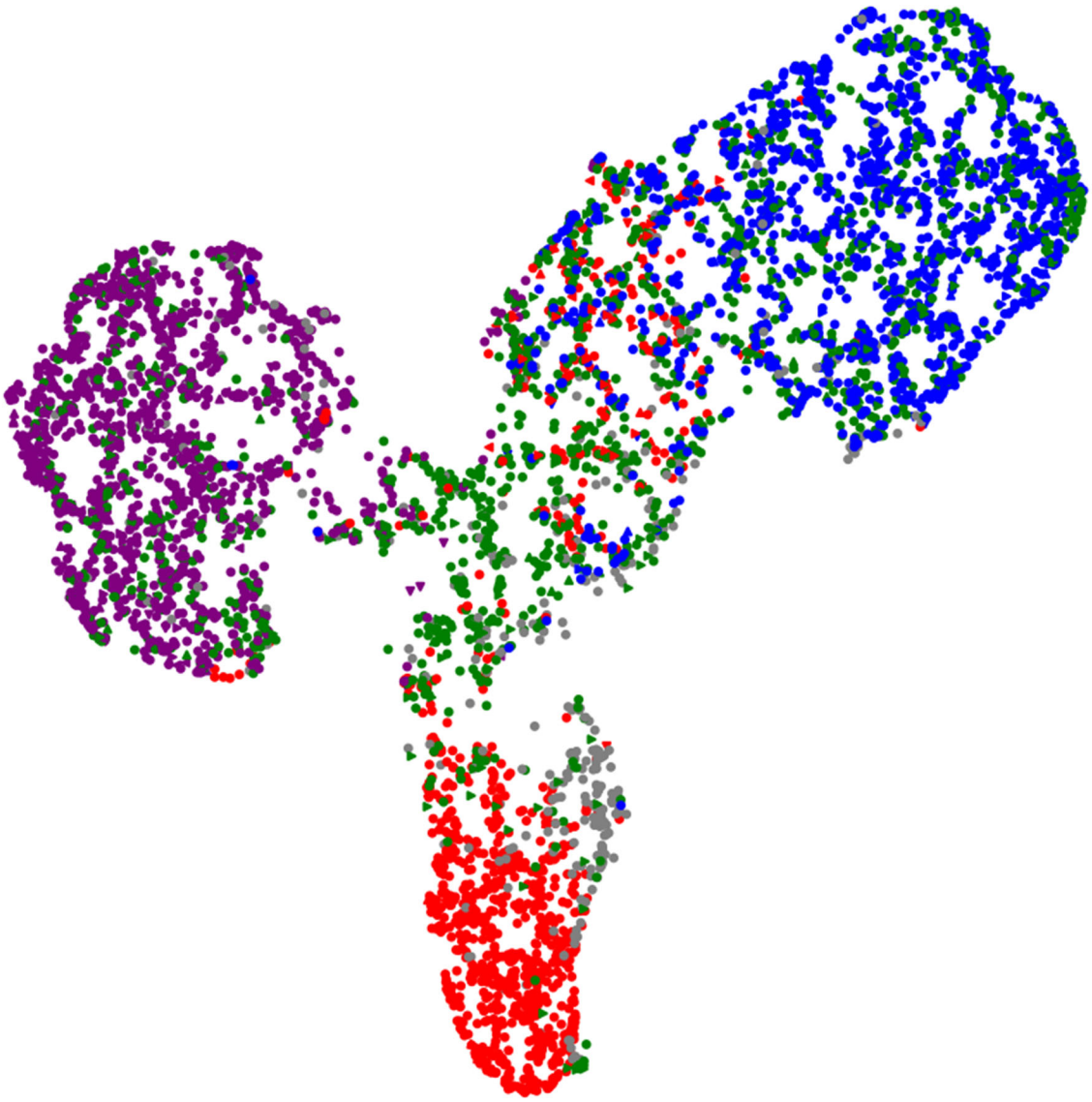
Abstract: Many processes in Parkinson's disease, despite a long history of research, are still not completely understood. In particular, there is no clear understanding of how Parkinson's disease rest tremor is generated and even how it can be detected and distinguished from voluntary movements in neural recordings. Such a distinction is important for developing for tremor-guided adaptive deep brain stimulation. Previous studies mostly used LFP from deep brain electrodes in basal ganglia to detect tremor (distinguishing tremor time periods from quiet time periods). However distinguishing tremor from voluntary movements has been proven to be challenging using only LFP data. Here we employ several machine learning methods on multimodal data from the cortex (MEG) and basal ganglia (subthalamic nucleus LFP) to successfully distinguish tremor, quiet periods and two types of voluntary movements in the PD patients displaying intermittent tremor. Moreover we also describe which data features (frequency bands bands, cortical regions, cross-channels couplings) contribute most to the classification. Figure shows a nonlinear projection to 2D of the data from one of the patients, transformed by a linear classifier, where different colors correspond to different ground truth behavioral states (determined from EMG recordings): red=tremor, purple=voluntary hold, blue=voluntary grasp, green=quiet rest.



Disclosures: **D. Todorov:** None. **A. Schnitzler:** None. **J. Hirschmann:** None.

Digital Abstract Session

P218. Advances in Decoding of Neuronal Activity

Program #/Poster #: P218.02

Topic: E.05. Brain-Machine Interface

Support: NSF Grant #1939987

Title: Does learning alter neural decoders of motor cortex?

Authors: *M. BHATTACHARYA[1], S. MEAMARDOOST[1], E. HWANG[2], T. KOMIYAMA[3], C. MEWES[4], L. WANG[5], Y. ZHANG[6], R. GUNAWAN[7];
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Abstract: The brain's motor cortex controls motor planning and motor tasks. Motor cortex has been shown to display a significant plasticity, in which neuronal activity of the motor cortex changes dramatically in a motor-skill learning. But, besides motor cortex, learning may also alter how motor cortex output is translated to voluntary movement - i.e., the motor cortex decoder. Here, we seek to elucidate alterations of the motor-cortex decoder in mice during new motor-skill by using long short-term memory (LSTM) recurrent neural network model. Deep neural network models have been demonstrated to be highly efficient in probing the functional outputs of the brain, and have been used as a powerful tool for Brain Computer Interfaces (BCIs). Here, we employ a data-driven approach to understand learning mechanisms. Neuronal activity of the motor cortex of mouse and the corresponding lever movement during a cue-driven reward-guided lever-press task has been used for the study. The mice are trained for 15 days to enable learning[1]. Preliminary analysis shows increasing trial-to-trial correlations between neuronal activity and lever movement[1] along with dynamic rewiring of connectome[2] inferred from neuronal activity, during motor skill learning. In this study, we use neuronal activity and corresponding lever movement data to model the motor cortex decoder. For elucidating the possible rewiring of the motor cortex decoder during learning, we built LSTM decoders for individual learning sessions and evaluated the accuracy of each session's decoder in predicting lever movements from motor cortex neuronal activity data from the corresponding session as well as other sessions. We further built a LSTM decoder using the cumulative data from all learning sessions and likewise, evaluated the performance of the cumulative decoder to predict lever movements. We observe stark changes from extreme naive to expert sessions and moderate alterations from one stage of learning to next with convergence towards the expert stages. The cumulative decoder is observed to be performing with comparable accuracy in all sessions as the individual decoders with performance improving towards later sessions due to highly correlated trial-trial neuronal activity and movements. Understanding the interplay of neural connectome rewiring across different brain's regions may lead to new architecture for interconnected AI.
REFERENCE: [1].Peters, Andrew J., Simon X. Chen, and Takaki Komiyama. "Emergence of reproducible spatiotemporal activity during motor learning." Nature 510.7504 (2014): 263-267., [2]. Meamardoost, Saber, et al. "FARCI: Fast and Robust Connectome Inference." bioRxiv (2020).

Disclosures: **M. Bhattacharya:** None. **S. Meamardoost:** None. **E. Hwang:** None. **T. Komiyama:** None. **C. Mewes:** None. **L. Wang:** None. **Y. Zhang:** None. **R. Gunawan:** None.

Digital Abstract Session

P218. Advances in Decoding of Neuronal Activity

Program #/Poster #: P218.03

Topic: E.05. Brain-Machine Interface

Support: University of Washington Computational Neuroscience Center Graduate Fellowship
Donald W. and Joan P. Baker Endowed Fellowship
Max E. Gellert Fellowship in Engineering
March Scholarship - Engineering Fund

Title: Electrocorticograph Signal Reconstruction with Recurrent Neural Network Autoencoders

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Abstract: Neural signal reconstruction and forecasting is a pragmatic means of approximating brain dynamics. Forecast signals can enable predictive algorithm design. Models of signal dynamics also enable unsupervised structure learning. We demonstrate electrocorticograph (ECoG) signal reconstruction using latent factor analysis for dynamical systems (LFADS) as a means of signal forecasting. LFADS has been used in prior studies to find task-relevant variable representation in latent factors within observed neural dynamics, but prior studies have done little to assess LFADS' neural signal reconstruction accuracy and have not yet applied the method to the reconstruction of field potential signals. Said studies have eschewed signal reconstruction performance assessments and instead focused on LFADS latent variable estimation capabilities, but signal reconstruction accuracy metrics are still needed for a robust model assessment. Seeking to bridge this gap, we report multichannel signal reconstruction using LFADS from non-human primate ECoG data collected continuously over a multiple day period and compare it to a best-fit linear autoregressive model. We find that LFADS provides substantially more accurate signal reconstructions than AR models in a relative prediction error (RPE) metric: AR models produce recreations with 0.827 +/- 0.094 RPE while LFADS model recreations achieve RPE values of 0.348 +/- 0.001. An RPE value of 0 indicates a perfect data reconstruction. While these results are promising, signals reconstructed by LFADS models were band-limited and incapable of recreating signal dynamics at frequencies higher than 40Hz (i.e. low and high gamma band frequencies). Further research into expanding LFADS representational capabilities will be needed for understanding how best to use LFADS-like approaches for reconstructing high-bandwidth neural data. We believe that these prediction accuracy improvements will enable novel predictive algorithm development and further enable unsupervised signal pattern learning through model latent space analysis.

Disclosures: **M. Nolan:** None. **B. Pesaran:** None. **E. Shlizerman:** None. **A.L. Orsborn:** None.

Digital Abstract Session

P218. Advances in Decoding of Neuronal Activity

Program #/Poster #: P218.04

Topic: E.05. Brain-Machine Interface

Support: U.S. NIH National Institute on Deafness and Other Communication (R01DC008358, R01DC018446, R01DC018055)
NIH National Institute of General Medical Sciences (R01GM134363)
National Science Foundation Grant BCS-1736028
Kavli Institute for the Brain and Mind (IRG #2016-004)
Office of Naval Research, Pew Latin American Fellowship in the Biomedical Sciences (E.A.)
National Science Foundation Graduate Research Fellowship under Grant No. DGE-1650112
IIE Fulbright (P.T)

Title: Latent state dynamics describe multiunit activity in premotor region of songbirds

Authors: *P. M. TOSTADO[1], D. E. BROWN, Jr.[2], E. M. ARNEODO[3], T. GENTNER[3], V. GILJA[2];
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Abstract: Brain Computer Interfaces (BCIs) hold promise to restore lost function to individuals with motor, speech and communication impairments due to injury or neurodegenerative disease. Traditionally, the primary research effort in this field has focused on studies of upper and lower limb motor control problems in rodents and non-human primates (NHPs). Despite recent heroic work in humans, more complex behaviors such as vocal production remain poorly understood. This is partly due to the lack of a well-established animal model for such behaviors. Thus, to better understand the neural mechanisms that enable complex motor-vocal behavior, we recorded the simultaneous activity of neurons in the sensory-motor nucleus HVC, a premotor region of the songbird brain, of male zebra finches (n=3) engaged in unconstrained vocal behavior during hours-long periods of time. We apply latent factor based models to describe the temporal dynamics of the recorded neural population. Complex, interdependent systems, such as biological neural networks, can often be captured by a lower-dimensional set of latent factors which describe how the observable output changes across time. Such latent representations of the system are typically robust to private noise present in measurements of its independent variables. Therefore, the understanding of the latent dynamics that emerge from activity across neural populations is crucially important in the design of robust, neurally-driven, chronic BCIs. We find that the temporal population dynamics of HVC correlate with specific features of the birdsong, including the onset of vocal production. Specifically, we observe a drop of up to 500% in the Fano factor in the majority of the channels recorded, which is time-locked to the start of periods

of vocalization and sustained across the song. The drop in neural variability happens in parallel with an expected x2 factor increase in the mean firing rates during vocal behavior relative to periods of inactivity. This visible reduction of neural variability prior to behavior onset is reinforced by a comparable reduction in shared variance in the latent space. Furthermore, we observe a temporal shift in the characteristic dynamics of the avian brain model and the behavior that may be informative of the functional connectivity across premotor and motor brain regions. These findings, originating from the latent-space modeling of brain activity in songbirds, are consistent with observations previously made in rodents and NHP during motor tasks.

Disclosures: P.M. Tostado: None. **D.E. Brown:** None. **E.M. Arneodo:** None. **T. Gentner:** None. **V. Gilja:** None.

Digital Abstract Session

P218. Advances in Decoding of Neuronal Activity

Program #/Poster #: P218.05

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01NS096971

Title: Trial-averaged neural trajectories display unique responses to unanticipated stimuli and can be used for online stimulus classification

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Abstract: Previous research into task-derived neural trajectories has often involved delayed-response tasks. However, motor brain machine interfaces (BMIs) must be able to react to complex, unpredictable scenarios. Thus, we sought to understand the low-dimensional neural dynamics associated with a task in which rats are subjected to unanticipated tilts. We then compared the performance of a classifier based on principal component analysis (PCA)-transformed data to that of a previously described classifier based on neural populations (i.e. peristimulus time histograms, PSTHs). **Methods:** Female Sprague-Dawley rats were placed on a platform that rotated along the frontal plane in two different directions and two different speeds to the same maximum angle at random intervals while neural activity was recorded in the hindlimb somatosensory cortex. Offline, we conducted PCA using the units as features and the first 200ms of spike data in 20ms bins from all trials as observations. Neural spike data was transformed using the output coefficients, and only the top three components were retained. Trajectories were found by plotting components against each other for each event type. Finally, to assess PCA-based classification of single trials, each event's trial-averaged PCA results were defined as templates, and a trial was assigned to the event with the smallest Euclidian distance to a given template. Classification performance was compared to that of a similar classifier that instead used PSTHs from all units. **Results:** Across animals, the first bin for each event trajectory was confined to a small state-space, suggesting similar baseline neural activity across

tilt types. However, these trajectories quickly diverged into four unique response profiles, with tilt direction being a large determinant of a trajectory's path and tilt speed determining trajectory length. These trajectories remained largely unchanged even after mid-thoracic spinal cord injury and over the course of 24 subsequent recording sessions. When comparing the classifier based off the first three components, offline performance remained largely unchanged compared to the full population, even when these first three components explained a small portion of the neural variance. **Conclusions:** Despite lacking an explicit motor planning component, visibly divergent, low-dimensional neural trajectories evolve over time when responding to unexpected postural perturbations, even after spinal cord injury. These trajectories can be used to classify single trials with a minimal loss of accuracy compared to classifiers based on full-population neural activity.

Disclosures: G.D. Disse: None. **K.A. Moxon:** None. **B. Nandakumar:** None.

Digital Abstract Session

P218. Advances in Decoding of Neuronal Activity

Program #/Poster #: P218.06

Topic: E.05. Brain-Machine Interface

Support: NSF Grant #1901492

Title: Action Selection for Heterologous Control of a Continuous-Output Myoelectric Prosthesis

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Abstract: Movement intent decoding algorithms can interpret human surface electromyography (sEMG) signals to control prosthetic limbs with many degrees of freedom (DOFs). In this work, machine-learning algorithms are used to decode the human subject's sEMG signals into a continuous output for each DOF of the prosthesis. This paper presents an Action Selection algorithm to improve the decoder training process. While generally applicable, it is motivated by the case of heterologous control of a robotic hand in which the subject cannot activate the forearm muscles originally used to control a hand. The subject therefore needs to create a new, unintuitive muscle-to-movement mapping by deciding which heterologous muscle actions to correspond to the DOFs of the prosthesis. The presented approach tackles this additional motor learning challenge efficiently.
Given a prosthesis with m DOFs and a set of available muscle actions A, the Action Selection algorithm determines the size-m subset of A that minimizes the expected error of a decoder trained on the actions in that subset. Various error metrics are combined to evaluate the decoder's accuracy, consistency, and responsiveness. The algorithm begins by collecting a small amount of training data from each action. Cross-validation of a machine-learning algorithm is run on the data from each individual action to eliminate poorly performing actions, and on data from each pairwise combination of actions to eliminate actions that are too similar for the decoder to differentiate. This process is repeated with stricter thresholds until the number of

subsets is less than a preselected threshold. Finally, the algorithm evaluates each remaining subset offline and outputs the ranking.
The algorithm was validated through several experiments. To validate the action removal step, inconsistently performed and repeated actions were added to an example action set. The individual-action and pairwise-action cross-validations successfully eliminated the extraneous actions. Next, in an example in which 3 actions were chosen from 6 (20 possible subsets), the algorithm's subset ranking was compared to the ranking when trained on larger datasets for each subset and tested online. The rankings correlated, indicating that the Action Selection algorithm can successfully predict online decoder performance from offline analysis of a limited training dataset. Finally, results from intact test subjects showed that action subsets chosen via the Action Selection algorithm outperformed those chosen by a naïve algorithm that ranks subsets based only on the feature data distributions.

Disclosures: S. Sylwester: None. **D.J. Warren:** None. **A. Fern:** None. **V.J. Mathews:** None.

Digital Abstract Session

P218. Advances in Decoding of Neuronal Activity

Program #/Poster #: P218.07

Topic: H.08. Learning and Memory

Support: NIH NINDS NS033221
NIH NINDS NS084017
NSF Center for Brains, Minds and Machines
McKnight Foundation

Title: Decoding of human identity by computer vision and neuronal vision

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Abstract: Computer vision (CV) guided by deep learning (DL) has made significant strides in the central challenge of recognizing a specific identity despite highly variable attributes. This indeed is the same challenge faced by the nervous system and partially addressed by the concept cells—neurons exhibiting invariant selective firing in response to specific persons/places, described in the human medial temporal lobe[1,2]. Given the firing pattern of such neurons, we can decode the concept presented in a discrete fashion[3]. Yet, the access to neurons representing a particular concept is limited due to these neurons' sparse coding. It is conceivable, however, that the information required for such decoding is present in small neuronal populations.

To address this question, we introduced an audiovisual sequence in the form of a movie, where a real-life changing narrative and several identities—movie characters—are presented with high variability. We recorded neural activity from multiple brain regions of ten neurosurgical epilepsy patients, implanted with depth electrodes, while they watched an episode of the TV series "24". We implemented two DL networks that used the time-varying population neural data as inputs and satisfactorily decoded the visual presence of the main characters in each frame. Before training and testing the DL models, we devised a minimally supervised CV algorithm (with comparable performance against manually labelled data[4]) to detect and label all the important characters in each frame. This methodology allowed us to compare "computer vision" with "neuronal vision", i.e., the footprints associated with each character present in the activity of a small number of neurons, and identify the brain regions that contributed to this decoding process. We then tested the DL models (trained on data during movie viewing) in a recognition memory task where subjects were asked to recognize clip segments from the presented episode and reject foil segments obtained from another episode they had not seen. Curiously, DL model activation was not only modulated by the presence of the corresponding characters but also by participants' subjective memory of whether they had seen the clip, and by the associative strengths of the characters in the narrative plot.
The described methodology can offer a novel way to probe the representation of concepts in time-evolving dynamic behavioral tasks. Further, the results suggest that the information required to robustly decode concepts is present in the population activity of a relatively small number of neurons in specific brain regions.
Refs:
[1]Quiroga et al., 2005
[2]Gelbard-Sagiv et al., 2008
[3]Quiroga et al., 2007
[4]Tang et al., 2016

Disclosures: **Y. Zhang:** None. **Z. M. Aghajan:** None. **M. Ison:** None. **Q. Lu:** None. **H. Tang:** None. **G. Kalender:** None. **T. Monsoor:** None. **J. Zheng:** None. **G. Kreiman:** None. **V. Roychowdhury:** None. **I. Fried:** None.

Digital Abstract Session

P219. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Program #/Poster #: P219.01

Topic: E.06. Posture and Gait

Title: The impact of dual task on step variability is influenced by stepping height during stepping in place without vision

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Abstract: Background. The variability of spatiotemporal gait parameters is altered when under dual task conditions (motor task + cognitive task) in young adults (e.g., Dubost et al., 2008, Decker et al., 2016). When variability is higher in dual task, it can indicate that the two tasks compete for attentional resources (Woollacott & Shumway-Cook, 2002), whereas when it is reduced, it may reveal a more automatic gait pattern promoted through a shift of attention away from movement control (Lövdén et al., 2008). Recently, we found that during stepping in place without vision, the coefficient of variation (CV) of stepping frequency was significantly larger in dual task than in the single stepping task. The question is whether the effect of dual task on the CV of stepping frequency and step height is modulated according to stepping height, i.e. comfortable step height (CStep) vs. high stepping (HStep). **Methods.** Fourteen young adults were blindfolded and stepped in place for 50 steps at a preferred pace. The protocol consisted of two single-task conditions CStep and HStep (approx. 45° and 90° of hip flexion, respectively) and two dual task conditions, (CStep+CT and HStep+CT). The cognitive task (CT) required participants to identify the total number of times that one pre-determined target digit occurred during a sequence of three-digit numbers presented every two seconds. In the dual task conditions, participants self-reported how much attention they had allotted to the CT relative to the stepping task. The Vicon512^{TM} System was used to record 3D kinematic data at a frequency of 200 Hz from markers placed on the heel (calcaneus) and big toe (distal phalanx) on both sides. Repeated measures ANOVA with two within-person factors (CT: with, without; Stepping Height: comfortable, high) were performed on the CV of stepping frequency and step height. **Results.** The CV of stepping frequency was lower for CStep and HStep+CT than for CStep+CT and HStep (significant interaction between CT and Stepping Height, $p < 0.01$). The CV of step height was lower at high stepping (HStep and HStep+CT) than at comfortable stepping (CStep and CStep+CT; significant main effect of Stepping Height, $p < 0.01$). The mean percentage of attentional focus that was allocated to the CT was lower at high (56%) than at comfortable stepping (68%, $p < 0.01$). **Conclusion.** We found that stepping height can modulate the dual task effect on the variability of spatiotemporal stepping parameters. Indeed, the stepping frequency and step height CVs were lower at HStep+CT than at CStep+CT. It suggests that lifting the knees high promoted a more stable stepping pattern, despite the shift in attentional focus towards the stepping task.

Disclosures: **N. Paquet:** None. **L. Michaud:** None. **N. Polskaia:** None. **Y. Lajoie:** None.

Digital Abstract Session

P219. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Program #/Poster #: P219.02

Topic: E.06. Posture and Gait

Support: SDSU University Grants Program

Title: Coordination of sensed state body state and foot placement related muscle activation

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Abstract: The dominant strategy for maintaining frontal plane walking balance appears to be control of lateral foot placement based on sensed body state. Previously, measured body state at mid-stance has best correlated with subsequent foot placement location, but visual information from the preceding step also appears to inform subsequent foot placement location. Therefore, it is unclear when body state is predominately sensed in order to devise a motor plan for controlling swing leg motion and subsequent foot placement. The purpose of this study was to establish timing of body state sensing in relation to foot placement related muscle activation and we hypothesized that this sensing would occur early in the gait cycle up until muscle activation. Six healthy adult subjects (age = 21.8 +/- 1.5 years, male = 3, female = 3) walked on a large treadmill at a comfortable speed for four minutes. Body segment positions were recorded with optical motion capture and used to estimate center-of-mass position and velocity (P_{com}, V_{com}) and swing foot position and velocity (P_{foot},V_{foot}). Surface electromyography (EMG) signals were recorded on four frontal plane hip muscles of the dominant leg: Gluteus Medius (GM), Tensor Fasciae Latae (TFL), Gracilis (Gr), and Adductor Longus (AL). Foot placement control timing was assessed by determining when body state was best correlated with foot placement related EMG. This was calculated by measuring and averaging the body states (P_{com}, V_{com}, P_{foot}, V_{foot}) and EMG of the GM, TFL, Gr, and AL within each 2% increment of the gait cycle (50 time windows per stride). Then, coefficient of determination (R^2) values describing the correlation between the average body states: P_{com}, V_{com}, P_{foot}, V_{foot} (independent variables) and average EMG for the GM (dependent variable) were assessed across 200 strides using a multiple linear regression. R^2 values were determined for each 50x50 sensory-motor time window combination. This analysis was repeated for each muscle tested. The correlation between body states and GM EMG was largest (R^2 highest) for body states at 20% [8,24] (peak [range]) and EMG at 20% [18,24] of the gait cycle. For TFL, the correlation was largest for body states at 18% [14,22] and EMG at 24% [20,24] of the gait cycle. For AL, the correlation was largest for body states at 20% [2,20] and EMG at 20% [20,22] of the gait cycle. For Gr, the correlation was largest for body states at 20% [14,22] and EMG at 22% [22,24] of the gait cycle. Our findings indicate that body states are broadly sensed from the beginning of double-support phase into early swing phase and inform foot placement related muscle activation focused around 30% of swing phase.

Disclosures: R.M. Sprague: None. **S.M. O'Connor:** None.

Digital Abstract Session

P219. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Program #/Poster #: P219.03

Topic: E.06. Posture and Gait

Barbour Foundation Studentship

Title: Differences in EMG activity of ankle plantarflexors and dorsiflexors during single and dual task walking in healthy older adults and people with Parkinson's disease

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Abstract: Background: Gait impairment in Parkinson's disease (PD) is characterised by decreased stride length, walking velocity and joint range of movement. Gait dysfunction is exacerbated during dual-task (DT) walking. Complex neural networks regulate activity and co-ordination of lower limb muscles essential for effective locomotion. Analysis of muscle activity may reveal underlying neural control mechanisms. However, current understanding of muscle activity during PD gait is limited (Islam et al. 2020).The aim of the study was to investigate: 1) distal lower limb muscle activity during walking in people with PD compared to healthy older adults (HOA) and; 2) the effect on muscle activity of adding an attentionally demanding cognitive task to single-task (ST) walking.**Methods**: We analysed muscle activity in 15 HOA (74 ± 6.9, m 8, f 7) and 30 people with idiopathic PD (71 ±5, m 19, f 11).Wireless surface electrodes recorded electromyographic (EMG) activity bilaterally from: tibialis anterior (TA), medial gastrocnemius (MG), lateral gastrocnemius (LG) and soleus (SO). Participants walked overground for 300 s (150 s ST, 150 s DT). Wavelet analysis was applied to the EMG signals to resolve them into time and frequency space. Eleven non-linearly spaced wavelets were used with centre frequencies from 7 Hz - 395 Hz (von Tscharner 2000). The wavelet transformed signals were segmented into gait cycles with gait events determined by a motion capture system and foot markers. EMG was time normalised to 100 timepoints and amplitude normalised to the mean maximum across gait cycles. EMG intensity and mean frequency (MF) were calculated. We applied linear mixed effect models which included walking task (ST/DT) and participant group (HOA/PD) as fixed effects.**Results:** Intensity of TA and SO decreased ($p<0.01$) during DT compared to ST whereas LG increased ($p<0.01$) in both groups. The PD group displayed a greater reduction in TA during DT walking ($p<0.01$) compared to the HOA. MF was lower during DT walking compared to ST in MG and SO for both groups with greater decrease for SO in the PD group.**Conclusions:** The decrease in EMG intensity during DT of uniarticular muscles TA and SO and increase in biarticular muscle LG may relate to the different motion demands and inhibitory effects of LG on SO (Prilutsky 2000). Greater reduction in TA activity in the PD group during DTW may be associated with changes in corticostriatal connectivity and sensorimotor impairment (Helich et al. 2015). As TA is important for foot placement, reduced activity during DTW may partially explain why people with PD experience greater falls. Reduction in MF during DT suggests changes in muscle fibre recruitment strategies.

Disclosures: K. Nazarpour: None. **L. Alcock:** None. **L. Rochester:** None. **A. Pantall:** None.

Digital Abstract Session

P219. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Program #/Poster #: P219.04

Topic: E.06. Posture and Gait

Support: NIH 2TL1TR001437

Title: Adaptive dynamic balance Response in Degenerative Cervical Myelopathy

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Abstract: Introduction Degenerative cervical myelopathy (DCM) is the most common form of spinal cord dysfunction in adults and frequently causes gait and balance impairments. Degenerative changes in the cervical spine may narrow the spinal canal and chronically and progressively compress the spinal cord. Despite surgical decompression, residual step width and balance impairments may persist. A better understanding of balance impairments and neuroadaptive capacity in this population will aid the development of rehabilitation interventions that improve balance in people with DCM (PwDCM) and inform whether rehabilitation strategies should focus on compensatory strategies or normalization of balance responses. The purpose of this study was to investigate the locomotor adaptability to repeated lateral waist pulls during walking among PwDCM compared to controls. Methods Ten PwDCM (7F/3M, 58.80±12.37y, modified Japanese Orthopedic Association Scale: 14.6±0.81, 3.20±3.96y post op) and 10 older controls without myelopathy (7F/3M, 55.78±10.09y) completed gait testing on an instrumented treadmill with an adjacent robotic cable pulley system. The primary outcome variable was step width pre-, early-, late-, and post-adaptation. Participants completed 624 steps per trial with 208 steps (104 left/104 right) pre adaptation, 208 steps within cable pull adaptation task, and 208 steps post adaptation. Four adaptation task conditions were used (5% body mass pulls in-phase with velocity of the center of mass, 2.5% body mass in-phase, 5% body mass anti-phase, 2.5% body mass anti-phase). Locomotion data was captured with 8 Vicon cameras and processed off line in Vicon Nexus (Oxford UK). Clinical outcome data collected were Berg Balance Scale, Functional Gait Assessment scores and walking speed. Statistical results were computed in SPSS (IBM, Chicago, USA) using a mixed effects model for fixed effects of myelopathy, pull magnitude, and pull phasing with Sidak corrected post-hoc tests and alpha set a priori at 0.05. Clinical tests were compared with independent t-tests. Results PwDCM walked at slower self-selected speed ($p=0.04$), had lower Berg Balance scores ($p<0.01$), and Functional Gait Assessment scores ($p=0.01$) than controls. For step width there was an interaction between pull phase and adaptation timepoint ($p<0.01$). In-phase pulls increased step width in early-adaptation and post-adaptation whereas anti-phase pulls decreased post-adaptation step width. PwDCM walked with wider steps regardless of pull phase, magnitude, or trial timepoint ($p<0.01$). Conclusions
Due to similar adaptability to controls, PwDCM may benefit from locomotor balance therapies.

Disclosures: T.F. Boerger: None. **L. McGinn:** None. **S. Lessila:** None. **M. Wang:** None. **B. Schmit:** None. **A. Hyngstrom:** None.

Digital Abstract Session

P219. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Program #/Poster #: P219.05

Topic: E.06. Posture and Gait

Title: Remote monitoring of motor performance via smartphone applications and markerless tracking

Authors: *E. QUARTA, G. VICHI, V. SORGENTE, R. BRAVI, E. J. COHEN, D. MINCIACCHI;
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Abstract: Motor control is usually studied by having test subjects coming to the lab to participate in experiments, often using dedicated instruments and setups. While this approach remains the gold standard for scientific experimentation, it is also important to develop methods for remote monitoring. This is especially relevant for populations with reduced mobility or that cannot easily access health professional consultancy. Moreover, due to the current pandemic, such a need is even more urging. Here we obtained movement data from healthy participants (n=9) that were either at their homes or, as a control condition, were tested in the lab. To collect data we leveraged on the fact that a large fraction of the general population (60.8%) owns a smartphone, especially those between the ages of 18 and 49 (more than 90%). In addition, appropriate data logger applications (app) are freely available. As a proof of concept, we tested postural control, which was performed remotely at the homes of the participants during a videoconference. The experimenter instructed the participant to install a free available app (i.e., AndroSensor, Fiv Asim, for Android devices or Physics Toolbox Sensor Suite, Vieyra Software, for iPhones) on their smartphone, which allowed to record tri-axial accelerations (sampling frequency 100Hz). Following that, participants were instructed to strap their phone to the lower back and “stand quietly while barefooted for 80 seconds”. The experiment was performed in several conditions in a randomised order which included: eyes open vs closed, bipodalic vs monopodalic stance. A control condition, in which the smartphone was placed on the ground, was also included. In selected trials, a concurrent video-recording of the trials (extracted from the videoconference; a sound cue was used for synchronisation) was performed by the experimenter, to be used for off-line markerless tracking of kinematic data which were later compared with the app data. Preliminary results indicate that remote monitoring of motor performance is similar to that obtained in the lab, with the great advantage of being low cost, time-effective and scalable which is a step toward a location-independent investigation of motor control/performance parameters.

Disclosures: E. Quarta: None. **G. Vichi:** None. **V. Sorgente:** None. **R. Bravi:** None. **E.J. Cohen:** None. **D. Minciacchi:** None.

Digital Abstract Session

P219. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Program #/Poster #: P219.06

Topic: E.06. Posture and Gait

Support: NIH UL1TR002538
NIH TL1TR002540
NIH 1DP5OD029571-01
DOD-CDMRP-PRORP-ARA W81XWH-16-1-0701

Title: Robust Electromyographic Control During Active Exoskeleton Assistance Despite Non-Linear Reorganization of Locomotor Output

Authors: *J. A. GEORGE[1], A. J. GUNNELL[2], D. ARCHANGELI[2], G. HUNT[2], M. ISHMAEL[2], K. B. FOREMAN[3], T. LENZI[2];
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Abstract: Robotic exoskeletons can assist humans with walking by providing supplemental torque at the joint level. Most assistive exoskeletons aim to provide supplemental torque proportionate to the user's muscle torque, which can be inferred by exploiting the relatively consistent torques generated during cyclic movements such as walking. Unfortunately, this control approach fails when the user performs non-cyclic movements (e.g., start walking, stop walking, change direction). In theory, electromyographic (EMG) control algorithms can overcome this challenge by estimating the user's muscle torque directly using EMG recordings from the muscles that generate the torque. However, EMG changes as a result of supplemental torque from an exoskeleton, resulting in unreliable estimates of the user's muscle torque during active exoskeleton assistance. Here, we present an EMG control framework for robotic exoskeletons that provides consistent muscle torque estimates across varying levels of assistance. Experiments with three healthy human subjects showed that using diverse training data (from different levels of assistance) enables robust control, and that a convolutional neural network (CNN), but not a Kalman filter (KF), can capture the non-linear transformations in EMG due to exoskeleton assistance. With diverse training, the CNN could reliably estimate muscle torque from EMG during zero, low, medium, and high levels of exoskeleton assistance (root mean squared error (RMSE) below 0.098). In contrast, without diverse training, RMSE of the CNN ranged from 0.103 to 0.147. RMSE of the KF ranged from 0.140 to 0.187 without diverse training, and did not improve with diverse training. When patient time is limited, training data should emphasize the highest levels of assistance first and utilize at least three full gait cycles. The average percent reduction in RMSE was 31.65% during the first three gait cycles and ~1% in all subsequent cycles (with performance still improving with 52 cycles). The results presented here constitute an important step towards adaptive and robust human augmentation via robotic exoskeletons. This work also highlights the non-linear reorganization of locomotor output when

using assistive exoskeletons; significant reductions in EMG were observed for the soleus and gastrocnemius, and a significant increase in EMG was observed for the spinae erector. Control algorithms that can accommodate spatiotemporal changes in muscle activity have broad implications for exoskeleton-based assistance and rehabilitation following neuromuscular injury.

Disclosures: J.A. George: None. **A.J. Gunnell:** None. **D. Archangeli:** None. **G. Hunt:** None. **M. Ishmael:** None. **K.B. Foreman:** None. **T. Lenzi:** None.

Digital Abstract Session

P219. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Program #/Poster #: P219.07

Topic: E.06. Posture and Gait

Title: Inter-limb coordination in chronic stroke survivors

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Abstract: Stroke is the leading cause of disability among adults and can cause major physical deficits including impaired gait, muscle weakness, and balance problems. Conventional rehabilitation practices often use assessments and therapy that target individual limb deficits and rarely does this therapy consider gait-coordination deficits. Although linear measurements have been the standard for measuring outcomes, they look at outcomes using averages and standard deviations, and in doing so only provides them with a snapshot of the behavior. Nonlinear measurements (e.g., cross recurrence quantification analysis (cRQA) and cross sample entropy (cSE), can be used to address this limitation, and complement current approaches by providing us a motion picture of inter-limb coordination. The goal of this project is to quantify the temporal structure of inter-limb coordination in chronic stroke survivors and healthy age-matched controls using nonlinear measurements. Thirteen chronic stroke survivors and thirteen healthy- age-matched controls walked on the treadmill for five minutes at their preferred walking speed, during which their kinematics were recorded via motion capture system. Inter-limb coordination was calculated via nonlinear methods cRQA (provides the variable 'Mean Diagonal Line Length' and corresponds to the average duration of coordination between the left- and right-legs) and cSE (describes the synchrony between the limbs during gait). Stroke survivors had significantly shorter mean diagonal line lengths than the healthy controls suggesting they had shorter duration of coupling between the two limbs. For cSE there were statistically significant differences between groups suggesting that the stroke survivors' gait was more repeatable, and possibly less adaptable to potential perturbations. Using cRQA and cSE, we have shown that the evolution of inter-limb coordination while walking is markedly different between healthy controls and chronic stroke survivors. Although even after a stroke, the two legs show coordinated behavior, the results show that this coordination is abnormal, may impede functional recovery and therefore could be a potential target when designing new rehabilitation paradigms.

Disclosures: **T. Sado:** None. **Z. Motz:** None. **M. Mukherjee:** None.

Digital Abstract Session

P219. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Program #/Poster #: P219.08

Topic: E.06. Posture and Gait

Support: NIH/NIGMS Award Number U54GM104942

Title: Step length adaptation imposes robust gait asymmetry in humans

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Abstract: Walking on a split-belt treadmill that imposes different speeds on each leg develops robust asymmetric aftereffects. The goal is to use this intervention as a rehabilitation approach. Yet, the difference in limb speeds is behaviorally related to turning and not to the limb preference, as seen in many gait pathologies. Here, we impose a stride length constraint using a wearable passive orthosis to modify limb dynamics independently from the limb speed. A group of young adults (N=3, 22.7±3 years) walked on a treadmill instrumented with force plates. Each session consisted of 3 blocks: unconstrained, constrained, and washout unconstrained locomotion at 0.8 m/s. The gait events corresponding to heel-contact and toe-off were detected from the ground reaction profiles for each limb, and each step cycle was described by four periods: leading double-stance, single stance, trailing double-stance, and swing. We observed temporal asymmetries during constrained stepping, shown by a large difference in magnitude between the leading and trailing double-stance compared to that in unconstrained stepping. To quantify this asymmetry, we calculated an asymmetric index (AI) as the difference between leading and trailing double-stance over the sum of the leading and trailing double-stance. A repeated measures ANOVA showed that there was a significant difference in mean AI between the unconstrained and constrained stepping, $F(1,2)=116.95$, $p<0.01$. The preliminary result indicates that this paradigm requires a period of deadaptation to return to symmetric locomotion. This supports the potential use of this task for the development of the intervention techniques for gait rehabilitation.

Disclosures: **E. Herrick:** None. **S. Yakovenko:** None.

Digital Abstract Session

P219. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Program #/Poster #: P219.09

Topic: E.06. Posture and Gait

Support: NREF Research Fellowship GR010471
NINDS Grant R01 NS089972
DOD Grant SCI140238/W81XWH1510584
NIH Grant 1S10OD023579-01

Title: Electrical Characterization of the Mesencephalic Locomotor Region in a Large Animal Model of Neuromodulation

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Abstract: Introduction: The mesencephalic locomotor region (MLR) is a functionally defined area in the midbrain, where electrical stimulation was discovered to initiate locomotion in several animals. Deep brain stimulation (DBS) of this region has been studied as a therapeutic target in rodent models of stroke, parkinsonism, and spinal cord injury. Clinical DBS trials have targeted the closely related pedunculopontine nucleus in patients with Parkinson's disease as a therapy for gait dysfunction, with mixed outcomes. Recent studies suggest that optimizing the MLR target could improve its effectiveness. **Objective:** We sought to determine if pigs have an MLR, and if stereotaxic targeting and DBS in the region was feasible to generate a large animal neuromodulation model of gait. We aimed to characterize optimal electrode positions and stimulation parameters to evoke locomotion, as well as any nearby off-target effects. **Methods:** We implanted electrodes into putative MLR structures in Yucatan micropigs to characterize the locomotor effects of acute DBS in this area, using EMG recordings, joint kinematics, and speed measurements on a manual treadmill. **Results:** MLR DBS robustly initiated and augmented locomotion in freely moving pigs. Effective locomotor sites centered around the Cuneiform nucleus and locomotor output depended on stimulation parameters. Off-target stimulation and high frequencies of stimulation evoked defensive and aversive behaviors in the animals. **Conclusion:** Pigs have an MLR and can be used to model neuromodulation of this gait-promoting center. Electrode position and stimulation parameters were critical to optimally evoke locomotor output while minimizing off-target effects. These results may provide insight to guide future clinical efforts.

Disclosures: S.J. Chang: None. **A.J. Santamaria:** None. **F.J. Sanchez:** None. **L.M. Villamil:** None. **P.M. Saraiva:** None. **Y. Nunez:** None. **J.P. Solano:** None. **J.D. Guest:** None. **B. Noga:** None.

Digital Abstract Session

P220. Posture and Gait: Afferent Control

Program #/Poster #: P220.01

Topic: E.06. Posture and Gait

Support: NIH Grant U54-GM-104942
NSF 2015317

Title: Evaluation and modeling of mechanosensory encoding of forces during walking using joint torques as 'naturalistic' stimuli

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Abstract: Control of adaptive walking requires the detection and regulation of muscle forces and loads. This information is thought be derived from sensory receptors of the legs but the specific parameters that are encoded in walking have not been determined. We have studied how forces are signaled by mechanoreceptors (campaniform sensilla) of the legs of insects. We have also recently developed a model of signal transduction that accurately simulates many characteristics of the receptors. Previous studies have shown that application of forces using 'naturalistic' stimuli (waveforms of joint torques of freely walking animals calculated by inverse dynamics) produced graded activation of leg muscles and substantially reduced motor adaptation seen to conventional ramp and hold functions. The goal of the present studies was to elucidate and model the sensory mechanisms underlying these effects. Experimental application of waveforms of torques of the femoro-tibial joint in stick insects elicited discharges in tibial campaniform sensilla that strongly reflected the rate of change of force (dF/dt) throughout the stance phase, not merely at initiation of force application. These waveforms show no 'hold phase' and initial, non-linear, graded increases are most often followed by periods in which variations can occur, potentially due to gait and load transfer. We studied the initial component by application of forces with varying exponential rates of increase that reached a plateau. These studies showed that the delay of onset of sensory adaptation was correlated with the duration of positive dF/dt. Sensory discharges also showed substantial hysteresis (similar to many other proprioceptors) and were inhibited during transient or prolonged periods of force decrease, even when they occurred in periods of high sustained forces. We postulate that hysteresis can aid in the control of residual muscle tensions. Many of these characteristics of responses to 'naturalistic' stimuli were accurately simulated by our model, including time dependent components. The model output is the comparison between the instantaneous force stimulus and a low pass filtered copy of the stimulus. As a result, the model is "self-calibrating"; that is, it adapts to tonic forces, but responds strongly to rapid changes in force. Future studies will examine the effects of receptor properties in motor control. Our working hypothesis is that sensory discharges are tuned for the regulation of skeletal muscles. The dynamic and static sensitivities form a continuum of signals that could aid in adjusting motor activities in the generation of natural movements of animals or in the control of walking machines.

Disclosures: **N. Szczecinski:** None. **S.N. Zill:** None. **C.J. Dallmann:** None.

Digital Abstract Session

P221. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Program #/Poster #: P221.01

Topic: E.06. Posture and Gait

Support: NASA EPSCoR grant 80NSSC18M0076
American Heart Association award 18AIREA33960251
American Heart Association (Pre-doctoral Fellowship) 19PRE34380881
NASA Nebraska Space grant fellowship

Title: Determining Transitions in Postural Sway Coupling between Visual Stimuli of Differing Complexities using Cross-Wavelet Coherence

Authors: Z. MOTZ[1], A. LIKENS[1], ***M. MUKHERJEE**[2];
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Abstract: Introduction: Postural sway is affected by environmental changes and adapting to these changes allow us to maintain our upright stance. The ability to couple with changing environmental conditions provides insights into our ability to flexibly adapt to dynamic environmental states. Previous studies have shown that participants can transition coupling their postural sway from one stimulus to another when such stimuli are presented in sequence. Such coupling behavior comprises of postural dynamics that shows complex patterns that are related to the complexity of the stimulus. However, it is not clear how the dynamics of postural control change at the transition points between different stimuli. In this study, we used cross-wavelet coherence to determine the common power between postural sway and visual stimuli to understand how participants transition coupling from one stimulus to another. **Methods*:*** Fourteen participants performed a postural task in which they shifted weight mediolaterally on the frontal plane while matching their sway to a moving visual stimulus presented on a 180° virtual reality screen. Both deterministic (periodic and chaotic) and non-deterministic (random) stimuli were provided individually (baseline) and in combination. The combination trials comprised of either 20 second sections of the random stimulus followed by 20 seconds of the periodic stimulus, repeating for three minutes or the same structure with the chaotic stimulus instead of the periodic. The degree of center-of-mass to target coupling for the five seconds before and five seconds after the stimulus transition was quantified using cross-wavelet coherence. Cross-wavelet coherence represented the power relation between two time series in the time-frequency space. **Results/Discussion:** Transition points in postural sway coupling when switching between two external visual stimuli, show characteristic behavioral patterns that are different from postural sway dynamics during steady state stimuli. These transitions display hallmark characteristics of dynamical systems in that participants seem to settle into attractor states before and after the transition, and complexity appears to be the control parameter, as evidenced by an apparent hysteresis effect concerning the direction of the transition. Moreover, coupling during transitions is context dependent and based on both the high- and low-frequency components of the stimuli.

Disclosures: **M. Mukherjee:** None. **Z. Motz:** None. **A. Likens:** None.

Digital Abstract Session

P221. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Program #/Poster #: P221.02

Topic: E.06. Posture and Gait

Support: NIH Grant EY026994

Title: Evaluating Associations Between Central Visual Field Loss and Conscious Movement Processing

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Abstract: Central visual field loss (CFL) due to diseases such as age-related macular degeneration (AMD) is a large and growing problem. While much is known about the visual limitations associated with this condition, one of its most dangerous and poorly understood outcomes is the increase in the risk of falls, which can be debilitating and even deadly. Recent studies suggest that older adults deemed to be at an increased risk of falls may alter their movement processing strategies (Wong et al. 2008). Specifically, heightened conscious movement processing and task-irrelevant ruminations are observed; both of which are linked to increased fall-related anxiety and increased movement errors when walking (Ellmers et al. 2019). These behaviors are also linked with maladaptive visual search strategies (i.e. reducing previewing of an intended walking path) during locomotion that may further increase the risk of trips, slips and falls. The current study sought to evaluate if similar conscious movement processes emerge in individuals with CFL and if these changes are related to the extent of visual deficit.
To assess changes in movement processing we used the Gait-Specific Attentional Profile (G-SAP, Young et al. 2020). Items on this scale were divided in to three sub-scales: conscious movement processing, anxiety, and rumination. The total score for each category was calculated separately for each participant and correlated with measures of binocular contrast sensitivity (MARS letter test) and visual acuity (VA) of the better eye. A total of 29 individuals with CFL (18 F) were surveyed for the study. We found binocular contrast sensitivity to be a significant predictor of increases in conscious movement processing during locomotion in individuals with CFL (linear regression, $p = 0.037$). Additionally, VA of the better eye was a strong predictor of an increase in anxious thoughts during locomotion ($p = 0.041$). The latter may be related to previously-reported increases in overall fear of falling in individuals with AMD (van Landingham et al. 2014). These findings suggest that further experiments are needed to investigate potentially dangerous behavioral changes in gaze strategies and posture in patients with CFL associated with increased conscious monitoring of movements during locomotion.

Disclosures: **N. Shanidze:** None. **R. Lee:** None. **W.R. Young:** None.

Digital Abstract Session

P221. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Program #/Poster #: P221.03

Topic: E.06. Posture and Gait

Support: CAPES Grant 88881.189725/2018-01
Barbour Foundation PhD studentship
Reece Foundation

Title: Changes in cortical activity and gait parameters during dual task walking and after bi-anodal tDCS and treadmill walking in young and older adults

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Abstract: Background: Gait dysfunction is common in older adults. Studies indicate that transcranial direct current stimulation (tDCS) combined with treadmill walking (tDCS_TW) has potential to decrease gait dysfunction, particularly during dual-task walking (DTW). **Objective**: We investigated the effect of tDCS_TW on cortical activity and gait parameters in young adults (YA) and OA during DTW. **Methods**: Twenty-three YA and 21 OA were randomly allocated to receive active or sham tDCS. Participants performed 5 mins of treadmill walking (alternating 30 s bouts of single-task walking and DTW) before and after a 20 min intervention of tDCS_TW. Active anodal stimulation was applied to the left prefrontal cortex (PFC) and primary motor cortex (M1) using 9 cm^2 electrodes at 0.6mA. Cortical activity of the PFC, M1, premotor cortex (PMC), and supplementary motor area (SMA) bilaterally were recorded using a tethered functional near infra-red spectroscopy (fNIRS) system. An accelerometer measured gait parameters. We applied linear mixed effects models which included group (YA, OA) and intervention (sham, active) as fixed effects. **Results**: OA displayed higher activity bilaterally in the PFC and M1, and unilaterally in the right PMC during DTW compared to YA ($p<0.05$). Cadence was lower and gait variability higher in OA than YA. M1 activity decreased in both YA and OA following active tDCS. **Conclusion**: Increased activity in multiple cortical areas during DTW in OA may act as a compensatory mechanism to deal with increased task complexity during gait. In addition, tDCS_TW decreased M1 activity in both groups, suggesting active tDCS combined with treadmill walking may improve neural efficiency.

Disclosures: **D. Orcioli-Silva:** None. **A. Islam:** None. **L. Rochester:** None. **L.T.B. Gobbi:** None. **A. Pantall:** None.

Digital Abstract Session

P221. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Program #/Poster #: P221.04

Topic: E.06. Posture and Gait

Support: NIH CoBRE P20GM109098

Title: Asymmetric precision walking task is controlled by the same synergies in rats

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Abstract: Walking with turning and precise limb placement is executed by a complex spatiotemporal pattern of muscle activations. The musculoskeletal high-dimensionality in walking is typically solved through the use of low-dimensional neuromuscular control signals, called synergies. Here, we test the composition of synergies in a precision walking task in symmetric and asymmetric conditions. Nine Sprague-Dawley rats were implanted with arrays of intramuscular electrodes to record EMG from the shoulder, elbow, and wrist flexors and extensors. Animals were trained to walk on a peg-way with movable bilateral individual pegs instrumented with force sensors. The asymmetric conditions were set so that animals walked either symmetrically with an overall preferred stride length of 15 cm or asymmetrically with either ipsilateral or contralateral limb forced to increase limb transfer during the swing and to exhibit limb preference during each stride. We extracted a low-dimensional structure in each condition from averaged EMG envelopes during the period from the onset of swing to foot contact identified from ground reaction forces. Using a dimensionality reduction algorithm, nonnegative matrix factorization (NNMF), we found four synergies explaining 99.95% of variability and temporally related to limb retraction, limb transfer, foot placement, and stance initiation. The weights representing the contribution of each temporal component are highly correlated across conditions ($r=0.96\pm0.04$) supporting the hypothesis that the same synergies generate movement across asymmetric conditions. This evidence provides further support that precise limb placement in symmetric and asymmetric stepping is controlled by the same control system in rodents, and it may be a therapeutic target for the rehabilitation of locomotor asymmetries.

Disclosures: K. Hanna: None. **S. Yakovenko:** None.

Digital Abstract Session

P221. Posture and Gait: Higher Order Control, Multi-Task Integration, and Theory

Program #/Poster #: P221.05

Topic: E.06. Posture and Gait

Support: SFARI-602705

Title: Predictive Impairments in Motor Skills in Children with Autism Spectrum Disorder

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Abstract: Anecdotal reports and selected research results suggest that individuals with autism spectrum disorder (ASD) exhibit difficulties in motor coordination, especially when interacting with dynamic objects, as in catching a ball. A theoretical framework from our group proposed that the distinct manifestations of ASD in multiple domains, including social interactions, cognition and motor coordination, have a common core: compromised temporal prediction (Sinha et al., 2014, PNAS). We tested this hypothesis in the motor domain: Would individuals with ASD show impaired motor coordination when intercepting a moving object as this task requires trajectory prediction? With this goal, we studied full body movements of ASD children while they attempted to catch balls launched towards them. To account for potential non-prediction related factors that could contribute to impaired catching performance, we conducted a series of matching control tasks. 16 children with ASD (aged 7-12 years) and 16 age- and IQ-matched neurotypical (NT) children participated in the experiment. They were asked to catch a ping-pong ball launched from a ball machine using one or two hands. 3D motion capture recorded joint kinematics of participants' arms and the flying ball. A set of control tasks assessed postural balance, reaction time when lifting both arms, and movement speed in reaching to a stationary ball. We hypothesized that in these control tasks, ASD and NT participants would not differ. Results showed that ASD participants indeed had fewer successful catches than NTs ($p=0.01$). Detailed kinematic analyses of ball and hand trajectories revealed that while NTs approached the ball with a relatively stable profile of hand velocity, ASDs exhibited a collision-like motion, suggesting insufficient prediction of the upcoming ball. In the control task of lifting both arms, ASDs and NTs showed similar reaction times ($p=0.18$). When reaching to a stationary ball, reaction times and movement speeds were also not significantly different ($p=0.789$). In addition, postural sway also did not differ ($p=0.45$). These lack of differences in basic aspects of motor coordination support the interpretation that it is the predictive demands that are likely responsible for the reduced performance of ASD participants when catching a flying ball. We conclude that in complex interactive motor tasks, performance of ASD children may be affected by reduced predictive motor control, consistent with our hypothesis. It remains to be seen whether this evidence of impairments in prediction will generalize beyond motor skills, enabling a more unified understanding of the complex autism phenotype.

Disclosures: S.F. Bond: None. **S. Park:** None. **M. Russo:** None. **A. Cardinaux:** None. **P. Sinha:** None. **D. Sternad:** None.

Digital Abstract Session

P222. Posture and Gait: Aging, Injury, and Disease

Program #/Poster #: P222.01

Topic: E.06. Posture and Gait

Title: Relationship between cognitive and gait recovery at 2- and 12- months post-Traumatic brain injury

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Abstract: Objectives/Rationale. This study examined the relationships between cognitive and motor functioning over time in a cohort of post-Traumatic Brain Injury (TBI) patients. Executive dysfunction is associated with slower walking speed (Yogev-Seligmann et al., 2008), increased incidence of falls (Persad et al., 2008), and increased stride time variability (Allali et al., 2008). However, investigations have yet to examine whether executive functioning predicts post-rehabilitation gait outcomes. We investigated if (1) measures of executive function correlate with gait outcomes and (2) if executive functioning at 2-months predicts gait at 12-months.**Methods.** N=93 TBI-patients underwent cognitive testing, Trail making B score (TMTB) and Digit Span Forward (DSF), and gait testing at 2- and 12-months post-injury. Six spatiotemporal variables were extracted: Step Length variability at Self Paced (SP) and Maximum Paced (MP), Step Time variability SP and MP, and Gait Velocity SP and MP. Spearman's correlation coefficients were calculated for gait and cognitive variables at concurrent time points and 2-month cognition with 12-month gait. Six Multiple Linear Regression models were fitted to assess whether 2-month cognition predicted 12-month gait. **Results.** 2-month TMTB correlated with 2-month Velocity MP ($r=0.28$, $p<0.01$) and SP ($r=0.33$, $p<0.01$), Step Time variability MP and SP ($r=-0.32$, $p=0.0326$; $r=-0.39$, $p<0.01$). 2-month Digit Span correlated with Velocity MP ($r=0.26$, $p<0.05$). At 12-months, TMT B and DSF were correlated with velocity SP. 2-month TMTB correlated with 12-month Step Length variability SP ($r=-0.51$, $p<0.01$), and Step Time variability SP (-0.53, $p<0.001$). 2-month Digit Span was correlated with 12-month Velocity SP and MP, ($r=-0.44$, $p<0.01$; $r=0.29$, $p<0.05$). 2-month TMTB was a significant predictor of 12-month gait in the models for Step Length variability SP ($p<0.05$), Step Time variability SP ($p<0.05$), and Velocity MP ($p<0.05$).**Conclusion**. Findings present evidence for correlations between the pre- and post-rehabilitation executive function and gait outcomes. Our prediction finding, TMTB (but not DSF) significantly predicted 12-month gait, suggests that future research in TBI rehabilitation should investigate motor and cognitive recovery in tandem rather than as distinct processes. Contrary to traditional approaches, an integrative approach to cognition and motor function should be considered when designing neurorehabilitation protocols.

Disclosures: V. Vuong: None. **K.K. Patterson:** None. **L. Cole:** None. **T.L. Henechowicz:** None. **C. Sheridan:** None. **R. Green:** None. **M.H. Thaut:** None.

Digital Abstract Session

P222. Posture and Gait: Aging, Injury, and Disease

Program #/Poster #: P222.02

Topic: E.06. Posture and Gait

Support: HMRF Grant 14150211

Title: Effects of lighting and visual search on dynamic postural control in patients with peripheral field loss

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Abstract: Introduction Due to reduced visual inputs, patients with vision loss have difficulty to maintain postural stability, in particular during challenging conditions. However, no studies have examined the impact of excessive lighting on dynamic balance function while engaging in a common daily task - visual search. This study was aimed to examine the effect of lighting and visual search on dynamic postural control in patients with and without peripheral field loss (PFL).
Methods Dynamic postural control for 10 patients with PFL due to retinitis pigmentosa (age: 61.1 ± 2.8) and 9 age-matched controls (age: 64.3 ± 0.8) were measured. Participants were required to walk a 4-metre pathway at self-pace, step onto a force platform (Bertec, OH, USA) at the end of walking path and maintain their posture for 20 sec for three levels of lighting (100 lux, 520 lux and 2100 lux). While walking, they were asked to either look at a stationary cross or perform a visual search task in which participants had to identify a target out of 5 distractors shown on 6 monitors located at 5-metre away, covering approximately 120 degree field of view. Each condition was repeated for 5 trials. Time to stabilize, maximal sway in anterior-posterior (AP) and medial-lateral (ML) directions, and total sway path length were analyzed.
Results Effect of lighting was only found in time-to-stabilize, in which significantly longer time was required to stabilize under the dimmest lighting condition (100 lux: 1.02s ± 0.06, 520 lux: 0.89s ± 0.05, 2100 lux: 0.91s ± 0.06; $p < 0.01$). The PFL group swayed significantly more than the control group, with significantly longer sway path length (148.0 cm ± 4.0 vs. 137.5 cm ± 2.8, $p<0.05$). Search task significantly affected their postural sway in AP (Fixation: 34.9 ± 1.5, Search: 37.5 ± 1.4; $p=0.02$) and ML (Fixation: 37.5 ± 1.8, Search: 52.3 ± 1.6; $p=0.02$) directions. However, no significant interaction effect of lighting-group, task-group and lighting-task-group was found ($p>0.05$).
Conclusion Our preliminary findings showed that the environmental changes (lighting), visual task (searching) and visual impairment (loss of peripheral vision) might cause detrimental impacts on dynamic postural control, but in different aspects. Changes in lighting (but not excessive lighting) affected the temporal domain of postural control (time-to-stabilize), while visual search and loss of visual input interrupted the spatial domain of postural control (sway path length, amplitudes in AP and ML sway). Further studies are needed to better understand the possible mechanisms of those environmental and daily life activities impact on dynamic postural control.

Disclosures: **A. Cheong:** None. **H. Lam:** None. **V. Suen:** None. **C. Tsang:** None. **G. Woo:** None. **J. Cho:** None. **J. Kwan:** None. **L.A. Abel:** None. **P. Lee:** None. **W. Tsang:** None. **M. Cheung:** None.

Digital Abstract Session

P222. Posture and Gait: Aging, Injury, and Disease

Program #/Poster #: P222.03

Topic: E.06. Posture and Gait

Support: NIH Grant R01AG033649
NIH Grant R01AG0033649-S1

Title: Intranasal insulin rapidly alters ambulatory activity and neuronal calcium network in aged somatosensory cortex

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Abstract: With a 30-40% annual rate of fall, individuals over the age of 65 represent a population target with elevated injury-related morbidity and mortality among older adults, yet it is surprising that few strategies are available for the prevention of falls in the elderly. Non-medicinal approaches such as physical therapy, exercise interventions, use of braces or mobility devices, and even stretching, all appear capable of improving ease of movement and reducing falls; however, these are only moderately effective. Further, lack of adherence to these programs, along with their cost, significantly reduce their potential impact. Here we investigated the hypothesis that acute intranasal insulin (INI) delivery could have an impact on ambulatory performance in the aged F344 rat when delivered at a dose previously shown to improve memory processes. We also monitored the impact of acute INI on neuronal calcium network within the primary somatosensory cortex (S1) in aged animals treated with either insulin aspart or saline. Compared to controls, animals treated with INI moved through our 3-plane visualization ambulatory task (cumulative time required to walk 1 m down a corridor, and across 4 different surfaces) at a faster rate. Based on several task indices (*i.e.,* deviation from the center line, left, and right side coordination based on variability in x and y distances, and other coordination measures) INI treated animals showed a significant improvement on this task. Measures of network activation, synchronization and size within S1 revealed a rapid and significant drug effect within ~15 minutes post-dose. Together, these results show that INI rapidly enhances network communication in S1 and also improves coordination in an animal model of aging. We provide evidence that improved motor coordination may represent a new functional and relevant modality that is sensitive to INI. Given the numerous positive outcomes seen in response to INI in the clinic, including improvements in memory, cognitive and emotional functions, it is not

surprising that increased physical activity could also be a target of the hormone in the brain. These results align well with preclinical and clinical findings, and suggest motor behavior may be a new target of insulin in the brain.

Disclosures: O. Thibault: None. **R. Lin:** None. **H.N. Frazier:** None. **K.L. Anderson:** None. **A.O. Ghoweri:** None.

Digital Abstract Session

P222. Posture and Gait: Aging, Injury, and Disease

Program #/Poster #: P222.04

Topic: E.06. Posture and Gait

Support: Supported by operating grants from the Canadian Institutes of Health Research (funding reference numbers AMG-100487, TIR-103945, and TEI-138295) and the AGE-WELL National Centre for Excellence (AW CRP 2015-WP5.2, AWCRP-2020-04).

Title: Upper limb protective responses during falls in complex environments by older adults

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Abstract: Falls cause up to 80% of traumatic brain injuries (TBI) in older adults (Fu et al. 2017 PLoS One). In the event of a fall, people often use upper limb protective responses to brace the fall and avoid impact and injury to the head. In laboratory studies, these responses are adjusted to the situational demands of the fall based on visual mapping of environmental features (Ghafouri et al. 2004 Exp Brain Res). However, it is unclear whether older adults, who are at the greatest risk for falls and TBI, tailor their protective responses to features of the environment in a functionally effective manner. In this study, we analyzed videos of real-life falls experienced by older adults in long-term care (LTC) to determine the portion of falls that involved object contacts (i.e. complex environments), and test the hypothesis that upper limb contacts to nearby objects reduced the risk of head impacts to objects.
We analyzed videos of 2746 falls using a valid questionnaire (Yang et al. 2013 BMC Geriatr) to characterize body part impacts, reach-to-grasp attempts and fall height. Falls were classified as occurring in complex environments if contact was initiated after fall onset between any body part with objects other than the floor. We used Chi-square to test whether, for falls in complex environments, the odds for head impact to objects was reduced by hand or elbow contact with objects.
We found that 46% of falls occurred in complex environments (n=1251), of which 51% involved upper limbs contacts with objects, and 33% involved head impacts with objects. When compared to falls from lower heights, falls from standing height (n=764) more often involved upper limb

contacts to objects (56 vs 44%), including successful reach-to-grasp of objects (35 vs 17%), elbow impact to objects (21 vs 28%) and hand impact to objects (14 vs 6%).
The probability of head impact to objects was lower in falls involving successful grasps compared to unsuccessful grasps or no attempt to grasp objects ($p<0.001$), in both falls from standing (17 vs 46 or 43%; $p<0.001$), and falls from lower height (12 vs 41 or 34%; $p<0.001$). In falls from lower than standing height, the probability of head impact to objects was reduced by hand or elbow impacts to objects compared to hand or elbow impacts with the ground or no hand and elbow impacts (19 vs 35 or 38%; $p<0.001$).
We found that nearly half of all falls by older adults in LTC occurred in complex environments, where upper limb interactions with objects reduced the risk for head impact to objects by nearly 3-fold. Our results suggest that older adults avoid head impact during falls through tailoring of upper limb protective responses to the immediate environment.

Disclosures: **N. Shishov:** None. **V. Komisar:** None. **D.S. Marigold:** None. **J. Blouin:** None. **S.N. Robinovitch:** None.

Digital Abstract Session

P222. Posture and Gait: Aging, Injury, and Disease

Program #/Poster #: P222.05

Topic: E.06. Posture and Gait

Support: Pitt Momentum Funds
Pittsburgh Claude Pepper Older Americans Independence Center (2P30AG024827-16)
Neurobiology T32 Training Grant (5T32NS086749-07)

Title: Cognitive and motor perseveration are associated in older adults

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Abstract: Aging causes perseveration (difficulty to switch between actions) in motor and cognitive tasks, suggesting that the same neural processes could govern these abilities in older adults. To test this, we evaluated the relation between independently measured motor and cognitive perseveration in young (21.4±3.7 y/o) and older participants (76.5±2.9 y/o). Motor perseveration was measured with a locomotor task in which participants had to transition between distinct walking patterns. Cognitive perseveration was measured with a card matching task in which participants had to switch between distinct matching rules. We found that perseveration in the cognitive and motor domains were positively related in older, but not younger individuals, such that participants exhibiting greater perseveration in the motor task also perseverated more in the cognitive task. Additionally, exposure reduces motor perseveration: older adults who had practiced the motor task could transition between walking patterns as

proficiently as naïve, young individuals. Our results suggest an overlap in neural processes governing cognitive and motor perseveration with aging and that exposure can counteract the age-related motor perseveration.

Disclosures: C.J. Sombric: None. **G. Torres-Oviedo:** None.

Digital Abstract Session

P222. Posture and Gait: Aging, Injury, and Disease

Program #/Poster #: P222.06

Topic: E.06. Posture and Gait

Title: Effect of kinesiology tape on static postural control in non-athlete young adults with functional ankle instability

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Abstract: Background**:** Kinesiology tape (KT) is often used to increase ankle stability and proprioception around the ankle of people with Functional Ankle Instability (FAI) during rehabilitation or physical activities (Williams et al., 2012). However, there is limited evidence of KT's effectiveness on static postural control, which can also be diminished by FAI. Furthermore, studies mainly focused on athletes thus limiting the possible inference toward a larger population. The effect of KT on static postural control is also mainly assessed using linear measures of the center of pressure (COP) displacement, which can be misleading since the COP displacement is nonlinear (Cavanaugh et al., 2005). Therefore, our objective was to determine if KT could improve static postural control of non-athletes with FAI using nonlinear COP measures. Method**:** Twenty young adults (23.4 ± 4.25 years) with FAI performed three standing tasks: unipedal stance on the leg with FAI, unipedal stance on the healthy leg and bipedal stance. Four trials of 45s were performed for each position and at three different times: before applying a KT on the unstable ankle (B), immediately after (I) and 24h later with the KT still on (24h). The COP displacement was obtained with an AMTI force platform at 500 Hz and analysed using two nonlinear COP measures: sample entropy and the contribution of specific frequency bands. The sample entropy measured the regularity of the signal in the anterior-posterior (AP) and medial-lateral (ML) direction. A discrete wavelet transform was applied to the signal, in both directions, to measure the contribution of four frequency bands related to different systems modulating the posture (medium: 1.56 to 6.25 Hz - proprioception, low: 0.39 to 1.56 Hz - cerebellum, very-low: 0.10 to 0.39 Hz - vestibular, ultralow: < 0.10 Hz - vision) (Quek et al., 2018). For the unipedal stances, two-way repeated measures ANOVA and MANOVA (leg x time) were performed on the sample entropy and wavelet data, respectively. For the bipedal stance, one-way repeated measures ANOVA and MANOVA (effect of time) were performed on the sample entropy and wavelet data, respectively. Result**:** Statistical analysis revealed only a main effect of time during the unipedal stance. Sample entropy was higher at B and 24h compared to I in both AP and ML. The low band in ML and the ultralow band in AP had higher contribution at I compared to 24h.

Conclusion**:** Our results suggest that KT didn't change the COP displacement dynamic during unipedal or bipedal stance. Although there was a main effect of time, there was no main effect of the leg side. This suggests that KT doesn't improve static postural control of non-athletes with FAI.

Disclosures: L. Michaud: None. **K. Ly:** None. **Y. Lajoie:** None.

Digital Abstract Session

P222. Posture and Gait: Aging, Injury, and Disease

Program #/Poster #: P222.07

Topic: E.06. Posture and Gait

Support: NIH Grant R01 AG057671
NIA Grant R01 AG045252
NIA Grant K24 AG057728

Title: Integrative measure of walking smoothness is associated with fear of falling, gait efficacy, and life-space in older adults

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Abstract: Gait speed is a prime indicator of mobility ability, function and independence, but it incompletely captures the motor control of walking or gait quality. Accelerometry and instrumented walkway methods provide objective measures of gait quality. We examined the association of gait variability and smoothness to self-reported fear of falling (FOF), walking confidence, and life-space mobility. We used baseline data from community-dwelling older adult participants (n=231; age 77 ± 6 years; 65% females; 89% whites; 44% had more than a high school education) in a clinical trial to improve walking. Gait quality at usual pace was quantified by 1) smoothness (Harmonic ratio) calculated as the vector sum of vertical (V), anterior-posterior (AP) and medio-lateral (ML) directions; 2) gait variability (CoV) for step time, step length and stride width; combined variability computed as sum of CoV (step time, step length and step width); and 3) gait speed. Smoothness was derived from a tri-axial accelerometer during over-ground six-minute walking. Variability and speed were calculated for 6 passes of walking over a 4-m instrumented walkway. The association of gait quality with FOF, walking confidence (modified Gait Efficacy Scale), and life-space mobility (Life Space Assessment scale) was assessed using walking speed, age, and sex as covariates. ANCOVA compared gait quality between those with and without FOF and spearman correlations examined relations of gait quality to walking confidence and mobility. Individuals without compared to those with FOF had better gait quality: smoothness V (2.38 ± .58 vs 2.14 ± .73), combined smoothness (3.84 ± 0.89 vs 3.55 ± 0.97), and faster speed (1.10 ± .15 m/s vs 1.04 ± .17 m/s), all $p<0.05$. Greater

smoothness V (ρ_P=0.23), AP(ρ_P=0.13) and combined (ρ_P=0.17) and gait speed (ρ=0.42), were associated with a greater walking confidence, all p<0.05. Greater smoothness ML (ρ_P=0.16), AP (ρ_P=0.15) and combined (ρ_P=0.14), and walking speed (ρ=0.21), related to greater life-space mobility, all p<0.05. Controlling for gait speed, age and sex, no association was found for gait variability with FOF, confidence or mobility. In addition to walking speed in the older adults studied, gait quality, particularly smoothness, reflects psychosocial aspects of walking and community mobility. Identification of relevant gait quality aspects related to clinically important outcomes may help inform rehabilitation.

Disclosures: A. Suri: None. **A.L. Rosso:** None. **J. VanSwearingen:** None. **L.M. Coffman:** None. **J.S. Brach:** None. **E. Sejdic:** None.

Digital Abstract Session

P222. Posture and Gait: Aging, Injury, and Disease

Program #/Poster #: P222.08

Topic: E.06. Posture and Gait

Support: Gondola Medical Technologies

Title: The effects of tactile somatosensory therapies on walking rehabilitation for neurological disorders: a systematic review

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Abstract: Introduction: With a neurological disorder, walking often requires compensatory strategies to overcome the disability, demanding increased executive control. On the contrary, a healthy walking pattern is predominantly driven by automatic patterns with minimal executive control. One way to enhance automatic circuits and to reduce the executive involvement during walking may be through somatosensory feedback. Given that somatosensory information is essential for body representation and walking control, somatosensory therapies may be efficient for walking rehabilitation. The objective of this systematic literature review is to understand the effects of tactile somatosensory therapies on walking abilities in patients with neurological disorders.
Methods: Articles were included if they tested mechanical, tactile, or sensory electrical stimulation for walking rehabilitation interventions and tested participants with neurological disorders affecting gait. The literature search of articles published in English was performed in 4 electronic databases. Article selection followed the PRISMA methodology.
Results: The search yielded 1269 potentially relevant articles, leading to 28 selected articles, (including 15 randomized controlled trials). Among the selected articles, 12 tested the Automated Mechanical Peripheral Stimulation (AMPS) treatment (1), 6 studies tested textured insoles, 2 vibrotactile stimulation, 7 sensory electrical stimulation and 1 neuromuscular taping. Study populations were 64% Parkinson’s disease, 14% stroke, and 14% multiple sclerosis; one

study included infants with myelomeningocele and one tested an adult with a spinal cord tumor. Eighteen studies (including 11 studies with AMPS) reported a significant positive effect of tactile somatosensory therapies on walking abilities. The remaining ten studies reported no significant effect. No study reported a negative effect. Eight studies showed a significant increase in walking speed due to the intervention, but only studies using AMPS (n= 5) showed a clinically important change in walking speed (threshold of 0.10 m/s (2)). Regarding the underlying mechanisms, two studies showed that AMPS significantly increased brain connectivity and one study demonstrated increased BDNF after AMPS, suggesting synaptic plasticity.
Discussion & Conclusion: This systematic review highlights the possibility of using tactile somatosensory therapies for walking rehabilitation. AMPS therapy seems to be especially promising to improve walking function in patients with a neurological disorder.
(1)Quattrocchi et al. 2015 PLoS ONE (2) Bohannon et al. 2014 Eval. Clin. Pract.

Disclosures: **T. Corbet:** A. Employment/Salary (full or part-time):; Gondola Medical Technologies. **S.N. Edd:** A. Employment/Salary (full or part-time):; Gondola Medical Technologies.

Digital Abstract Session

P222. Posture and Gait: Aging, Injury, and Disease

Program #/Poster #: P222.09

Topic: E.06. Posture and Gait

Support: NIH/NIA U01AG061393

Title: Mild Parkinsonian signs are associated with decreased cortical-striatal connectivity in executive control networks in healthy older adults

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Abstract: Background: Mild Parkinsonian signs (MPS) may precede Parkinson's Disease (PD), but it is not known whether patients with MPS exhibit similar alterations of functional connectivity (FC) to those observed in PD. We hypothesize that MPS are associated with PD-like alterations of FC in the cortical-striatal-thalamic dopaminergic networks regulating sensorimotor, executive, and reward functions. Methods: Participants (N=272; mean age 83; 57% female; 41% African American) without PD completed structural and resting-state functional magnetic resonance imaging followed by the motor component of the Unified Parkinsonian Disease Rating Scale (UPDRS) within 6 months of scan. Risk factors for MPS included: gait speed, quadriceps strength, joint pain, body mass index, vision, lung function, and proprioception. To measure the FC between striatum and cortex in each network, we segmented both the striatum and cortex into sensorimotor, executive, and limbic regions using extant atlases. The thalamus (without functional subdivisions) was defined by existing atlas. After

regressing out mean white matter and cerebrospinal fluid signals, we computed correlations within each cortical-striatal network (e.g., between mean of sensorimotor cortex and mean of sensorimotor striatum). Total brain white matter hyperintensities (WMH) and gray matter volume (GMV) were normalized by total brain volume. Logistic regression tested the association of FC of each cortical-striatal network with MPS (UPDRS>0), adjusted for MPS risk factors first, then additionally for WMH and GMV. WMH by FC interactions were tested and analyses were repeated stratified by burden of WMH (median value cutoff). Results: Compared to those without MPS, those with MPS had significantly lower cortical-striatal FC in the left executive control function network (adjusted Odds Ratio [95% CI], p value: 0.188 [0.043, 0.824], 0.027). Association survived adjusting for MPS risk factors (0.151 [0.027, 0.860], 0.033) but was attenuated after adjusting for WMH (0.209 [0.036, 1.200], 0.079). In models stratified by WMH, left executive cortical-striatal FC was significantly associated with MPS for those with high WMH (0.077 [0.010, 0.599], 0.014) but not for those with low WMH (1.245 [0.128, 12.132], 0.850). Associations of MPS with FC in other networks were not significant. Conclusion: Cortical-striatal FC, especially in executive control networks, is lower for those with MPS. The association is independent of other risk factors but was stronger for those with high WMH, another central nervous system source of MPS. Future work should examine whether higher FC may protect against the influence of WMH on MPS.

Disclosures: J.B. Hengenius: None. **A.L. Rosso:** None. **C. Rosano:** None. **T.J. Huppert:** None.

Digital Abstract Session

P223. Reflexes and Reflex Modulation

Program #/Poster #: P223.01

Topic: E.06. Posture and Gait

Support: NIH Grant NS097781
VA Grant B2316

Title: Distributed force feedback modulates the dependence of limb stiffness and inter-joint coordination on perturbation direction: a simulation study

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Abstract: Force feedback from Golgi tendon organs is more widely distributed than length-dependent feedback from muscle spindles, so force-dependent pathways are likely to have a more global effect on limb stiffness and inter-joint coordination. Limb stiffness is task-dependent and is decreased in downhill walking. To understand the role of force feedback in regulating limb mechanics for different terrains, we are developing a three segment feline hindlimb model

in Matlab, with realistic lengths and masses and rotational springs and dampers at all the joints to represent the intrinsic properties of muscles with integral stretch reflexes. Based on the known distribution of muscular mass, we set joint impedance to decrease from hip to ankle. In this initial model version, there is no provision for bi-articular muscles. Force feedback is represented by a matrix multiplying the joint torques. To simulate limb behavior during weight acceptance, a step input force equivalent to twice the body weight of a 3.5 kg cat is applied over one second, which is long enough for the model to settle to steady state. To simulate different kinds of terrain, the direction of the endpoint force is varied in even increments from the rostral to caudal aspects of the limb. For each perturbation, the stiffness, Kleg, is calculated along the direction of the limb for the following experimentally-observed inhibitory force feedback distributions: no force feedback, weak homonymous (i. e., each joint onto itself), strong homonymous, and three heteronymous (i.e., inter-joint) force feedback distributions (proximal to distal, distal to proximal, and balanced). Our hypothesis is that for each force feedback distribution, Kleg will be maximum for endpoint forces pointing directly to the hip (0 deg), and decrease for endpoint forces directed caudally or rostrally from the hip. We expect that Kleg will depend to the greatest extent on the direction of the endpoint force with homonymous feedback, but that the directional dependence will be attenuated significantly by inter-joint (i.e., heteronymous) force feedback. Preliminary results from a two segment model show that Kleg changes with the direction of the endpoint force in the expected manner, but the peak stiffness occurs for a rostrally-directed endpoint force (~-15 deg). Our results also indicate that inter-joint force feedback distributions significantly reduce the dependence of Kleg on the direction of an endpoint force, and that this effect is sensitive to the details of the feedback topology. However, in addition to the inter-joint distributions the results suggest that strong homonymous feedback attenuates the directional dependence of Kleg.

Disclosures: **T. Govindaraj:** None. **D. Howland:** None. **G. Sawicki:** None. **T. Nichols:** None.

Digital Abstract Session

P223. Reflexes and Reflex Modulation

Program #/Poster #: P223.02

Topic: E.06. Posture and Gait

Support: VA P01 HD32 (JRW)
NIH (HD36020(XYC)
NS22189(JRW)
NS061823(XYC&JRW)
HD032571(AWE)
NICHD/1P41EB018783(JRW)

Title: Combining H-reflex conditioning and locomotor training enhances locomotor recovery in rats with incomplete spinal cord injury

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Abstract: Operant conditioning of the spinal stretch reflex or its electrical analog, the H-reflex (HR), changes brain and spinal cord (Curr Op Behav Sci 20:138-144, 2018 for review). In rats and humans with incomplete spinal cord injury (SCI), appropriate reflex conditioning improves locomotion (J Nsci 26:12537-43, 2006 & 33:2365-75, 2013). We are exploring the therapeutic impact of combining H-reflex conditioning with locomotor training (LT) after SCI. Under anesthesia, SD rats were implanted with EMG recording and nerve stimulating electrodes, and received a right lateral column lesion at T9; 20 d later, each was exposed for 60 d to right soleus HR up-conditioning plus 5 d/wk LT (CB rats, n=9), or LT alone (LT rats, n=7). Locomotor EMG and HRs, horizontal ladder crossing, locomotor kinematics were assessed before and after SCI, at the end of treatment (Rx), and 50 ds later. CB Rx increased the protocol HR (HR in the conditioning protocol); LT Rx did not. In CB rats, the protocol HR appeared to increase more quickly and more than in normal rats exposed to up-conditioning. CB, but not LT Rx also increased the locomotor HR (HR during locomotion). Horizontal ladder missteps were 0.11(±0.08SE) and 0.24(±0.10)/crossing for CB & LT rats, respectively (p=0.32, CB vs LT, t-test) before SCI, 6.24(±0.82) and 5.34(±0.58) (p=0.41) after SCI but pre-Rx, and 1.80(±0.51) and 5.14(±0.57) (p<0.001) post-Rx. In CB rats, missteps remained low 50 d post-Rx. Left and right hip heights (mm) were 53.6(±1.4) and 53.9(±1.6), respectively (p=0.20, right vs left, paired t-test) for CB rats and 55.0(±2.3) and 54.9(±2.2) (p=0.39) for LT rats before SCI, 58.1(±1.3) and 54.4(±1.1) (p<0.001) for CB rats and 57.3(±1.9) and 53.9(±1.9) (p=0.016) for LT rats after SCI and pre-Rx, and 56.8(±1.8) and 56.9(±1.9) (p=0.76) for CB rats and 61.6(±0.76) and 57.5(±1.0) (p=0.010) for LT rats post-Rx. In CB rats, hip heights remained symmetrical 50 d post-Rx. Step symmetry (right step duration as % of left) was 101.1(±0.4) and 100.5(±0.9) in CB and LT rats, respectively (p=0.99, CB vs LT, ANOVA) before SCI, 91.3(±1.0) and 88.5(±0.5) (p=0.99) after SCI and pre-Rx, and 97.9(±1.1) and 92.2(±0.5) post-Rx (p=0.001 CB vs LT; p<0.001 and p=0.11; pre- vs. post-Rx). In CB rats, steps remained symmetrical 50 d post-Rx. In rats with SCI, HR conditioning plus LT produces recovery superior to that of LT alone. Horizontal-ladder performance is better; right/left step and hip-height symmetries are restored. Improvements persist after Rx ends. The results are consistent with current human studies of therapeutic combinations (e.g., J Physiol 2019, https://doi.org/10.1113/JP278173), and can help guide their further development.

Disclosures: Y. Chen: None. **L. Chen:** None. **X. Yang:** None. **J.R. Wolpaw:** None. **X. Chen:** None.

Digital Abstract Session

P223. Reflexes and Reflex Modulation

Program #/Poster #: P223.03

Topic: E.06. Posture and Gait

Support: NIH Grant P41 EB018783

VA Merit I01 CX001812
NYS SCIRB C32236GG and C33279GG

Title: H-reflex operant conditioning of the flexor carpi radialis

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Abstract: H-reflex operant conditioning is a targeted new approach for rehabilitation of motor function following spinal cord injury and potentially stroke (Thompson et al., J Neurosci, 2013). In this protocol, participants learn to increase or decrease the size of abnormal reflex responses (as needed) triggering widespread beneficial plasticity that improves movement. H-reflex operant conditioning can complement existing therapies, is non-invasive, and has no known adverse side effects (Norton and Wolpaw, COBS, 2018). Previous investigations of H-reflex operant conditioning have focused on the soleus muscle in the leg (Thompson et al., J Neurosci, 2009; Thompson et al., J Neurosci, 2013). Here, we report on an ongoing research extending H-reflex operant conditioning to the flexor carpi radialis (FCR) in the arm and examining concurrent changes in brain activity—as measured using electroencephalography (EEG)—that occur during H-reflex operant conditioning.

The conditioning protocol we use in our study is based on the one described by Thompson et al. (J Neurosci; 2009). Participants are asked to complete 6 baseline and 24 conditioning sessions (3/wk over ~10 wks; EEG data recorded at ~2 wk intervals). During each session, participants complete a sequence of trials where they maintain a predefined level of FCR activity and H-reflexes are elicited using transcutaneous electrical stimulation. During conditioning sessions, immediate rewards occur when H-reflex size is below (down-conditioning) or above (up-conditioning) a criterion.

To date, 10 healthy participants (7 up- and 3 down-conditioned) have completed the protocol. For these participants, we compared average H-reflex size from the 6 baseline sessions to the average H-reflex size from the last 6 conditioning sessions. In 6 participants, H-reflex size changed in the rewarded direction (two-sample, one-sided t-test; $p<0.05$). In the other 4 participants, H-reflex size did not change. Analysis of the EEG data is underway; it is focusing on 8-13 Hz (*alpha*) and 20-30 Hz (*beta*) activity recorded over the contralateral sensorimotor cortex. These frequencies and locations are being targeted because the sensorimotor cortex is critical to H-reflex operant conditioning (Chen et al., J Neurophysiol, 2006) and there is a correlation between the size of sensorimotor rhythms in the alpha and beta bands and H-reflex amplitude (Thompson et al., Front Neurosci, 2018). Initial data indicate that operant conditioning of the FCR H-reflex is possible. Following confirmation of these initial results and further development we plan to investigate FCR H-reflex conditioning for motor recovery in Veterans who have had a stroke.

Disclosures: **J.J.S. Norton:** None. **S. Devetzoglou-Toliou:** None. **J. Brangaccio:** None. **D.E. Gemoets:** None. **S.M. Heckman:** None. **T.M. Vaughan:** None. **N.J. Hill:** None. **J.S. Carp:** None. **J.R. Wolpaw:** None.

Digital Abstract Session

P223. Reflexes and Reflex Modulation

Program #/Poster #: P223.04

Topic: E.06. Posture and Gait

Support: NIH Grant P41 EB018783
VA Merit I01 CX001812

Title: Enhancing H-reflex operant conditioning using brain-computer interface (BCI)-based feedback

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Abstract: We present ongoing research on the development of an H-reflex operant conditioning protocol that is enhanced with brain-computer interface (BCI)-based feedback. H-reflex operant conditioning is a promising new therapeutic approach for motor function recovery following injury or illness from spinal cord injury (SCI), stroke, or other neuromuscular disorders. In this paradigm, participants learn to change the size of their abnormal reflexes (either increasing or decreasing them as needed), triggering widespread beneficial plasticity that improves movement (Thompson, Chen, and Wolpaw, J Neurosci, 2009; Chen et al., Ann. N. Y. Acad. Sci., 2010). Present models of the neural mechanisms of H-reflex conditioning describe this beneficial plasticity as operating hierarchically. When participants learn to modify their H-reflex size, it generates plasticity in the sensorimotor cortex, which subsequently guides and maintains plasticity in the spinal cord (reviewed in Norton and Wolpaw, COBS, 2018). Thus, we hypothesize that guiding this critical first stage of plasticity in the sensorimotor cortex should improve the speed and efficacy of the entire H-reflex conditioning protocol. To investigate this, we have developed a sequential H-reflex conditioning protocol. In this protocol, participants are first trained to use a sensorimotor rhythm (SMR)-based BCI to control the amplitude of activity in their sensorimotor cortex (Wolpaw et al., Clin Neurophysiol, 1991). Then, they complete H-reflex operant conditioning enhanced with additional feedback on the amplitude of their SMRs. To date, we have identified seven participants with measurable flexor carpi radialis H-reflexes to complete a study of our enhanced H-reflex conditioning protocol. Of these seven participants, five have completed SMR-based BCI training (~10 sessions); four of these participants learned to use the BCI with better than 70% accuracy, and three significantly improved their accuracy with the BCI after training (Mann-Whitney U test; $p < 0.05$). In addition, we have completed the

development of a new version of the Evoked Potential Operant Conditioning System (EPOCS) that includes SMR-based BCI feedback. Healthy participants are now ready to complete the enhanced H-reflex conditioning protocol as soon as human research resumes. Pending positive experimental results with healthy participants, this enhanced H-reflex conditioning protocol will be investigated in Veterans with upper extremity motor impairments due to stroke.

Disclosures: S. Devetzoglou-Toliou: None. **M. Adamek:** None. **D.J. McFarland:** None. **J.R. Wolpaw:** None. **J.J.S. Norton:** None.

Digital Abstract Session

P223. Reflexes and Reflex Modulation

Program #/Poster #: P223.05

Topic: E.06. Posture and Gait

Support: the Physical Therapy and Human Movement Sciences departmental research support

Title: Neural activation of brainstem modulated by shoulder abduction loads during prepared arm lifting

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Abstract: Motor preparation is task-specific. If the brainstem is involved in motor preparation of upper extremity movements, it is possible to modulate the brainstem's activity during the preparation phase by altering the motor task effort level at proximal joints. To demonstrate this, we used the startle responses to probe the brainstem's activity during shoulder abduction (SABD) against various loads (15 to 55% of a subject's maximal voluntary SABD load). We hypothesize that increasing shoulder load magnitude will result in an enhanced brainstem activity, as demonstrated by greater startle responses.
Four able-bodied subjects with right-hand dominance, no known hearing or movement deficits (2 females, 2males, aged 32± 10.6 years) were recruited to perform prepared shoulder abduction against five load levels (15%, 25%, 35%, 45%, and 55% of maximal voluntary SABD load) respectively. Each loading condition was performed in one block. The order of blocks was random. Each trial started with a 'ready' sound indicating subjects to prepare for a shoulder abduction effort after 5-6s. In 90% of all trials, a second sound either at 80dB (~65% trials as the 'go' condition) or at 115dB (~35% trials as the 'startle' condition) was presented randomly at 1.5-2s following the first "ready" sound, indicating subjects to perform the required task as quickly as possible. EMG data were recorded from the right biceps, triceps, intermediate deltoid, and sternocleidomastoid muscles. The root mean square (RMS) ratio of EMG data between the biceps and intermediate deltoid was calculated to quantify the responses evoked by different

sounds with various loadings. The results showed that the EMG ratio between biceps and intermediate deltoid increased as the loading was getting heavier under both the 'startle' and 'go' conditions. The startle-induced EMG ratio increased faster than induced by the ‘go’ sound resulting in a strong and positive linear relationship (R^2=0.9188). As startle responses reflect the activation level of the reticular formation, our results indicate that shoulder load magnitude can modulate the neural activation of the brainstem during a prepared arm lifting task.

Disclosures: Y. Zhao: None. **K. Wright:** None. **J.P. Dewald:** None. **J. Yao:** None.

Digital Abstract Session

P223. Reflexes and Reflex Modulation

Program #/Poster #: P223.06

Topic: E.06. Posture and Gait

Support: NYSDOH Contract C33276GG
NYSDOH Contract C32095GG
NYSDOH Contract C32248GG

Title: Transspinal stimulation downregulates flexor locomotor networks during stepping in humans

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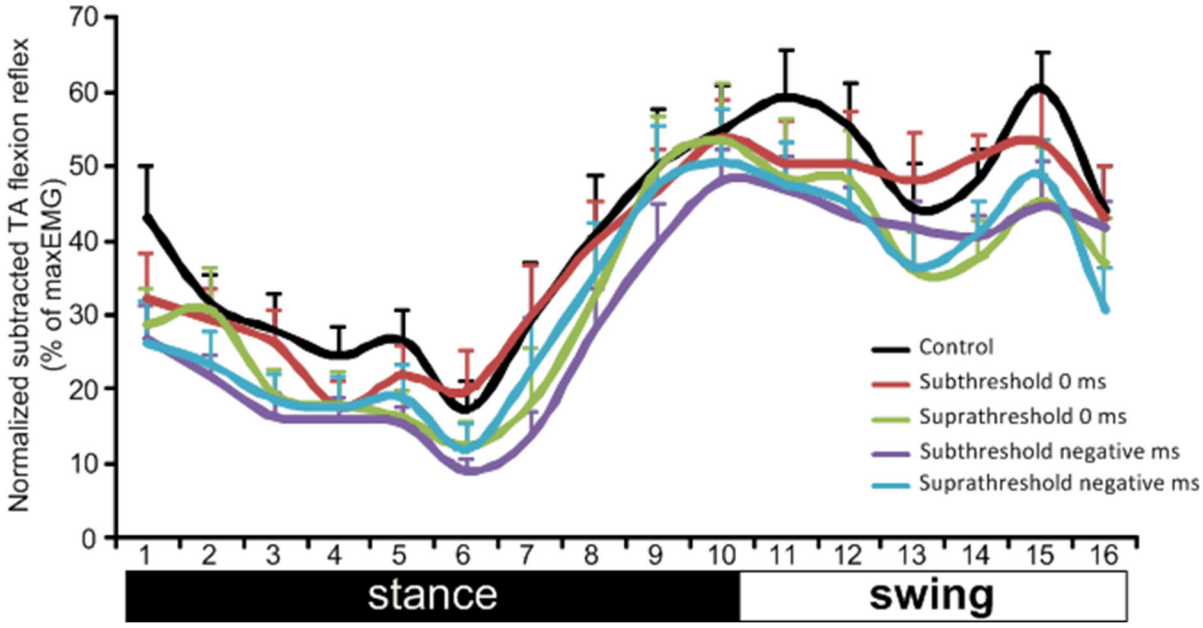
Abstract: Non-invasive transspinal stimulation is a promising approach to augment movement-based rehabilitation following neurological injury. However, the knowledge regarding the effects of transspinal stimulation on different spinal networks during locomotion remains limited. We looked into the effects of transspinal stimulation on tibialis anterior (TA) flexion reflex during stepping in healthy individuals. The TA flexion reflex was evoked with a 30 ms pulse-train delivered to the medial arch of the right foot. This reflex was conditioned by a single pulse transspinal stimulation delivered at a conditioning-test (C-T) interval either after (~20 ms) or at the same time (0 ms) with the last pulse of the pulse train. Stimulation was delivered randomly at different phases of the step cycle, which was divided into 16 equal bins, based on the foot switch threshold signal. In this study we observed that short-latency TA flexion reflex was depressed throughout the step cycle at both sub- and supra-threshold transspinal intensities. Non-invasive transspinal stimulation over the thoracolumbar region of the spinal cord in healthy subjects during stepping depressed flexion reflexes which are considered part of the spinal locomotor networks. Thus, transspinal stimulation could be potentially used to decrease the excitability of the flexion reflex that is usually exaggerated following a central nervous system disorder.



Disclosures: **M. Zaaya:** None. **T. Pulverenti:** None. **M. Islam:** None. **M. Knikou:** None.

Digital Abstract Session

P223. Reflexes and Reflex Modulation

Program #/Poster #: P223.07

Topic: E.06. Posture and Gait

Support: Physical Therapy and Human Movement Sciences Departmental Research Support

Title: Startling Acoustic Stimulus Increases Activity in Flexors but Not Extensors During Lifting: A Preliminary Study

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Abstract: Startling acoustic stimulus (SAS) has been shown to elicit a quick release of a prepared movement via the reticulospinal tract (RST), making the SAS a useful tool to investigate the RST-innervated muscles in various movements. Previous work in primates has demonstrated that the RST favors flexor muscles over extensor muscles. In humans, earlier findings under pathological conditions evidenced the higher innervation of RST to flexors versus extensors. For instance, stroke survivors usually have strong upper extremity flexor muscle recovery, while the extensor muscles remain weak. However, we still lack evidence to support that RST favors the activation of flexors muscles in an intact human nervous system. To

determine this, our study investigates the effect of the startle response on elbow flexor and extensor muscles during the generation of various shoulder abductions (SABD) loads.
We recruited four able-bodied participants with right-hand dominance, no known hearing or movement deficits to perform SABD over five different loads (i.e., 15, 25, 35, 45, 55% of a subject's maximum voluntary force in the SABD direction). Each loading condition was assigned to a trial block, and the condition order was randomized. At the beginning of each trial, the subjects heard a 'ready' sound to prepare them for a self-initiated SABD after 5-6 s. In 90% of the trials, a second sound was delivered randomly at 1.5-2 s after the 'ready' sound. When hearing the 2nd sound, the subjects were requested to lift the arm as quickly as possible. 65% of the 2nd sounds were at 80dB (the 'go' sound), and 35% at 115dB (the 'startle' sound). EMGs were recorded from the right sternocleidomastoid, biceps (BIC), triceps (TRI), and intermediate deltoid (IDL). Muscle co-activation patterns were quantified to reflect the level of brainstem activity, which was calculated as the ratio of BIC or TRI activity to IDL activity.
Our results showed that EMG ratios of the BIC and TRI increased as a linear function of shoulder load. Furthermore, the BIC ratios were consistently greater than the TRI ratios. The slope of BIC data for the 'startle' condition was greater than the slope for the 'go' condition. However, the 'startle' and 'go' induced EMG ratios for the TRI were almost identical, suggesting the TRI was not reacting to the SAS. These preliminary findings imply that elbow flexors are preferentially activated by RST in an intact human nervous system and confirms earlier findings in the monkey as well as in individuals with hemiparetic stroke.

Disclosures: K. Wright: None. **Y. Zhao:** None. **J.P. Dewald:** None. **J. Yao:** None.

Digital Abstract Session

P224. Motor Neurons: Activity, Sensory, and Central Control - Exercise, Injury, and Disease

Program #/Poster #: P224.01

Topic: E.09. Motor Neurons and Muscle

Title: Neurophysiology of cognitive load during physical performance

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Abstract: INTRODUCTION: The interaction of workload and fatigue in physical and mental domains has recently aroused interest although no specific procedure and measures are available. Moreover, the detrimental effects of mental fatigue has been recently studied and research is still in progress to fully understand the neurophysiological mechanisms occurring in the brain. In the present study we aimed to evaluate the relationship between workload and fatigue during traditional physical training (CON) vs. combined aerobic exercise and demanding cognitive tasks (EXP) to assess their effects on behavioural, physiological and cortical measures. **METHODS:** Twenty healthy and fit participants were tested in a randomized cross over order to compare the

acute psychological, physiological and neurophysiological effects of CON and EXP conditions . After a familiarization, each participant was asked to cycle for 1 hour in 2 different occasions at 75% percent of their maximal capacity. During EXP participants were asked to engage in highly demanding cognitive task, while they were cycling. Before and after the cycling trial, they were completing a psychomotor vigilant test (PVT). Pre, during and after cycling test Brunel Mood Scale, NASA Task Load Index, Rating of Perceived Exertion, heart rate, blood lactate, electromyography (EMG) and electroencephalographic recordings were used to evaluate workload and fatigue. **RESULTS:** There were no significant differences between EXP and CON conditions for of HR, blood lactate and EMG during cycling. HR ($P = 0.001$), blood lactate ($P = 0.003$) and EMG ($P = 0.001$) increased significantly over time in both conditions. The NASA TLX revealed significant differences between EXP and CON during cycling in Mental Demands ($P = 0.001$), Temporal Demands ($P = 0.02$), Performance ($P = 0.032$), and Effort ($P = 0.012$). Rating of perceived exertion was significant higher in the EXP condition ($P = 0.001$) during cycling. The Alpha/Beta ratio ($P = 0.022$) and Beta power/(theta power + alpha power) ratio, ($P = 0.031$) were higher during the EXP condition during cycling. Subjective feelings of fatigue increased significantly in EXP after the cycling ($P = 0.001$) compared to CON while vigour significantly decreased. Alpha/Beta ratio during the PVT increased significantly after EXP condition ($P = 0.001$). Average reaction time during the PVT increased significantly after EXP ($P = 0.021$). **DISCUSSION:** The increase of workload, adding cognitive tasks to exercise, required more mental effort than traditional physical training, as assessed by behavioural and electrophysiological measures of fatigue while no difference were detected in physiological variables.

Disclosures: W. Staiano: None. **E.J. Gibbins:** None. **F.J. Romero Garcia:** None. **M. Romagnoli:** None.

Digital Abstract Session

P224. Motor Neurons: Activity, Sensory, and Central Control - Exercise, Injury, and Disease

Program #/Poster #: P224.02

Topic: E.09. Motor Neurons and Muscle

Title: Characterizing REST/NRSF expression in motor neurons after peripheral nerve injury

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Abstract: Introduction: Peripheral nerve injuries (PNIs) present complex challenges for repair as they typically include long-gap nerve injuries with surrounding trauma to the muscle and vasculature. Re-innervation of muscle tissue after injury is necessary for functional recovery, but motor neurons may not regenerate sufficiently to provide for complete recovery which can result in permanent loss of function. A deficiency in neurotrophic factors (NTFs) or the nuclear

programming of regenerative-associated genes can limit the capacity of axonal regeneration after PNI. Increased expression of the Repressor Element-1 Silencing Transcription factor (REST) may play a central role in transcriptional repression of the regenerating neuronal phenotype. Changes in REST expression have been shown in sensory neurons in models of neuropathic pain, but little is known about whether there is altered REST expression after injury in motor neurons. We are developing a novel approach to peripheral nerve regeneration by targeting the motor neuron nucleus to enhance the natural proximal to distal degeneration pattern. We have established a rodent model of lesion of the facial motor nerve to characterize potential alterations in REST expression specifically in motor neurons. **We hypothesize that a similarly induced upregulation of REST expression exists in motor neurons and will result in transcriptional repression of NTFs and other regenerative-associated genes.** Assessing whether REST activity after injury occurs in motor neurons may identify potential therapeutic targets for functional recovery after PNI.
Methods: Male Sprage-Dawely rats, 8-9 weeks old at the time of surgery were grouped into facial nerve transection or sham injury groups at 6-hour and 48-hour timepoints (n=12/group). The facial nerve transection injury involved a complete transection at the nerve trunk exiting the stylomastoid foramen, sham surgery received a skin incision wit the nerve trunk exposed but left intact. Protein and gene expression levels were assessed via immunohistochemistry and RT-PCR, respectively. Injury and sham groups were compared as well as the ipsilateral and contralateral sides in each animal as an internal control.
Results: There is significant upregulation of REST protein expression in injured animals compared to sham and naïve groups at both time points. No significant changes were present in mRNA expression in either timepoints, however samples are still in process.
Discussion: Facial nerve transection injury is an effective model for assessing functional mechanisms in PNI and injury-induced expression of REST exists at acute timepoints after facial nerve transection.

Disclosures: A.K. Yaszemski: None. **N. Madigan:** None. **A.J. Windebank:** None. **L. Nesti:** None.

Digital Abstract Session

P224. Motor Neurons: Activity, Sensory, and Central Control - Exercise, Injury, and Disease

Program #/Poster #: P224.03

Topic: E.09. Motor Neurons and Muscle

Support: N66001-10-C-4056, DARPA

Title: Supraspinal Mechanisms of Fatigue and Fatigability

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Abstract: Fatigue is the subjective sensation of weariness, increased sense of effort, or exhaustion and is one of the most common and debilitating symptoms in neurological illnesses such as multiple sclerosis. Despite its epidemiological importance, there is a limited understanding of the neurophysiological mechanisms of fatigue driven by two major shortcomings in the current literature. First, most studies investigate the factors that impair cognitive or motor function (e.g. reduced force capacity, also known as fatigability) without addressing fatigue itself (the percept of weariness, etc.) - even though evidence shows fatigue is often unrelated to fatigability. Second, despite our extensive understanding of spinal and peripheral (i.e. neuromuscular junction and muscle) mechanisms of fatigue, we have a limited knowledge of its supraspinal mechanisms. Here we aim to disambiguate the supraspinal changes related to fatigue versus fatigability by characterizing the changes in primary motor cortex (M1) and somatosensory cortex (S1) at the single- and multi-unit activity (SUA/MUA) level and how they relate to *both* increased perception of fatigue and altered neuromuscular function. *We* ***hypothesize*** *that S1 activity will correlate with changes in the participant's percept of fatigue whereas M1 activity will correlate with increases in EMG activation and coactivation (i.e. fatigability).* To do this we will concurrently measure percepts of fatigue, neuromuscular activity via electromyography (EMG), and SUA/MUA of M1 and S1 before, during, and after a fatiguing task in a human spinal cord injury (SCI) participant implanted with microelectrode arrays (MEAs) in his sensorimotor cortex. Importantly, these MEAs were implanted in bilateral areas of M1 and S1 that control/project to areas of our participants upper limbs where he retains some motor abilities and sensory perception. The fatiguing task will require the participant to press against a force transducer, using his wrist extensor, and exert 60% of his maximum voluntary contraction (MVC) for 4 seconds. Critically, the task will be repeated until the participant is fatigued but before neuromuscular failure is induced. These results will allow us to better understand the supraspinal neurophysiological mechanisms of fatigue at the neuronal level for the first time in a human and disentangle it from peripheral mechanisms of fatigue. This knowledge will be fundamental for identifying biomarkers of fatigue and will aid in the development of effective treatments for this prevalent and debilitating symptom.

Disclosures: A. Casamento Moran: None. **R. Nickl:** None. **M. Singhala:** None. **T. George:** None. **V. Chib:** None. **J. Brown:** None. **P. Celnik:** None. **G. Cantarero:** None.

Digital Abstract Session

P225. Motor System Neurophysiology

Program #/Poster #: P225.01

Topic: E.05. Brain-Machine Interface

Support: SPARC Grant OT2 OD025340

Title: High frequency ultrasound for the visualization of vagotopy in a pig model

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Abstract: Vagus nerve stimulation (VNS) is an FDA approved treatment for epilepsy and depression and is currently being evaluated for treatment of numerous other disorders. Intraoperative placement of the clinical vagus nerve stimulating cuff is based on anatomical landmarks, with very little patient specificity in terms of vagal anatomy such as fascicular organization. As currently administered, VNS is associated with limited therapeutic effects and many intolerable side effects, such as dyspnea and cough, from off-target activation of motor fibers. These motor fibers extend into the superior and recurrent laryngeal branches of the vagus nerve, which innervate key muscles of the throat implicated in many of these therapy-limiting side effects.Previous histological studies in a pig model, similar in size and organization to that of humans, have shown bimodal organization between sensory and motor fascicles within the vagus nerve, otherwise known as vagotopy. Clinical outcomes could be improved by individualizing cuff placement using this organization. Intraoperative ultrasound imaging of vagotopy may lead to avoiding off target effects and improve efficacy. Ultrasound is traditionally used non-invasively at the surface of the skin to image peripheral nerves. However, non-invasive ultrasound does not offer the resolution required to visualize fascicular organization. In this study, we take advantage of the access provided during the surgical implant of the cuff by placing the ultrasound transducer within the surgical pocket of a pig model to increase resolution.High frequency ultrasound was found to accurately depict the bimodal organization of the pig vagus nerve intra-operatively, as confirmed via post-mortem histology. This bimodal organization was observed in the nodose ganglion with the grouping of motor fibers opposite a large fascicle containing pseudo-unipolar cell bodies. The pseudo-unipolar cell region indicated the origination of fascicle groupings containing sensory fibers. These groupings could be followed down the length of the vagus nerve to aid in informing cuff placement. Additionally, the superior and recurrent laryngeal nerves were identified to aid in characterizing the surgical window.Visualization of vagus nerve fascicular organization, or vagotopy, using ultrasound offers a minimally invasive approach during clinical procedure. This approach could be used to optimizeelectrode cuff placement in order to avoid unwanted activation of surrounding nerves and nerve fibers implicated in off-target effects and improve patient outcomes.

Disclosures: **M. Kasole:** None. **A. Skubal:** None. **M.L. Settell:** None. **B.E. Knudsen:** None. **N.A. Pelot:** None. **J. Trevathan:** None. **E.N. Nicolai:** None. **C. Huang:** None. **C. Zhou:** None. **W.M. Grill:** None. **S. Chen:** None. **K.A. Ludwig:** None.

Digital Abstract Session

P225. Motor System Neurophysiology

Program #/Poster #: P225.02

Topic: E.05. Brain-Machine Interface

Title: Sex differences in vasti synergistic control patterns for neuromuscular endurance

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Abstract: There are sex differences in synergistic control patterns among the upper, middle, and lower subdivisions of trapezius during shoulder elevation. However, this have yet to be investigated in quadriceps subdivisions, especially between vastus medialis oblique (VMO) and vastus lateralis (VL). Different muscle synergistic patterns may project different degrees of neuromuscular fatigue on upstream central nervous system (CNS). The purpose of this study is to investigate sex differences in VMO-VL synergistic control patterns and their relationship with endurance time of sustained fatiguing tasks. Ten healthy females and ten healthy males were instructed to perform sustained isometric leg-extension until task failure at an intensity of 20% maximal voluntary contraction (MVC). Surface EMG of the VMO and VL was recorded. The endurance time was calculated as the time from the start of contraction until the force dropped by 5% below the target force. The VMO-VL synergistic control patterns were evaluated by averaging relative normalized EMG root mean square (RMS) ratios within each quarter of endurance time, i.e., (RMS_{VMO} / MVC RMS_{VMO}) / (RMS_{VL} / MVC RMS_{VL}) × 100%. Independent-samples t tests showed that the endurance time of females was significantly longer than that of males (F vs. M, 305. 7 ± 96.8 s vs. 204.8 ± 53.8 s, $p < 0.05$). A repeated-measures two-way ANOVA with sex as the between-group factor and fatigue phase as within-group factors revealed that the VMO/VL RMS ratios of males were significantly higher than those of females across all four fatigue phases (F vs. M, 73.1 ± 8.7 % vs. 103.4 ± 8.7 %, $p < 0.05$) and were close to 100%, whereas the VMO/VL RMS ratios of females were significantly lower than 100% (one-sample t test, $p < 0.05$). Pearson correlation analyses revealed that there was a significantly negative correlation between VMO/VL RMS ratios and the endurance time in females ($r = - 0.570$, $p < 0.05$) but not in males. This may be because higher VMO/VL RMS ratios require greater common drive from CNS to both VMO and VL and may incur greater fatigability in CNS and lead to shorter endurance in males. It may help to prevent the overuse of patellofemoral joints whose disorders are much more prevalent in females.

Disclosures: Z. Wang: None. **A. Johnson:** None. **L. Griffin:** None.

Digital Abstract Session

P226. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Program #/Poster #: P226.01

Topic: E.09. Motor Neurons and Muscle

Support: NIH1R01 NS110577-01A1(JRW, YW, JSC)
NS22189(JRW)
NIH/NIBIB/P41/EB018783(JRW)
NYS SCI Research Trust Fund

Title: Electro-cortical stimulation increases Gad1 transcription of ventral spinal cord GABAergic interneurons but does not change Gabbr1 expression of soleus motoneuron

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Abstract: Weak electrocortical stimulation (ECS) of rat sensorimotor cortex (SMC) over several weeks increases soleus H-reflex (HR) size; the increase persists for months. This increase correlates with GABA receptor decrease and GAD_{67}^{+} terminal increase on soleus motoneurons (SOL MN), showing that ECS affects spinal cord GABAergic function (Chen et al. 2007; Wang et al. 2012). We are studying the transcriptional mechanisms of these effects by determining *Gad1* and *Gabbr1* mRNA levels by single molecule in situ hybridization in GAD_{67}^{+} interneurons in spinal cord ventral horn ($Gad1^{+}$-INs) and SOL MN respectively. Rats are implanted with SOL EMG electrodes and a posterior tibial nerve stimulation cuff in the right leg and ECS electrodes over left hindlimb SMC. ECS (25-Hz train of 0.1-ms biphasic pulses for 1 s every 10 s) was delivered 24 hr/d X 30 days; it produced a small SOL motor evoked potential and no visible response. The SOL HR was recorded throughout; it increased to 243(±61 SE) % of initial value. At the end of ECS, ECS rats and naïve control (NC) rats are injected with cholera toxin subunit B (CTB) in SOL muscles and perfused 3 days later. L4-S1 lumbar cord is sectioned and hybridized with RNAscope™ probes targeting *Gad1* and *Gabbr1*. Images for $Gad1^{+}$-INs in ventral horn and CTB-labeled SOL MN are prepared; somatic *Gad1* and *Gabbr1* puncta are quantified; area fractions are calculated for cell images in a double-blind manner. Intergroup differences are assessed by one-way ANOVA. $Gad1^{+}$-INs are widespread in the spinal cord, including lamina VII (LVII) where most inhibitory pre-motor neurons are. In results to date, $Gad1^{+}$ puncta in LVII are significantly increased in the ECS group; they average 117±6/cell in NC rats vs 168±6 in right and left sides of ECS rats ($p<0.001$ vs NC). mRNA density/100 μm^2 somatic area was also increased; it averaged 39±2 for NC rats and 47±2 (left) and 45±2 (right) for ECS rats ($p<0.001$ (left) and $p< 0.01$ (right) vs NC). In SOL MNs, *Gabbr*1 mRNA puncta were similar between groups averaging 74±6 in NC rats and 80±5 in ECS rats ($p=0.3$ vs NC); mRNA densities were also similar, averaging 103±4 (left) & 104±3 (right) % of NC ($p=0.2$ and 0.3 vs NC, respectively). Combined with previous studies (Refs above), these initial results suggest two novel insights. (1) ECS significantly increases *Gad*1 mRNA in ventral spinal cord GABAergic interneurons, consistent with previous findings of increased GAD_{67}^{+} immunoreactivity; this implies a pre-translational mechanism. (2) The GABA receptor decrease in SOL MNs found previously is not associated with a decrease in *Gabbr*1 mRNA in SOL MNs; this implies a post-translational mechanism.

Disclosures: **Y. Wang:** None. **B. Herron:** None. **Y. Chen:** None. **X. Chen:** None. **J. Carp:** None. **A. vato:** None. **T. Fake:** None. **J. Wolpaw:** None.

Digital Abstract Session

P226. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Program #/Poster #: P226.02

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant F32NS112556

Title: Axon initial segment distance is a determinant of motoneuron excitability

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Abstract: The axon initial segment (AIS) is emerging as a contributor in regulating neuronal excitability and action potential initiation. Manipulating the somatic proximity and/or length of the AIS segment impacts neuronal excitability by varying threshold for action potential initiation. This study was motivated, in part, by continued interest in determining the biophysical and anatomical contributors to intrinsic motoneuron excitability which is essential for understanding rank ordering in recruitment of motoneurons within a motor pool, i.e. motoneurons (MNs) innervating the same muscle. A variety of parameters have been implicated in determining MN excitability such as input conductance, subthreshold voltage-sensitive currents, and channel properties underlying spike generation, however a definitive explanation for the diversity in MN excitability, and ultimately recruitment, remains incomplete. Furthermore, the possible role of the AIS as a contributing factor in addition to these biophysical parameters has not been studied for individual MNs. In our studies, we began by utilizing immunohistochemistry to identify and compare the AIS length and distance to soma between two retrogradely labeled motor pools known to differ in their relative recruitability; the medial gastrocnemius (MG, approximately 80%F/20%S-type) and the soleus (SOL, majority S-type) motor pools. The MG motor pool is known to have a much wider range in excitability as defined by rheobase which spans 20-30-fold, while the SOL motor pool is known to have a much narrower and lower rheobase on average compared to the MG MNs. We found that the MG MN population has, on average, a longer distance in AIS proximity compared to the SOL pool. We did not find any difference in AIS length between the two pools. However, these anatomical experiments did not directly measure excitability of individual MNs. In order to accomplish this, we recorded and intracellularly filled MG MNs *in vivo* in combination with anatomical measurements of the AIS. Using this combinatorial approach, we found evidence consistent with the idea that a more distal AIS associates with lower MN excitability. In fact, when compared to several other anatomical and biophysical measures obtained from the intracellularly filled MNs, AIS distance was the dominant determinant of intrinsic excitability.

Disclosures: T.M. Rotterman: None. **D.I. Carrasco:** None. **S.N. Housley:** None. **P. Nardelli:** None. **T.C. Cope:** None.

Digital Abstract Session

P226. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Program #/Poster #: P226.03

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant NS110577
VA P01-HD32
NIH Grant NS22189
NIH/NIBIB Grant P41 EB018783
NYS SCIRB Trust Fund C32241GG

Title: H-reflex up- versus down-conditioning effects on NMDA receptor 1, protein kinase C, and potassium-chloride cotransporter activation in soleus motoneurons of spinal cord-injured rats

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Abstract: H-reflex (HR) operant conditioning can increase (HRup mode) or decrease (HRdown mode) the soleus HR (Curr Opin Behav Sci 20:138-144, 2018 for review). Appropriate HR conditioning can improve locomotion in rats or people with incomplete spinal cord injury (SCI) (J Neurosci 26:12537-12543, 2006 & 33:2365-2375, 2013). Up- and down-conditioning are not mirror images; they appear to have different mechanisms. We are studying in SCI rats the effects of up- or down-conditioning on soleus motoneuron (SOL MN) NMDA receptor 1 (NR1), protein kinase Cα phosphorylation (p-PKC), and potassium-chloride cotransporter (KCC2).
Under anesthesia, 15 SD rats received a right T9 lateral column lesion. Ten of them were implanted in right leg with SOL EMG electrodes and a posterior tibial nerve stimulating cuff. After 30 days, the implanted SCI rats underwent HR down-conditioning (n=5, SCI-down) or up-conditioning (n=5, SCI-up) for 60 days; the other 5 SCI rats were maintained without implantation or conditioning (SCI-control) for 60 days. At the end of the protocol, all SCI rats and 5 body weight-matched naïve control rats (NC rats) received CTB-Alexa Fluor 647 injection in the right SOL muscle and were perfused 3 days later. Lumbar 4-5 spinal cords were isolated and cut for anti-NR1, anti-p-PKC, and anti-KCC2 immunofluorescence labeling. SOL MN 3-D image stacks were prepared, coded, and quantified double-blindly. SOL MN IR data were expressed in % of NC mean values; intergroup differences were assessed by one-way ANOVA. In SCI-control rats, NR1-, p-PKC-, and KCC2-IR intensity on SOL MNs averaged 94±2(SE)%, 98±4%, and 94±3% of NC ($p>0.05$ vs NC for all). In SCI-down rats, final SOL HR size was 62±11% of its initial value, NR1-IR was 93±2% ($p<0.01$ vs NC, $p=0.3$ vs SCI-control), p-PKC-IR was 94±2% ($p=0.02$ vs NC; $p=0.4$ vs SCI-control), and KCC2-IR was 91±4 % ($p=0.3$ vs NC; $p=0.7$ vs SCI-control). In SCI-up rats, final SOL HR size was 241±34% of initial ($p<0.01$), NR1-IR and p-PKC-IR on SOL MNs increased to 149±6% and 146±4%, respectively (both $p<0.0001$ vs either NC or SCI-control), while KCC2-IR decreased to 73±2% ($p<0.001$ vs either NC or

SCI-control).
In SCI rats, HRdown conditioning did not appear to affect SOL MN NR1, PKC phosphorylation, or KCC2; in contrast, HRup conditioning was associated with increased NR1 and PKC phosphorylation and reduced KCC2. These results provide further insight into the differing mechanisms of HRup and HRdown conditioning, and may be relevant to their potential therapeutic applications.

Disclosures: J.S. Carp: None. **Y. Wang:** None. **Y. Chen:** None. **X.Y. Chen:** None. **B. Herron:** None. **A. Vato:** None. **J.R. Wolpaw:** None.

Digital Abstract Session

P226. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Program #/Poster #: P226.04

Topic: E.09. Motor Neurons and Muscle

Title: Cross-frequency dependencies in sensorimotor processes after monetary feedback

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Abstract: EEG/ERP research on reward feedback processing has reported important relationships between monetary gains/losses and oscillatory activity occurring within the delta (1-4Hz), theta (3-7Hz), and high beta (20-30Hz) frequency bands. However, there is a paucity of information about alpha (8-12Hz) and low-beta (13-20Hz) oscillatory activities during basic reward feedback processing. Previous research using performance feedback-locked epochs describes a desynchronization in sensorimotor alpha (SMα) and sensorimotor low-beta (SMb) power after accurate button presses (Luft, Takase & Bhattacharya, 2014). Literature on alpha and low-beta rhythms in motor mirror neurons suggests that the motor cortex is activated by observing rewarding stimuli and suppressed by observing punishment (Brown et al., 2013; Gros, Panasiti & Chakrabarti, 2015), but has not always found significant results in both frequency bands. Therefore, our study sought to determine how monetary feedback modulates SMα and SMb, and to assess their relationships to more widely-studied measures from gambling feedback tasks (i.e. delta, theta, and high beta). 145 participants completed a gambling task (Gehring & Willoughby, 2002) as EEG data were recorded. Centroparietal delta, midline medial frontal theta, bilateral SMα, bilateral SMb, and medial frontal high-beta amplitudes were band-pass filtered and each assessed for loss-gain differences with principal components analyses (see Watts et al. 2018). Delta (t=4.75, p<.001, Cohen's d=.39) and high-beta (t=4.14, p<.001, d=.34) power were both greater after gains than losses. Theta power was greater after losses than gains (t=-10.56, p<.001, d=-.88). SMα (t=-3.86, p<.001, d=-.32) and SMb power (t=-2.72, p<.01, d=-.23) were both attenuated after gains relative to losses. Neither delta nor theta nor high-beta loss-gain differences were significantly associated with one another (rs<.08, ps>.05), so all were used

to predict SMα and then SMb loss-gain differences in separate multiple regressions. Delta (β=.17, t=2.05, p<.05) and theta (β=.21, t=2.53, p=.01) power significantly predicted SMα power, but high-beta power did not (p=.61). Theta (β=.21, t=1.19, p<.01) and high beta power (β=.26, t=3.31, p=.001) predicted SMb power, but delta power did not (p=.24). These results replicate effects in delta, theta, and high-beta, and implicate the sensorimotor cortex in processing both gain and losses: loss-gain effects in SMα and SMb are similar in latency and direction and both depend on medial frontal theta, but are each associated with different gain-related signals.

Disclosures: A.A. Vivino: None. **D. Butler:** None. **P. Whittaker:** None. **E.M. Bernat:** None. **S. Aviyente:** None.

Digital Abstract Session

P226. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Program #/Poster #: P226.05

Topic: E.09. Motor Neurons and Muscle

Support: ZIA-HD008966 (NICHD)

Title: Single nucleus RNA-sequencing defines unexpected diversity of cholinergic neuron types in the adult mouse spinal cord

Authors: *M. R. ALKASLASI, Z. E. PICCUS, S. HAREENDRAN, H. SILBERBERG, L. CHEN, Y. ZHANG, T. J. PETROS, C. E. LE PICHON;
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Abstract: In vertebrates, motor control relies on cholinergic neurons in the spinal cord that have been extensively studied over the past hundred years, yet the full heterogeneity of these neurons and their different functional roles in the adult remain to be defined. Here, we developed a targeted single nuclear RNA sequencing approach and used it to identify an array of cholinergic interneurons, visceral and skeletal motor neurons. Our data expose markers for distinguishing these classes of cholinergic neurons and their extremely rich diversity. Specifically, visceral motor neurons, which provide autonomic control, could be divided into more than a dozen transcriptomic classes with anatomically restricted localization along the spinal cord. The complexity of the skeletal motor neurons was also reflected in our analysis with alpha, beta, and gamma subtypes clearly distinguished. In combination, our data provide a comprehensive transcriptomic description of this important population of neurons that control many aspects of physiology and movement and encompass the cellular substrates for debilitating degenerative disorders.

Disclosures: M.R. Alkaslasi: None. **Z.E. Piccus:** None. **S. Hareendran:** None. **H. Silberberg:** None. **L. Chen:** None. **Y. Zhang:** None. **T.J. Petros:** None. **C.E. Le Pichon:** None.

Digital Abstract Session

P227. Motor Unit Recordings, Kinematics, and EMG

Program #/Poster #: P227.01

Topic: E.09. Motor Neurons and Muscle

Support: Israeli Ministry of Science and Technology Grant 3-12072
Israel Science Fund Grant 3-14527
Ben-Gurion University of the Negev

Title: Recovery steps from unannounced perturbations while walking: does physiology reflect behavior?

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Abstract: To avoid falling, consequential to unexpected balance loss i.e., perturbation, require people to readjust their footing rapidly and effectively (i.e., recovery stepping response). For this study, we describe lower limb muscle activation and differences between ankle and knee muscle recruitment due to unexpected perturbations during walking as measured by surface electromyography (sEMG). We aimed to explore sEMG frequency content changes before and after unannounced perturbations. Twenty young adults (27.00±2.79 years, 10 females) were exposed to unannounced surface horizontal translation perturbations while walking on a treadmill in a virtual reality environment. Perturbations were applied randomly under 2 conditions (e.g., single vs. dual task), in different gait phases in 4 directions (i.e., anterior/posterior/ right/left). sEMG signals from both lower extremities were recorded from the tibialis anterior (TA), gastrocnemius lateralis (GC), vastus lateralis (VL) and biceps femoris (BF) muscles. sEMG total spectral power for all signal frequencies and for specific bands (40-150 Hz - Low, 150-250 Hz - Medium, 250-400 Hz - High) were compared between 4 seconds baseline walking prior to perturbation and four seconds after 457 unannounced perturbations. We found that compared to baseline in the early phase post perturbation, the total spectral power of lower-extremity muscles, for all frequencies, increased significantly ($p<0.001$). The highest spectral power increase was found for 40-150 Hz and the lowest for 250-400 Hz, which is consistent with the literature. Additionally, we found that TA had significant change in frequency bands: Medium > High > Low ($p<0.001$). The higher frequency elevation response lasted for the first second after perturbation followed by gradual return to baseline total spectral power subsiding after 3 seconds. Interestingly, VL demonstrated a different response (i.e., Low > Medium > High, $p<0.001$). Our finding suggests that recruitment of muscle fiber sub-types (i.e., slow and fast twitch muscle fibers) is modulated in real time to fit functional goal requirements i.e., rapid change of footing response/recovery stepping response.

Disclosures: **U. Rosenblum:** None. **I. Melzer:** None. **O. Ben-Gal:** None. **G. Zeilig:** None. **M. Plotnik:** None.

Digital Abstract Session

P227. Motor Unit Recordings, Kinematics, and EMG

Program #/Poster #: P227.02

Topic: E.09. Motor Neurons and Muscle

Support: Natural Sciences and Engineering Research Council of Canada. Grant Number: 180970

Title: Firing rate trajectories of human motor units during isometric ramp contractions

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Abstract: During small increments of voluntary force in isometric contractions motor units (MU) exhibit initial firing rate acceleration followed by saturation demonstrating non-linear responses. Saturation of firing rates during increasing force may represent a decreased sensitivity to further input, with persistent inward currents (PICs) acting as a mechanism responsible for firing rate acceleration. However, there is evidence that PIC characteristics differ in relation to motoneuron size, such that smaller motoneurons (i.e. lower-threshold MUs) have a relatively greater normalized PIC response than larger motoneurons as well as having longer activation duration. In humans, evidence is limited as to whether later recruited (i.e. higher-threshold) MU firing rate saturation occurs and follows a trajectory like low-threshold units. Five participants were provided force targets of 10, 25 and 50% maximum voluntary contraction (MVC) for visual feedback in order to produce smooth 10s dorsiflexion ramp contractions to each level. During these contractions single MUs were recorded in the tibialis anterior using tungsten microelectrodes. To characterize firing rate trajectory, each MU sample was fit by firing rate as a function of force by both an exponential (i.e. saturated) and linear function, based on prior analysis methods (Fuglevand et al, 2015). This approach statistically compared the sum of squared errors (SSE) between models to determine if the exponential trajectory better fit the data than the linear trajectory. From recruitment thresholds ranging from 0.02 - 41% MVC, 261 MUs were collected ranging from 19-323 spikes per MU train. Linear regression provided a better fit for 94% of MUs, whereas the remaining MUs (6%) were fit better with an exponential (saturated) firing rate trajectory. As MU recruitment threshold (RT) increased the occurrence of firing rate saturation decreased. Greater rates of torque development (RTD) also resulted in a decline in saturation occurrence. As higher RT or RTD increased firing rates and variability, the SSE for both the linear and exponential trajectory also were increased. The likely lesser synaptic drive during low force levels when the lowest-threshold MUs are firing may not overcome the decreased sensitivity presented by the PIC leading to firing rate saturation. Conversely, the combination of greater synaptic drive and variability during higher intensity contractions

(increased RT and RTD), with presumed lesser PIC activity and activation of larger motoneurons leads to a linear increase in firing rates but with more rate variability. Supported by NSERC

Disclosures: **A.M. Zero:** None. **E. Kirk:** None. **K. Hali:** None. **C. Rice:** None.

Digital Abstract Session

P227. Motor Unit Recordings, Kinematics, and EMG

Program #/Poster #: P227.03

Topic: E.09. Motor Neurons and Muscle

Title: Multiscale Entropy of Lower Limb EMG During Walking Tasks in People with Parkinson's Disease: An Indicator of Altered Sensory-Motor Control

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Abstract: Background: Falls are a major health concern for older adults (OA) particularly in individuals with neurodegenerative diseases. Dual-task walking (DTW) presents an additional falls risk. Greater understanding of motor control of gait is essential for reducing falls. The purpose of this study was to investigate motor control through analysis of complexity of surface EMG signals. Complexity of EMG may indicate the richness of interactions within neural networks. The aims were to identify the effect of ageing and Parkinson's disease (PD) on complexity and how complexity is affected by walking tasks. Gender, muscle and symmetry were also examined. Based on the loss of complexity hypothesis and findings of current studies, it was hypothesized that people with PD have less complexity than healthy young adults (YA) and OA, and this complexity would further decrease during DTW due to cognitive-motor interference. Conversely, complexity was anticipated to increase during audio-cued walking (ACW) which decreases gait variability. Methods: A subsample of 15 individuals from a larger study group were randomly selected: 5 healthy YA, 5 healthy OA and 5 people with PD. Demographic and neuropsychological data were collected. Surface electrodes recorded EMG bilaterally from tibialis anterior (TA) and soleus (SO) using wireless EMG sampling at 1000 Hz. EMG was recorded during normal walking (NW), DTW and ACW. A 30 second block of EMG was analyzed for each muscle for each walking task. Surrogate analysis tested nonlinearity of EMG time-series. Optimal parameter calculation was undertaken yielding m = 2 and r = 0.25 (Lake et al. 2002). Multi scale entropy was determined and complexity index (CI) calculated across scales 4 to 40 (25-250 Hz) (Busa & van Emmerik, 2016) with lower complexity representing more regular movement. Statistical significance testing was carried out using the Kruskal-Wallis test. Results: People with PD had a lower CI across muscles compared to OA, although greater than YA. No walking task effect was observed. Females had a significantly higher CI compared to males. The CI was greater in the left side compared to the right side. Discussion: The lower CI in PD compared to age-matched controls supports our hypothesis. However, ageing was associated with greater CI. Older adults recruiting additional motor areas

resulting in more complex neuronal motor control (Vitorio et al. 2018) may partially explain this. The simplicity of the cognitive memory task in DT may account for CI similarity between conditions. The asymmetry may be linked to limb dominance or the clockwise walking track. The greater complexity in females is intriguing and needs further investigation

Disclosures: **A. Amrapala:** None. **C. Watson:** None. **A. Pantall:** None.

Digital Abstract Session

P227. Motor Unit Recordings, Kinematics, and EMG

Program #/Poster #: P227.04

Topic: E.09. Motor Neurons and Muscle

Support: NSERC PDF (GEP)
NIH: R01NS098509 (CJH)
NIH: F32HL151251 (OUK)
NIH: F31NS120500 (JAB)

Title: Does intrinsic motoneuron excitability contribute to torque generating capacity at various joint angles?

Authors: *G. E. PEARCEY, J. A. BEAUCHAMP, O. U. KHURRAM, C. J. HECKMAN; Northwestern Univ., Chicago, IL

Abstract: All motor behaviours involve complex interactions between excitatory, inhibitory and neuromodulatory commands. We can readily measure motoneuron firing patterns from the muscle fibers they innervate, collectively known as motor units (MUs), due to their one-to-one spike ratio. Neuromodulatory commands set the intrinsic excitability of the motoneuron through dendritic persistent inward currents (PICs), which contribute to the amplification of motor commands. PICs decrease when the antagonist muscle is stretched in the decerebrate cat, and we have recently shown that estimates of PICs are also reduced with vibratory input to antagonist tendons in humans. Here, we examined whether changes in joint angle altered estimates of PICs and whether these changes were associated with the changes observed in maximum voluntary torque at the various joint angles. MU firing patterns of the tibialis anterior (TA), soleus (SOL) and medial gastrocnemius (MG) were discriminated using high-density surface electromyography array electrodes and a convolutive blind source separation algorithm. We estimated PICs using the paired MU analysis technique, which quantifies discharge rate hysteresis (ΔF) by comparing the onset and offset of a high-threshold MU with respect to the firing rate of a low-threshold MU, providing an estimate of intrinsic excitability of the MU. Participants performed isometric plantarflexion and dorsiflexion triangular ramp contractions to a peak of 30% of maximal voluntary contraction, and 10 s ascending and descending phases. In a random order, the ankle joint was set in a 1) neutral (90°), 2) dorsiflexion (70°), and plantarflexion (110°) position. Preliminary analysis suggests that putting the ankle into dorsiflexion reduces TA estimates of PICs and maximum dorsiflexion torque. On the contrary,

putting the ankle into plantarflexion reduces SOL and MG estimates of PICs and maximum plantarflexion torque. This suggests that sensory feedback from the antagonist muscle plays a role in the decrements in torque generating capacity at various joint positions by altering intrinsic excitability of motoneurons.

Disclosures: G.E. Pearcey: None. **J.A. Beauchamp:** None. **O.U. Khurram:** None. **C.J. Heckman:** None.

Digital Abstract Session

P227. Motor Unit Recordings, Kinematics, and EMG

Program #/Poster #: P227.05

Topic: E.09. Motor Neurons and Muscle

Support: NIH R01NS085122
NSF CBET 991804550

Title: Effect of single and double pulse TMS on the relationship between MEP amplitude and cortical silent period

Authors: *K. LOCKWOOD[1,2], N. PINKES[2], S. JACOBS-SKOLIK[1,2], M. FURMANEK[2], M. YAROSSI[1,2], E. TUNIK[2];
[1]SPIRAL Center, Electrical and Computer Engin., [2]Movement Neurosci. Lab, Physical Therapy, Movement, and Rehabil. Sci., Northeastern Univ., Boston, MA

Abstract: Transcranial magnetic stimulation (TMS) can be used, as a "virtual lesion", to transiently disrupt function of a target brain area in order to test its role in a given task. When a long period of disrupted activity is desired, a second TMS pulse may be given at a latency of less than 100ms from the first pulse. Despite the popularity of this approach, rarely is the effectiveness of a virtual lesion in disrupting information reported. The cortical silent period (CSP), the silencing of EMG activity following a stimulation of the motor cortex during active contraction, can be used as a proxy to assess the length of disrupted cortical activity. In this study we examined the duration of disruption caused by single and double pulse TMS to M1 by measuring associated cortical silent periods. Ten young healthy right handed individuals participated in the study following institutionally approved informed consent. EMG was recorded in the right first dorsal interosseous muscle. Single pulse TMS, and double pulse TMS (50 ms inter-stimulus interval), were delivered to the left motor cortex at 110% of an individual's resting motor threshold, while the participant held a voluntary contraction equivalent to 25% of their maximum finger flexion force. CSP duration and MEP amplitude were labeled during post-processing. We compared the amplitudes of MEPs and durations of CSPs produced in the single pulse and double pulse TMS conditions. Greater MEP amplitude of the second pulse in the double pulse condition was associated with greater prolongation of the CSP, with respect to the single pulse condition ($r = 0.8414$, $p < 0.01$). These results have implications for the use of

virtual lesions for analysis of cortical involvement in behavior, and for understanding of inhibitory circuits in the motor cortex.

Disclosures: K. Lockwood: None. **N. Pinkes:** None. **S. Jacobs-Skolik:** None. **M. Furmanek:** None. **M. Yarossi:** None. **E. Tunik:** None.

Digital Abstract Session

P228. Sensory Systems: Behavior or Manipulation

Program #/Poster #: P228.01

Topic: F.01. Neuroethology

Support: HFSP LT001049/2015

Title: The emergence of a collective sensory threshold in the response of ant colonies to temperature perturbations

Authors: *A. GAL, D. KRONAUER;
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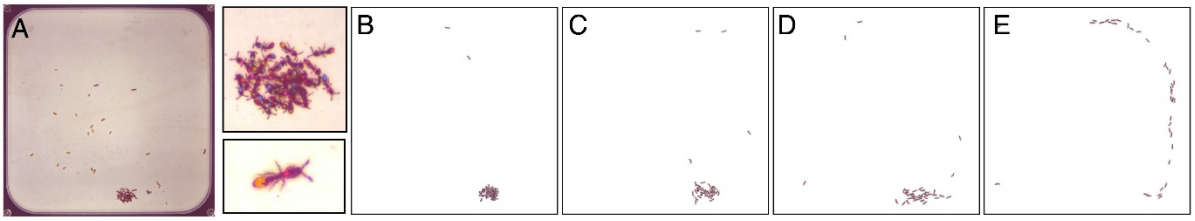
Abstract: The sensory threshold is one of the most fundamental and well-studied computational primitives organisms perform, both as a standalone computation, and as a component of more complex tasks. In social organisms such as ant colonies, which perform computational tasks at the group level, the collective sensory threshold is an emergent property that depends on the responses of individuals in the group and on the interactions between them. Here we study this emergence in the clonal raider ant (*Ooceraea biroi*), a model system that provides convenient and precise control over the properties of the colony. We use automated individual tracking to show that an ant colony indeed responds collectively to step changes in temperature by initiating a coordinated and synchronized movement (Figure). We further show that this collective response is characterized by a threshold, and that this threshold is sensitive to the size of the colony. This implies that interactions play an important role in the response dynamics of the colony, and that the collective threshold is indeed an emergent property distinct from the sensory threshold of the individual ants. We then use a mathematical model to study how collective threshold can emerge in an interacting group of agents and show that an asymmetrical and change resisting interaction between the ants is required to replicate the experimental observation. Inspired by the history of computational neuroscience, we argue that studying simple responses to well controlled stimuli can advance our understanding of how sophisticated cognitive-like function emerge in complex social groups.
Figure: (**A**) A snapshot from a raw experimental video. (**B**) *Baseline state*. Before the onset of the perturbation most ants reside in the nest, with few scout ants wondering around the arena. (**C**) *Excitement*. Following the onset of the perturbation, the ants first respond by increasing their activity level around the nest. (**D**) *Moving out*. After a delay, the ants suddenly begin to leave the nest to a well-defined direction. (**E**) *Full emigration*. The colony forms a organised emigration column.



Disclosures: **A. Gal:** None. **D. Kronauer:** None.

Digital Abstract Session

P228. Sensory Systems: Behavior or Manipulation

Program #/Poster #: P228.02

Topic: F.01. Neuroethology

Support: NIH Grant 1K12NS098482-01
NIH Grant T32NS043124-15
The Charif Souki Fund
NIH Grant RO2NS078294
The McNair Medical Institute

Title: Co-transmitting inhibitory interneurons drive olfactory behaviors through convergent and divergent pathways

Authors: *A. M. LYONS-WARREN[1], E. HANSON[2], M. KOCHUKOV[2], B. ARENKIEL[2];
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Abstract: Sensory processing abnormalities are a common feature of neurodevelopmental disorders. Co-transmission is an important but not fully understood mechanism that contributes to sensory processing. One example of co-transmitting cells are superficial short axon cells (sSAC) in the olfactory bulb which release both GABA and dopamine. The aim of this study was to elucidate the role of co-transmission from sSAC in olfactory processing. First, we implemented whole cell electrophysiological recordings and immunohistochemistry to identify the dopaminergic and GABAergic targets of sSAC. We found that external tufted cells (ETC) receive both GABAergic and dopaminergic input from sSAC as part of a dopamine modulated feedback loop with projection neurons called Mitral cells. More specifically, in 76% of recordings we observed monosynaptic, GABAergic signals from sSAC onto ETC. Further, ETC that receive GABAergic input express dopamine receptor type 1 (D1) and exhibit decreased responses in the presence of a pharmacological D1 blocker. In contrast, less than 8% of Mitral cells, and no D1 expressing granule cells, received GABAergic input from sSAC. Next, we recorded monosynaptic, glutamatergic currents from labeled sSAC while stimulating Mitral cells. Finally, we found dopamine receptor type 2 (D2) labeling was restricted to olfactory sensory neurons, and that Mitral cells express dopamine receptor type 3 (D3). Thus, we conclude that GABA and dopamine from sSAC converge on ETC as part of a feedback loop but divergent pathways exist in which GABA and dopamine provide independent signals. Having identified

the GABAergic and dopaminergic targets of sSAC, we next measured odor discrimination via novelty response tasks and odor detection, and found that sSAC are necessary for both odor detection and discrimination. Interestingly, we found these behaviors are mediated via distinct pathways, in which loss of both GABA and dopamine impairs odor detection, but loss of either GABA or dopamine signaling alone disrupts odor discrimination. In conclusion, we have revealed a critical role for sSAC in odor detection and discrimination, and have begun to elucidate the circuit mechanisms underlying those functions. Namely, we identified a convergent pathway in which ETC receive GABAergic and dopaminergic signaling from sSAC as part of a dopamine modulated feedback loop with Mitral cells, as well as divergent dopaminergic targets that do not receive GABAergic input from sSAC including olfactory sensory neurons, granule cells, and Mitral cells. Together, these results improve our understanding of circuit mechanisms underlying sensory processing disorders.

Disclosures: A.M. Lyons-Warren: None. **E. Hanson:** None. **M. Kochukov:** None. **B. Arenkiel:** None.

Digital Abstract Session

P228. Sensory Systems: Behavior or Manipulation

Program #/Poster #: P228.03

Topic: F.01. Neuroethology

Support: NIH Grant R34NS111669 (Niell)
NIH F32 EY027696 (Parker)
NIH R01-NS118461 (Wyrick, Mazzucato, Niell)

Title: An ethological model of visual distance estimation in the mouse

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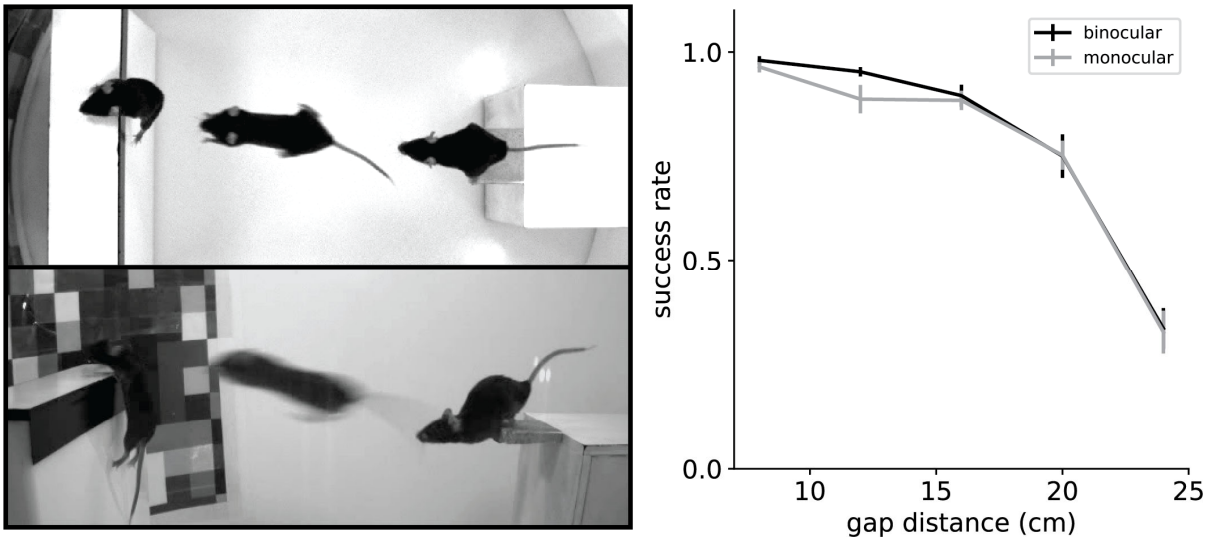
Abstract: Distance estimation is a core function of visual systems across animal species, and is critical for behaviors ranging from navigation to prey capture. There are multiple strategies to obtain depth information, including pictorial cues, binocular stereopsis, and motion parallax. The latter of these requires integrating visual and self-motion signals, but its neural circuit mechanisms are not understood. Mouse visual cortex is strongly modulated by self-motion, including head movement and vestibular signals. Could these signals be integrated with visual input to compute depth? We sought to determine if mice judge distance using self-motion, which would permit the use of genetic tools to measure and manipulate neural activity during behavior. We adapted a gerbil/rat distance estimation task to mice, in which animals jump across a variable gap in order to obtain a reward. Mice are recorded with high-speed cameras from the top and side, and videos are tracked using DeepLabCut. We found that mice accurately jump to distant platforms across gaps up to 30 cm, and performance is similar under monocular vision,

demonstrating that binocular cues such as stereopsis are not necessary. Landing platform size is varied to prevent the use of retinal image size as a cue, and performance is equivalent across platforms. Analysis of behavior before jumping showed that mice perform a variety of head movements that could be used to generate motion parallax cues. The presence, frequency, and amplitude of movements vary with experimental condition and are correlated with performance on the task. Simultaneous recording of eye movements with miniature head-mounted cameras showed that gaze is maintained toward the platform throughout large-amplitude head movements. Finally, optogenetic suppression of primary visual cortex impairs performance on the task, suggesting a critical role in the neural computations underlying estimation of depth from motion. Together, these findings establish an ethological paradigm for the investigation of cortical sensory computations utilizing self-motion in mice.



Disclosures: **P.R.L. Parker:** None. **E.T.T. Abe:** None. **N.T. Beatie:** None. **E.S.P. Leonard:** None. **D. Martins:** None. **S. Sharp:** None. **D. Wyrick:** None. **L. Mazzucato:** None. **C.M. Niell:** None.

Digital Abstract Session

P228. Sensory Systems: Behavior or Manipulation

Program #/Poster #: P228.04

Topic: F.01. Neuroethology

Support: PAPPIT IN306918
PAPIME PE306318

Title: Behavioral description of O. maya: Locomotion and stress behaviors.

Authors: *D. A. GONZÁLEZ-NAVARRETE[1], F. VERGARA-OVALLE[2], P. GARCÍA-ANDALUZ[2], F. AYALA-GUERRERO[2], D. B. PAZ-TREJO[3], H. SANCHEZ-CASTILLO[4]; [1]Univ. Nacional Autónoma De México, Ciudad de México, Mexico; [2]Univ. Nacional Autónoma

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Abstract: Behavioral description of *O. maya*: Locomotion and stress behaviors.
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Behavior is the part of the functioning of an organism dedicated to acting with the outside world. Historically, behavior studies have focused mainly on vertebrates but research on invertebrate behavior has been increasing because the potential for research of those models. Nowadays when talking about mollusk behavior, cephalopods are the first group that stands out, however, there is a lack of research with the endemic species from México, particularly with *Octopus maya.* This species of octopus has shown to adapt well to laboratory conditions since breeding in captivity has been successfully achieved through several generations, giving it a great advantage as a research model. This allows reducing the number of variables that cannot be controlled, inherent in the use of captured specimens like other research groups do. However, to be able to work properly with a species in the laboratory it is necessary to know in detail its behavioral repertoire, that is why the main goal of this study was to make a detailed description of some behaviors that *O. maya* can display in controlled laboratory conditions, specifically the related to locomotion and stress. *O. maya* presents four behaviors related to locomotion: Crawling, jet propulsion, hovering and escalation; and three behavior related to stress during and after a tank cleaning procedure: Isolation, Ink ejection, and protection. Our results show that *O. maya* raised in captivity can display behaviors comparable to other species of octopus and support that it is an organism that can be proposed as a study model. Also, knowing the behavioral repertoire of this species allows greater control in subsequent investigations related to behavior and provide a great tool to constantly monitor the health status of organisms and its use for research, being a precedent for future behavioral research in this species related to neuroscience and cognition.
Key Words: Octopus, stress, locomotion, behavior, response, cephalopod**Supported by:**
PAPPIT IN306918, PAPIME PE306318

Disclosures: D.A. González-Navarrete: None. **F. Vergara-Ovalle:** None. **P. García-Andaluz:** None. **F. Ayala-Guerrero:** None. **D.B. Paz-Trejo:** None. **H. Sanchez-Castillo:** None.

Digital Abstract Session

P228. Sensory Systems: Behavior or Manipulation

Program #/Poster #: P228.05

Topic: F.01. Neuroethology

Title: Modulation of vestibular-sensitive neurons in deep mesecephalic nucleus to locomotion in walking monkeys

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Abstract: Locomotion is crucial for even the most basic needs. Successfully maintaining balance during locomotion requires incorporating self-motion cues such as vestibular information. The deep mesencephalic nucleus (DpMe) is a large the midbrain reticular area which plays a key role in integrating and processing sensory, attention, and limbic inputs. Its diverse connections include spinal, cortical, basal ganglia, and limbic inputs as well as outputs to thalamic nuclei and reticulospinal areas. DpMe cells have been shown to respond to passive vestibular (rotational) stimuli, which suggests that this region plays an important role in vestibulo-motor control. However, DpMe responses to locomotion have not yet been characterized. Here we analyzed single-unit extracellular activities from the DpMe in rhesus monkeys during passive vestibular stimulation (translations and rotation) and locomotion. Head-mounted 3Dgyroscopes/3Daccelerometers were used to record the head motion. High-speed cameras were used for motion recording synchronously. DeepLabCut were used to extract the animals' 3D posture for gait analysis. We identified that DpMe cells demonstrated a significant increase in activity during head-fixed treadmill walking, head-fixed and head-free ground walking when compared to resting activities. Notably, DpMe cells demonstrated phase-dependent modulation during walking, even in the absence of vestibular stimulation. Modulation patterns varied between DpMe cells- some cells demonstrated peak activity during swing phase, and others during stance. Taken together these results suggest that DpMe neurons play a critical role in processing multi-sensory input during the complex sensorimotor activities generated in everyday life, such as walking. In addition, our findings also advance our knowledge of the neural circuits that coordinate gait and balance and thus have important implications for the development of novel treatments and interventions.

Disclosures: R. Wei: None. **E. Gugig:** None. **O. Stanley:** None. **K. Cullen:** None.

Digital Abstract Session

P228. Sensory Systems: Behavior or Manipulation

Program #/Poster #: P228.06

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Non-invasive receptor-specific millimeter-precision manipulation of brain circuits

Authors: M. S. OZDAS[1,2], A. S. SHAH[1,2], P. M. JOHNSON[1,2], N. PATEL[1], M. MARKS[1,2], T. B. YASAR[1,2], U. STALDER[3], L. BIGLER[3], W. VON DER BEHRENS[1,2], S. R. SIRSI[1,4], M. F. YANIK[1,2];
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Abstract: Non-invasive, receptor-specific, focal modulation of different brain circuits in a controlled, reliablemanner can lead to breakthroughs in future treatments of brain disorders. To achieve this, wesystemically deliver engineered ultrasound-controllable therapeutic drug carriers. We thenrepeatedly apply a two-component focused ultrasound pulse sequence at a region of interestinside the brain; the first sequence concentrates the drug carriers with millimeter precision byorders of magnitude. The second sequence uncages the carrier's cargo locally to achieve hightarget specificity and low off-target effects. Upon release from the carriers, the drug locally crossesthe intact BBB. As a proof of concept, we test this method in the rat brain where we inhibitinformation flow from vibrissae sensory cortex (vS1) to vibrissae motor cortex (vM1). We load theultrasound-controlled drug carriers with muscimol (a GABAA receptor agonist) which readilycrosses the BBB, we sonicate in vS1 and record whisker evoked neural activity with a penetratingmulti-electrode array from vM1. We prove that the drug delivery is confined to a small area byrecording visually evoked activity from primary visual cortex (V1), a cortical circuit which is notdirectly involved with the information flow between vS1 and vM1. The method uses orders ofmagnitude lower amount of drug than is otherwise required by systemic injection and very lowultrasound powers (20-fold below FDA limits for diagnostic ultrasound imaging). We show thatBBB remains intact using MRI-contrast agents and, sensitive fluorescent dye extravasation,immunohistochemistry and passive cavitation detection.

Disclosures: **M.S. Ozdas:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EP20191365. **A.S. Shah:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EP20191365. **P.M. Johnson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EP20191365. **N. Patel:** None. **M. Marks:** None. **T.B. Yasar:** None. **U. Stalder:** None. **L. Bigler:** None. **W. von der Behrens:** None. **S.R. Sirsi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EP20191365. **M.F. Yanik:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EP20191365.

Digital Abstract Session

P229. Vocal and Social Communication: Avian

Program #/Poster #: P229.01

Topic: F.01. Neuroethology

Support: UMD BBI Seed Grant
NIH T32 DC00046
NIH F31 DC017884

Title: Sequence processing in the auditory forebrain of the zebra finch (Taeniopygia guttata) and the budgerigar (Melopsittacus undulatus)

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Abstract: Human speech and birdsong both rely on central processing of acoustic sequences for vocal communication. Several lines of work show that humans surpass other primates in processing auditory sequences, raising the question of what neural mechanisms evolved that could explain such species differences. Interestingly, recent behavioral experiments in birds have shown that budgerigars, a parrot species, exceed zebra finches, a songbird species, in perceiving differences in auditory sequences. Comparative neurophysiological studies in these avian species can thus help illuminate differential mechanisms of sequence processing in the central nervous system. Here, we measured neural responses to sequence changes by inserting a 24-channel linear array (Plexon U-Probe) into the caudomedial nidopallium (NCM) of adult zebra finches and adult budgerigars. Neurons in the secondary areas of the auditory forebrain of birds are known to habituate in spike rate to repeated stimuli and dishabituate to novel stimuli. Awake, head-fixed birds were passively exposed to sequences composed of synthetic song elements differing in fundamental frequency (FF) and in conditions differing in the duration of each element (40, 80, 120, and 200 ms). Birds heard 20 repetitions of a sequence, followed by blocks of 20 repetitions of the same elements in a reordered sequence. We measured the change in spike rate after each change in sequence, using dishabituation as an index for sequence sensitivity. Additionally, we investigated what properties of the sequences the neurons may be encoding and conducted behavioral experiments using the same synthetic song sequences.We present the results of >100 neurons for each condition in the zebra finch (n = 27, 13 male, 14 female) and preliminary results from >40 neurons for each condition in the budgerigar (n = 6, 4 male, 2 female). We found significant sensitivity to sequence changes in both species but stronger dishabituation in budgerigar neurons than zebra finch neurons for the shortest elements (40 ms). Additionally, we provide evidence that budgerigar neurons more strongly encode changes in FF in terms of element position than do zebra finch neurons. Preliminary behavioral results (3 zebra finches, 2 budgerigars) show weak sequence sensitivity for these stimuli in both species, but a trend towards better performance in budgerigars than zebra finches for the shortest stimuli. These results suggest that neurons in NCM of the zebra finch and budgerigar differ in temporal sensitivity to sequence information, though future work is necessary to relate physiological responses to behavior.

Disclosures: **A. Fishbein:** None. **K. Lu:** None. **W. Liu:** None. **W. Idsardi:** None. **J. Fritz:** None. **S. Shamma:** None. **R. Dooling:** None.

Digital Abstract Session

P229. Vocal and Social Communication: Avian

Program #/Poster #: P229.02

Topic: F.01. Neuroethology

Support: NIH GRANT R15HD085143
Carol Angle Fund for Faculty Research,Wellesley College
Brachman-Hoffman fellowship

Title: Reorganization of the neural substrate for tutor song memory after exposure to a novel song model

Authors: *P. ARYA[1], S. PETKOVA[2], P. P. KULKARNI[4], N. H. KOLODNY[3], *S. M. H. GOBES[1];
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Abstract: Imitating the behavior of adults is critical for the development of some complex social behaviors, such as speaking in humans and singing in birds. Zebra finches (*Taeniopygia guttata)* normally learn to sing from their father but if they are exposed to a novel song model during the sensitive period, are able to imitate the song from their second tutor. The lateral magnocellular nucleus of the anterior nidopallium (LMAN), responds to the song from the first tutor in juvenile zebra finches, but adult LMAN neurons respond to second-tutor song. On the other hand, the caudomedial nidopallium (NCM) of adult zebra finches is activated in response to auditory playbacks of both tutors, and activity is related to the strength of song learning. However, how neural selectivity for the tutor song changes while learning a second song remains unknown. We investigated changes in neural activity during acquisition of two songs using blood-oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI). Both fMR imaging and behavioral analysis were performed before (55 dph) and after (90 dph) exposure to the second tutor. We found that experience with a novel, tutor during the sensitive period for learning changes the BOLD response in both the auditory midbrain (mesencephalicus lateralis dorsalis [MLd]) and higher auditory regions (Field L, NCM, caudomedial mesopallium [CMM], caudolateral nidopallium [NCL]). Young birds (55 dph) exhibited a higher BOLD response to the novel second tutor song (TUT2) as compared to the learned first tutor song (TUT1) in the entire auditory cortex. After learning (90 dph), TUT2 song selectivity was visible in MLd while TUT1 song selectivity emerged in the left NCM. When comparing good and poor learners (based on the similarity with the second tutor's song), learning-related changes were apparent in the NCL. Even before learning the second song, a higher BOLD response in the entire auditory cortex distinguished good from poor learners. After learning the second song, only in good learners, the previously diffuse BOLD activity in the auditory cortex became localized to the NCL. Interestingly, the more the birds had learned from second tutor, the higher the BOLD response was in the NCL. Thus, we found neural response to song in several auditory regions is altered as a result of learning two songs. The modification of activity found in the NCL of good

learners is reminiscent of neural activity involved in language acquisition in human prefrontal cortex. Executive control from the prefrontal cortex could be a key mechanism directing attention necessary for successful multiple-model imitation in both birds and humans.

Disclosures: P. Arya: None. **S. Petkova:** None. **P.P. Kulkarni:** None. **N.H. Kolodny:** None. **S.M.H. Gobes:** None.

Digital Abstract Session

P229. Vocal and Social Communication: Avian

Program #/Poster #: P229.03

Topic: F.01. Neuroethology

Support: NSF-DEB 1557151
NSF Bio Anthro DDRIG 1613709
Coordination for Improvement of Higher Education Personnel (CAPES), Brazil
Brown Univ. and UC Merced start up fund
Interdepartmental Program in Molecular, Cellular and Integrative Physiology – UCLA
Eureka and Hyde Fellowships - Department of Integrative Biology and Physiology – UCLA
Will Rogers Scholarship - Center for Accessible Education – UCLA

Title: Evolutionary Forces in the Bengalese Finch Song: Parallels and Implications for Study of Human Language Evolution

Authors: *M. FARIAS-VIRGENS[1], T. DEACON[2], K. OKANOYA[3], S. WHITE[1], E. HUERTA-SANCHEZ[4];
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Abstract: The ability of learning how to produce sounds, in addition to associating innate sounds with external events or objects, enables speech and language acquisition in humans. Despite being quite rare or rudimentary among mammals, vocal production learning is very prominent in three bird groups: songbirds, parrots, and hummingbirds. In this work, we identify genes and biological pathways of importance for functional aspects of vocal production learning in the Bengalese finch (*Lonchura striata domestica*), a domesticated songbird commonly found in pet shops, but also a popular animal model in the study of learned vocal behaviors. The Bengalese finch has a remarkably complex song, in which transitions between vocal units are not fixed, introducing variability in song sequencing. This vocal complexity evolved during its domestication from the white-backed munia (*Lonchura striata*), a wild songbird easily found throughout East Asia. We use whole-genome sequencing data and analytical tools from population genomics to assess the contributions of selection processes (such as female choice for more complex songs) and demographic events (such as the major population bottleneck during

domestication) in shaping the Bengalese finch's genetic variation. Using genome-wide Fst scans, we identify several differentiated genomic regions between domesticated and wild songbirds, with the sex chromosome Z showing the greater proportion of highly differentiated genes. We also find that, as many domesticated animals, Bengalese finches are overall less genetically diverse than their wild ancestors, as shown by reduced average heterozygosity per sampled individual. However, genome-wide Tajimas'D scans show that genetic diversity in munias deviates less from expected across the genome, while diversity deviates more from the expected in Bengalese finches, with long stretches of the genome showing either considerable loss or gain of variability. Interestingly, domesticated and wild songbirds differ in multiple components of the dopamine system, a biopathway fundamental to vocal learning. Our results serve to guide further comparative efforts toward identifying convergent patterns of evolutionary change leading to vocal learning in our own species.

Disclosures: **M. Farias-Virgens:** None. **T. Deacon:** None. **K. Okanoya:** None. **S. White:** None. **E. Huerta-Sanchez:** None.

Digital Abstract Session

P229. Vocal and Social Communication: Avian

Program #/Poster #: P229.04

Topic: F.01. Neuroethology

Support: NIH F31-MH110209
UCLA Council on Research

Title: Inhibition of miR-128 rescues deficits in learned vocal communication by normalizing microglia

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Abstract: Novel approaches for identifying autism drug targets are urgently needed to develop therapeutics for patients with severe communication deficits. Autism risk genes are enriched in microRNA-128 targets, and miR-128 is also aberrantly upregulated in postmortem tissue from autism patients. Given its relevance to the disorder, miR-128 may be a broadly relevant target for therapeutic development.
The zebra finch songbird is a widely used animal model for speech and language. Previously our lab generated an activity-dependent gene expression network in the adult songbird striatopallidal song nucleus Area X to identify master regulators of learned vocal behavior. Using this dataset we discovered that the two host genes for miR-128 are among the top genes correlated to singing. We hypothesized that focal reduction of miR-128 in Area X after critical period closure would restore the capacity for improving song.
Zebra finches were raised in isolation (isolates) to generate developmental communication deficits, then bilaterally injected in Area X with a miR-128 siRNA sponge or scramble control

sequence. After recovery they were returned to a social learning environment for four weeks. We found that inhibition of miR-128 was sufficient to rescue deficits in learned vocal sequence organization relative to scramble controls.
To determine the cellular and molecular effects of miR-128 inhibition we used MERFISH to characterize the spatial location of 173 transcripts of interest in three sets of sibling-matched zebra finches: siRNA- treated isolates, untreated isolates, and typically reared animals. Strikingly, we identified a unique microglial cell type in untreated isolates that was not present in siRNA-treated isolates or typically reared siblings. These results dovetail with work in autism patients indicating that microglial activation directly correlates with severity of communication deficits. Inhibition of miR-128 may therefore be a viable therapeutic strategy for enhancing the efficacy of speech therapy in patients with severe autism.

Disclosures: C.M. Aamodt: None. **Z.E. Hemminger:** None. **R. Wollman:** None. **S.A. White:** None.

Digital Abstract Session

P229. Vocal and Social Communication: Avian

Program #/Poster #: P229.06

Topic: F.01. Neuroethology

Support: HFSP Long Term Fellowship (LT00759/2007-L) to RR
DBT Ramalingaswami Fellowship (BT/HRD/35/02/2006) to RR
SERB (EMR/2015/000829) to RR
Department of Science and Technology, India (DST/CSRI/2017/163) to RR, DR
NIH R01 (MH55987) to Allison Doupe

Title: Repeats of vocalizations with unique temporal progression, accompanied by HVC activity representing past and future events, initiate the zebra finch song.

Authors: *D. RAO, S. CHITNIS, R. RAJAN;
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Abstract: The naturally learned song of the zebra finch is a widely established model to study complex motor sequences. While the song sequence is stereotyped, song bouts begin with repeats of the same syllable called Introductory Notes (INs) that vary considerably in their number across bouts. Further, INs are associated with temporal and acoustic progression, where intervals between successive INs become shorter and less variable, and acoustic properties change for successive INs as they approach the song. (Rajan and Doupe, 2013). Also, the progression is independent of real-time sensory feedback from the external periphery, suggesting internal processes in the brain drive these changes towards song initiation (Rao et. al 2019). What drives INs to song and how complex motor sequences like song are initiated remains poorly understood. Here, we address the question of how repeats of INs transition to song by using a combination of behavioral analysis (19 birds), electrophysiological recordings of

interneurons in premotor nucleus HVC during singing (13 neurons, 2 birds), and computational modeling of the HVC network. First, we show that temporal progression is not a property of syllable repetition, but a property unique to INs before the song. In addition to previous work showing distinct HVC interneuron activity for the last IN (Rajan and Doupe, 2013), we show that individual interneurons show activity selective for the first and middle INs, middle and last INs, or last INs. Thus, for a given repeat position during INs, we find that the activity across HVC interneurons may be: 1. similar for first and middle INs, predicting the next syllable is an IN, 2. similar for middle and last INs, predicting the previous syllable was an IN, or 3. distinct for the last IN, predicting the next syllable is the start of the song. Finally, we explore how such responses in HVC may be generated using a computational model of a network of neurons within HVC. Taken together, our results show that the variable INs speed up to the song as activity in HVC changes across neurons representing past and future events. This suggests that the zebra finch HVC also represents variable elements in song by representing information about past and future syllable, much like what has been shown in songbirds with more complex vocal sequences.

Disclosures: **D. Rao:** None. **S. Chitnis:** None. **R. Rajan:** None.

Digital Abstract Session

P229. Vocal and Social Communication: Avian

Program #/Poster #: P229.07

Topic: F.01. Neuroethology

Support: NGP T32 Predoctoral Training in Interdisciplinary Neurosciences
Hearing and Communication Neuroscience Fellowship

Title: Songbird basal ganglia neurons respond differentially to stimuli of varying goal behavior-similarity

Authors: *L. E. EISENMAN, S. W. BOTTJER;
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Abstract: Procedural learning is necessary for the acquisition of vital skills such as speech. Vocal learning in songbirds is a powerful model for procedural learning because basal ganglia circuitry is highly similar between mammals and songbirds, and localized circuits in songbirds are solely dedicated to functions related to vocal learning and behavior. In both humans and songbirds, juveniles first memorize vocal sounds of a social tutor (the goal behavior) and then produce variable utterances during a phase of sensorimotor integration. To improve performance, these utterances must be evaluated against an internal representation of the goal behavior. Although cortico-basal ganglia circuitry has long been implicated in procedural learning, the mechanisms underlying the evaluation of comparisons between self-generated behavior and goal behaviors remain unclear. Previous research in the Bottjer lab has found that many neurons located in the cortical region LMAN-shell alter their firing rate only during playback of the

learned tutor song in juvenile birds. These "tutor-tuned" neurons provide the requisite internal representation of the goal tutor song. LMAN-shell neurons project to Area X-shell, a region of basal ganglia that is necessary for song learning. However, it is unknown how X-shell circuitry contributes to the evaluation of song performance. To determine the role of X-shell in the evaluation of song performance, I am recording neurons from both X-core (a sensorimotor region) and X-shell in anesthetized juvenile male zebra finches during early stages of vocal practice while presenting the following song stimuli: tutor song, self-generated songs that are either similar (OWN-SIM) or dissimilar (OWN-DSIM) to tutor song, and conspecific adult and age-matched conspecific control songs. Since LMAN-shell projects to X-shell, I hypothesized that tutor-tuned neurons were also present in X-shell. Unexpectedly, I found a small population of neurons that responded only to self-generated song that is similar to tutor song. In addition, neurons in X-shell responded more selectively, on average, to OWN-SIM stimuli compared to tutor song, OWN-DSIM, and conspecific juvenile stimuli. This suggests that neurons in X-shell can distinguish between tutor-similar self-generated song and actual tutor song and between "good" and "poor" matches to the tutor song. These neurons may be used as a "benchmark" of most recent, best performances. Understanding how cortico-basal ganglia circuitry evaluates comparisons between self-generated motor output and goal behaviors is critical to uncovering the fundamental building blocks of procedural learning across taxa.

Disclosures: L.E. Eisenman: None. **S.W. Bottjer:** None.

Digital Abstract Session

P230. Social Interaction

Program #/Poster #: P230.01

Topic: F.01. Neuroethology

Support: NIH 2P2G0GM103653
NIMH R01MH122752

Title: Experience dependent behavioral flexibility during social interaction

Authors: *R. S. CLEIN[1], M. WARREN[2], D. SANGIAMO[1], J. P. NEUNUEBEL[2];
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Abstract: Animals living in groups frequently interact with conspecifics and use social feedback to modify their behavior. While social status strongly influences how individuals modify their behavior in groups, other factors may also impact group dynamics. In particular, the role that an animal's current behavioral state plays in future interactions with other group members is less clear. To explore this question, we recorded unrestricted social interactions for 5 hours in mixed-sex groups (n = 11; two males and two females in each group) of adult mice (13-21 weeks old; B6.CAST-Cdh23Ahl+/Kjn) and automatically extracted distinct behaviors using a machine-based learning system (Kabra et al., 2013). For every recording, we computed an aggression score based on the number of aggressive behaviors performed by each male, with scores ranging

from 0.02 to 0.97. The overall level of a male's aggression did not affect the number or duration of male-female interactions (Mann-Whitney, all p values > 0.05). However, we found that a male's aggressive state profoundly influenced subsequent male-female interactions. Surprisingly, animals in a submissive state interacted with a female more frequently, for shorter durations, and sooner after the aggressive encounter than animals in an aggressive state (Mann Whitney, all p values < 0.04). In contrast, the number, duration, and temporal delay of interactions with a female were similar between males after non-social or non-aggressive behaviors (Mann-Whitney, all p's > 0.05). When looking at the movement patterns across different post-aggression behavioral sequences, the kinematics were strikingly different between males in submissive and aggressive states (Mann-Whitney, $p < 0.05$). The movement patterns of males with higher or lower aggression levels were similar (Mann-Whitney, $p > 0.05$), suggesting that a male's role in aggressive behavior strongly influenced subsequent male-female interactions. Additionally, movement patterns were highly predictive of a male's aggressive state and subsequent social engagement (Z-test, $p < 0.01$). Our results demonstrate that mice rapidly update their behavior in response to changes in aggressive social contexts, indicating that an animal's behavioral state influences overall group dynamics.

Disclosures: R.S. Clein: None. **M. Warren:** None. **D. Sangiamo:** None. **J.P. Neunuebel:** None.

Digital Abstract Session

P230. Social Interaction

Program #/Poster #: P230.02

Topic: F.01. Neuroethology

Title: Measuring voluntary behaviors in gerbil families

Authors: C. MITELUT, R. E. PETERSON, M. H. GAMER, L. DIEZ, *D. H. SANES;
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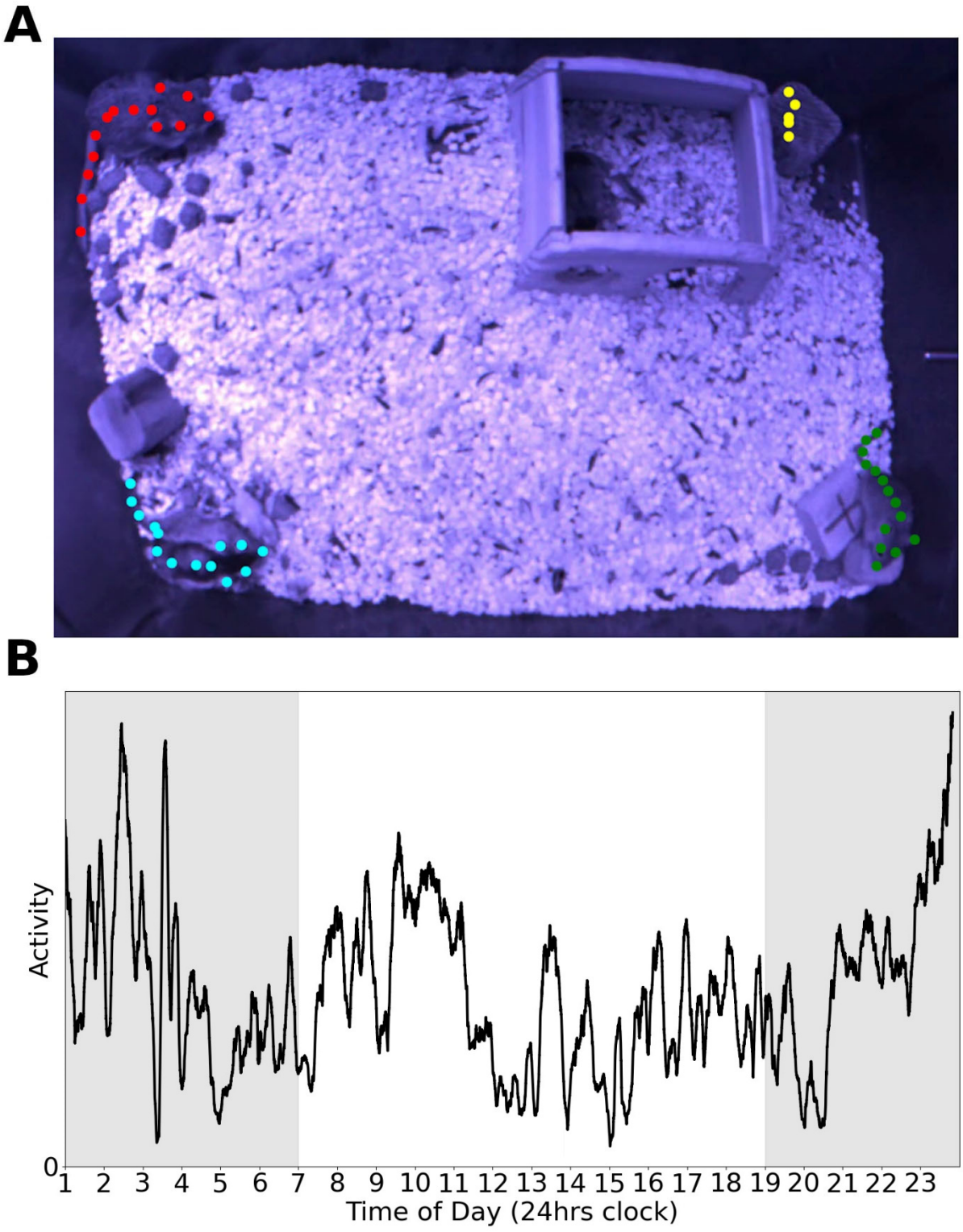
Abstract: Social interactions facilitate learning across a broad range of species, particularly during development, yet research has historically focused on short-term, laboratory-designed tests between adult dyads. However, outside of vocal learning in juvenile male songbirds (e.g. Slagsvold & Wiebe 2011), the impact of social learning, especially sex as a biological variable, remains uncertain. Importantly, historical studies have not characterized uninterrupted behaviours during developmental periods while each member of a family behaves freely in a homecage setting.To address this we established a habitat in which gerbil family cohorts (2 adults and 2-4 pups) could be monitored from birth to weaning (P1-P30). Audio and video recordings were obtained continuously to characterize the social behavioural repertoire of individual gerbils. We combined open source markerless pose estimation methods (Mathis et al 2018) with improvements to identify individual animals throughout day/night cycles and across weeks (Mitelut et al, Cosyne, in submission) (Fig A). Reviewing a single 24 hour recording, we found that we could track the circadian cycles of behaviour (Fig B). We also found that

interactions between animals varied with some animals preferring interaction with specific individuals. Lastly, we show the presence of social interaction biases where some animals are more likely than others to initiate movement. Our findings show that spontaneous behaviour can be tracked for highly social animals over periods of weeks pointing the way to future studies focusing on the neuroethology of social behaviours.



Disclosures: **C. Mitelut:** None. **R.E. Peterson:** None. **M.H. Gamer:** None. **L. Diez:** None. **D.H. Sanes:** None.

Digital Abstract Session

P231. Hormones and Behavior

Program #/Poster #: P231.01

Topic: F.02. Behavioral Neuroendocrinology

Title: Daily Administration of the Gonadotropin-Releasing Hormone Agonist Leuprolide Acetate Throughout Adolescence Delays the Development of Reproductive Physiology and Behavior in Male Rats.

Authors: *F. A. GUARRACI[1], L. K. DAVIS[2], E. L. HENNEMAN[2], E. TORO[2], S. E. ODELL[2], N. LE[2], J. M. NAVARRO[2], H. S. VALDIVIA[2], I. WILLIAMS[2], M. CREDEUR[2], A. C. GORE[3]; [1]Psychology, [2]Southwestern Univ., Georgetown, TX; [3]The Univ. of Texas, at Austin, AUSTIN, TX

Abstract: The present study was designed to examine the long-term effects of suppressing pubertal onset with leuprolide acetate, a gonadotropin releasing hormone (GnRH) agonist. Starting on postnatal day (PD) 25, male Long-Evans rats were injected daily with either leuprolide acetate (25 µg/kg dissolved in 0.9% sterile physiological saline; n=13) or sterile physiological saline (1.0 ml/kg 0.9% NaCl; n=14) for a total of 25 days. Males were monitored daily for signs of puberty (i.e., preputial separation). On the last day of leuprolide treatment (PD 50), half of each treatment group was injected with 10.0 µg of estradiol benzoate (EB) daily for three consecutive days (PD 50-52) and 1.0 mg of progesterone (P) on the 4th day (PD 53), whereas the other half of each treatment group received oil injections. Four hours after P injections, all subjects were given the opportunity to interact with a gonadally intact male and a sexually receptive female rat (i.e., a partner-preference test with and without physical contact). Copulatory behavior and sexual motivation were measured. Hormone injections and mating tests were repeated weekly for a total of 3 consecutive weeks. Leuprolide delayed puberty as well as the development of copulatory behavior and the expression of sexual motivation. By the last test, the leuprolide-treated subjects showed signs of catching up, however, some continued to be delayed. Estrogen and progesterone may have mildly feminized male physiology and behavior, but did not interact with leuprolide treatment. These findings can inform the delay of puberty in adolescents questioning their gender identity.

Disclosures: F.A. Guarraci: None. **L.K. Davis:** None. **E.L. Henneman:** None. **E. Toro:** None. **S.E. Odell:** None. **N. Le:** None. **J.M. Navarro:** None. **H.S. Valdivia:** None. **I. Williams:** None. **M. Credeur:** None. **A.C. Gore:** None.

Digital Abstract Session

P231. Hormones and Behavior

Program #/Poster #: P231.02

Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC Grant 2019-04999

Title: Contributions of androgen action within the vomeronasal organ for sexual differentiation of the brain and behaviour

Authors: *Y. H. MARTIN[1], S. SALIA[1], S. CROSS[2], A. SWIFT-GALLANT[1];
[1]Dept. of Psychology, [2]Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: The vomeronasal organ (VNO) situated in the nasal cavity is largely responsible for pheromone processing and plays a critical role in mediating socio-sexual behaviors in mice. Testosterone masculinizes and defeminizes the brain and socio-sexual behavior during early development by acting directly on androgen receptors (AR) or indirectly via estrogen receptors (ER), following aromatization to estradiol. We asked whether androgens can act via the VNO in early development to affect the display of socio-sexual behaviors in adulthood. In Experiment 1, we administered a microinjection of testosterone locally to the VNO on the day of birth (PND1) in C57BL/6 male and female mice. In Experiment 2, we asked whether testosterone acts directly on AR or indirectly via ER by injecting the VNO directly with a vehicle, estradiol or the non-aromatizable androgen, dihydrotestosterone (DHT) on PND1. Socio-sexual behaviors of experimental mice were measured in two separate tests, once in response to an estrus-induced female and another in response to a castrated male mouse swabbed with the urine of sexually experienced male. In Experiment 1, we found that a single microinjection of testosterone on PND1 was sufficient to increase male territorial aggression but did not affect the behavior of female mice. In Experiment 2, our preliminary results suggest that both estradiol and DHT differentially affect behavior in male and female mice. Males exposed to DHT demonstrated increased anogenital investigation and territorial aggression to a male intruder, whereas both males and females exposed to estradiol demonstrated an increase in investigation and self-grooming in response to a female intruder. The results suggest that testosterone may be acting both directly on AR and indirectly through ER in critical periods in development within the VNO to affect adult socio-sexual behavior. Further analyses will assess whether androgen action via the VNO affects the sexual differentiation of downstream neural targets.

Disclosures: Y.H. Martin: None. **S. Salia:** None. **A. Swift-Gallant:** None. **S. Cross:** None.

Digital Abstract Session

P231. Hormones and Behavior

Program #/Poster #: P231.03

Topic: F.02. Behavioral Neuroendocrinology

Support: DGAPA-UNAM IA207520

Title: Kisspeptin induces a conditioned place preference and sexual receptivity in female rats

Authors: *J. E. HERNÁNDEZ[1], R. G. PAREDES[1,2], M. BEDOS[2];
[1]Inst. de Neurobiología, Querétaro, Mexico; [2]Escuela Nacional de Estudios Superiores, Unidad Juriquilla, Querétaro, Mexico

Abstract: ABSTRACT

Kisspeptin induces a conditioned place preference and sexualreceptivity in female rats

J. Hernandez[1], R. Paredes[1,2], M. Bedos[2].1 Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, Mexico2 Escuela Nacional de Estudios Superiores, Unidad Juriquilla, Querétaro, Mexico

Paced mating induces a positive affective reward state as revealed by the conditioned place preference (CPP) paradigm. Using this methodology, our group showed that opioids are involved in the activation of the reward system associated with sexual behavior. Kisspeptin (Kiss), a potent regulator of the hypothalamic-pituitary-gonad axis, is also involved in other processes outside the hypothalamus, namely, the integration of sensory signals such as olfactory signals in rodents and the processing of the sexual and emotional information in humans. Interestingly, Kiss and its receptor (GPR54) are expressed in different brain regions known for their participation in the processing of reward and motivation of reinforcing behaviors. Therefore, in the present study we evaluated if Kiss induces a reward state and its role in the sexual behavior. We used sexually naïve Wistar female rats in all experiments. First, the paced mating behavior of 9 rats was evaluated in a Latin square design in 30min-sessions with sexually experienced males. All females received a s.c. injection of estradiol benzoate (E2, 2.5 µg/rat) 48h before the tests and either Progesterone (P4) 4h before the tests (s.c., 500µg/rat, E2+P4), Kiss 1h before the tests (i.p., 14nmol/rat, E2+Kiss), P4 and Kiss (E2+P4+Kiss) or vehicle (E2), depending on the session. The following parameters were recorded: lordosis intensity and quotient, exits from the male chamber and the time that the female took to return to the male chamber after a copulatory event. We didn't find any significative difference between groups except for the E2 group, which showed an increase in the number of exits from the male chamber and a decrease in the lordosis quotient and intensity. Secondly, we evaluated the effect of Kiss with the CPP paradigm. Rats were assigned to the following groups: 7 and 14 nmol of Kiss and Controls that were conditioned with saline (n=10 each). In the CPP test we found a significant increase in the time spent in the reinforced chamber between pretest and test after the administration of 7 or 14 nmol of Kiss (P=0.0012 and P=0.0049, respectively). Our findings provide evidence that Kiss administrated with E2 induces a similar receptivity and response to males during paced mating as with the classic E2+P4 treatment. Moreover, females injected with Kiss developed a clear CPP, demonstrating its reinforcing properties.

Disclosures: J.E. Hernández: None. **R.G. Paredes:** None. **M. Bedos:** None.

Digital Abstract Session

P232. Parental Behavior

Program #/Poster #: P232.01

Topic: F.02. Behavioral Neuroendocrinology

Support: UMass Amherst Startup to MP
NIH PREP to KPC

Title: Role of the medial Preoptic Area in tailoring maternal behavior to match offspring needs

Authors: K. PIZARRO-COLON, K. COPELAS, ***M. PEREIRA**;
Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Maternal behavior that is sensitive to the needs of the young is essential for the healthy development and wellbeing of all mammals. Our prior research suggests that the medial Preoptic Area (mPOA), a critical node of the circuitry regulating maternal behavior, plays a significant role in coupling appropriate caregiving with the needs of the young. Maternal sensitivity refers to this maternal ability to link sensory cues with the underlying needs of the young and to respond appropriately (i.e., promptly and contingently) to meet those needs. The present study investigated the role of the mPOA in maternal sensitivity. To this aim, we used Gi-coupled Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to selectively inhibit the mPOA of mother rats during maternal interaction with pups with varying needs. In order to examine the role of the mPOA in maternal sensitivity (versus maternal behavior), we used multiparous mother rats, as their caregiving behavior is more resilient to mPOA functional inactivation, and a within-subject design to examine the behavioral effect of chemogenetic inhibition of the mPOA. As expected, vehicle-treated mothers interacting with demanding pups adjusted their caregiving behavior to match their increased needs. However, chemogenetic inhibition of the mPOA following treatment with the DREADD agonist clozapine-N-oxide in these same mothers disrupted maternal sensitivity, as reflected by a similar expression of maternal behavior regardless of the offspring's needs. Additional studies combining RetroBeads retrograde tracing with immunostaining for the activity marker cFos detected differential patterns of responsive mPOA neurons projecting to the infralimbic cortex and the ventral tegmental area following maternal interaction with offspring with varying needs. Together, results from this study demonstrate that the mPOA orchestrates appropriate maternal caregiving with the needs of the pups.

Disclosures: **M. Pereira:** None. **K. Copelas:** None. **K. Pizarro-Colon:** None.

Digital Abstract Session

P232. Parental Behavior

Program #/Poster #: P232.02

Topic: F.02. Behavioral Neuroendocrinology

Support: 5R03HD097085-02

Title: Nucleus accumbens serotonin 1A receptor expression is critical for normal postpartum social motivation

Authors: *E. M. VITALE[1], T. A. MEINHARDT[2], E. G. FORD[3], J. S. LONSTEIN[4];
[1]Pharmacol. and Toxicology, Univ. of Kansas, Lawrence, KS; [2]Psychology, [4]Neurosci.,
[3]Michigan State Univ., East Lansing, MI

Abstract: Mammalian mothers show a unique suite of behaviors beginning around the time of parturition that are necessary for rearing the young. This transition to motherhood requires changes in many neurochemicals, including classic neurotransmitters such as serotonin. Our lab recently found reproductive state-related changes in the expression of a number of serotonin receptor subtypes in the female rat forebrain and midbrain. The most robust of these changes was a 3-fold increase in the expression of 5-HT1A receptor mRNA in the nucleus accumbens shell (NAcSh) on the day of parturition compared to before mating or during mid-pregnancy. We also found that repeated variable stress during pregnancy prevented this parturitional increase in NAcSh 5-HT1A expression, as well as reduced maternal care and increased depression-like behaviors. The current experiment directly tested whether high 5-HT1A receptor expression in the NAcSh is necessary for the normal display of postpartum maternal caregiving and affective behaviors. Long-term knock down of 5-HT1A was established by infusing an adeno-associated virus promoting shRNA against 5-HT1A mRNA into the NAcSh during early pregnancy. In some ways similar to what we found after pregnancy stress, 5-HT1A knock down in the NAcSh resulted in higher frequencies of off-nest behaviors, delayed retrieval of displaced pups back to the nest, and increased anxiety-like behavior. These results further indicate that the periparturitional rise that we found in NAcSh 5-HT1A receptors is critical for postpartum caregiving and affective behaviors, and provide a potential mechanism via the brain's reward system through which pharmacological treatments that target the central serotonin system (e.g., SSRIs) may alleviate postpartum affective disorders.

Disclosures: E.M. Vitale: None. **T.A. Meinhardt:** None. **E.G. Ford:** None. **J.S. Lonstein:** None.

Digital Abstract Session

P232. Parental Behavior

Program #/Poster #: P232.03

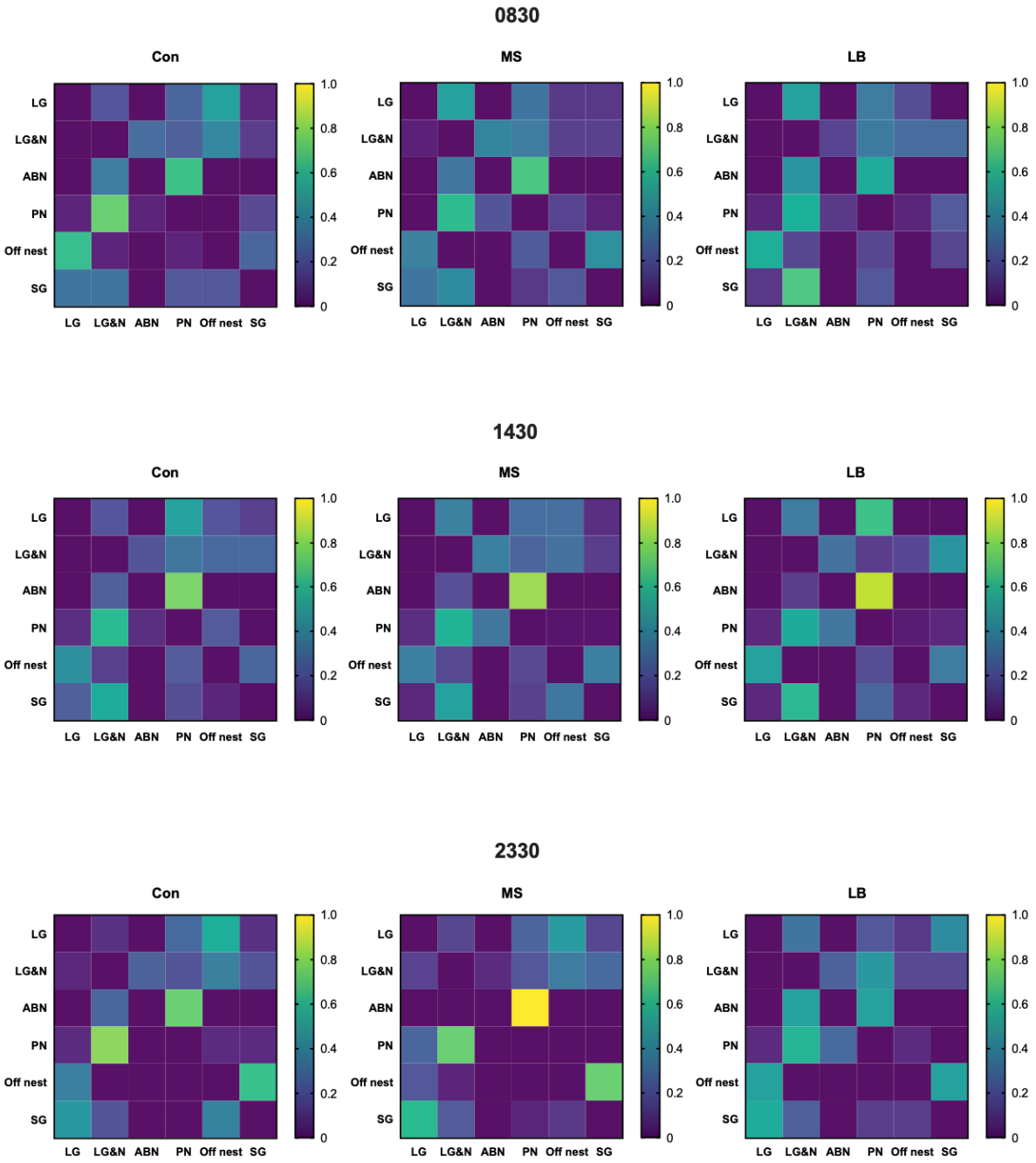
Topic: F.02. Behavioral Neuroendocrinology

Title: Differential impacts of early life stress paradigms on maternal care

Authors: *A. VALENTINE, L. GRANATA, H. BRENHOUSE;
Northeastern Univ., Boston, MA

Abstract: Animal models can elucidate the interactions between early life environment and brain development, with maternal care being a critical aspect of both. Poor quality and limited quantity of maternal behaviors carry a negative impact on emerging brain networks in rat pups. Maternal exposure to stress has been found to increase the unpredictability or fragmentation of maternal care, magnifying pup exposure to stress. Researchers report paradigms of early life

stress (ELS) initiate poor maternal care, but limited studies uncover the differential effects of the two most common ELS models: maternal separation (MS) and limited bedding (LB). Thus, we unobtrusively recorded MS, LB, and control litters (n = 10-12 dams/group) on postnatal day 8-9 for 30 minutes every 4 hours over 24 hours. We manually scored maternal behavior at three of the time points (1430, 2330, 0830) and categorized behaviors: nursing (none, passive, or arched back), nest (on or off), licking and grooming (none, licking and grooming, or self-grooming). Dams in the LB condition exhibited increased passive nursing and time on nest compared to other groups at 1430pm and 0830am. MS dams showed increased duration of arched back nursing at 1430pm compared to other conditions, possibly due to separations ending at 1400pm. Patterns of behavior were explored via transition matrices (see Figure), demonstrating small variances in predictability of maternal care amongst each paradigm and time point. Taken together, our results suggest that slight alterations in maternal care exist amidst ELS models; MS dams compensate for separation from their pups with more active nursing, and LB litters show increased quantity but poorer quality of maternal behaviors. However, fragmentation was not a characteristic of either ELS paradigm, possibly due to inadequate length of sampling periods. Next steps include analyzing additional time points to further reveal nuanced effects of models. Our results suggest researchers should consider how their ELS paradigm of choice differentially impacts maternal care and its implications on neurodevelopment.



Disclosures: **A. Valentine:** None. **L. Granata:** None. **H. Brenhouse:** None.

Digital Abstract Session

P233. Neurobiology of Social Behavior

Program #/Poster #: P233.01

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01DA0396062-01
NIH Grant 1R24MH114815-01A1
NIH Grant 1F31MH123025-01A1

Title: Sex differences in the transcriptional networks underlying playfulness suggest a distinct function for play in males compared to females

Authors: *A. E. MARQUARDT[1], J. W. VANRYZIN[2], S. A. AMENT[3], M. M. MCCARTHY[2]; [1]Program in Neurosci., [2]Dept. of Pharmacol., [3]Inst. for Genome Sci. and Dept. of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Social play, or rough-and-tumble play, is a dynamic behavior expressed by most juvenile mammals. While its function is debated, a prevailing hypothesis suggests play serves to sculpt and refine neural circuitry enabling expression of appropriate adult behavior. Importantly, in most species, males play more than females, a sex difference driven by the medial amygdala (MeA). To investigate whether the transcriptional signatures underlying play also differ by sex, we performed RNA-sequencing of MeA samples from high- and low-playing juvenile rats of both sexes. Using Weighted Gene Co-expression Network Analysis (WGCNA), we identified 22 co-expression modules, or networks of genes highly correlated in expression across samples. Of the 12 modules (for $p<0.05$) associated with play, almost all (11/12; ~92%) are sex-specific in expression, correlating with expression of play in one sex only. These data suggest there is a distinct transcriptomic profile associated with playfulness in the MeA of males compared to females, a noteworthy finding given the MeA regulates many sex-typical adult social behaviors. We propose that this is no coincidence: play-associated gene networks in the MeA are sex-specific because play modulates circuitry driving different adult behaviors depending on sex. To investigate this, we are currently exploring whether preventing play has distinct functional consequences on various adult behaviors in males and females. We created perforated Plexiglass cage dividers to separate animals during the juvenile period and thus prevent play, while still allowing for various other social interactions to occur. Preliminary data support our hypothesis, indicating a sex-specific effect of preventing juvenile play on adult copulatory behavior, social preference, and sex preference in males but not females. Surprisingly, we have observed no effects of this manipulation in females. Future experiments will also examine the effects of modulating expression of our identified sex-specific gene modules on juvenile playfulness and later-life behavior using a viral approach. Together, these analyses will provide novel insight into the ultimate function of play and how and why this may differ by sex.

Disclosures: A.E. Marquardt: None. **J.W. Vanryzin:** None. **S.A. Ament:** None. **M.M. McCarthy:** None.

Digital Abstract Session

P233. Neurobiology of Social Behavior

Program #/Poster #: P233.02

Topic: F.02. Behavioral Neuroendocrinology

Support: NIAAA T32 Training Grant
NIAAA Grant R01 AA019793

Title: Assessing the oxytocin system as a target for pharmacotherapy of alcohol use disorder using socially housed prairie voles (Microtus ochrogaster).

Authors: *S. POTRETZKE, A. E. RYABININ;
Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: The prevalence and devastating impact of alcohol use disorder (AUD) necessitate the development of efficacious treatments. Oxytocin (oxt), a hormone with crucial roles in a variety of social behaviors, has drawn interest as a promising pharmacotherapy for AUD with its implicated role in mediating the processes associated with drug use and its social effects which may serve to bolster abstinence. Oxt has been shown to be effective in decreasing alcohol craving in humans and consumption in rodents, including results from our own laboratory with repeated (intraperitoneal, i.p.) treatments in both prairie voles and mice. Of crucial importance in designing therapies are the complex interactions of alcohol-related behaviors with the social environment, demonstrating the need for incorporating social paradigms in alcohol consumption studies. Our laboratory utilizes the "Herdsman" cage system which incorporates RFID tracking and precision balances to allow for individualized measurements of both consumption and behavior in a truly social setting. In this investigation we tested whether intranasal (i.n.) oxt can decrease alcohol consumption in prairie voles (*Microtus ochrogaster*) - a socially monogamous rodent species with demonstrated translational validity to humans through common mechanisms regulating social behaviors. Adult male and female same-sex cagemates were socially housed and underwent a continuous access two-bottle choice (5% alcohol, water) procedure for 5 days to establish baseline measures of consumption. Oxt (5mg/kg or 10mg/kg, i.n.) or saline was then administered and various measures of consumption and behavior were collected. Oxt decreased alcohol consumption and preference during the first hour following treatment. Our ongoing experiments examine the effects of a small molecule oxt receptor agonist, LIT-001, which displays a more favorable pharmacokinetic profile. These studies will also characterize the behavioral mechanisms to establish whether oxt's effects on alcohol consumption are direct or mediated by increased social interaction. Together, these results will explicate oxt's role in mediating the reward processes associated with drug use and social behavior and serve to clarify whether oxt is a promising target for pharmacotherapy for AUD.

Disclosures: A.E. Ryabinin: None. **S. Potretzke:** None.

Digital Abstract Session

P233. Neurobiology of Social Behavior

Program #/Poster #: P233.03

Topic: F.02. Behavioral Neuroendocrinology

Support: Kent State University Undergraduate Research Scholars Program

Title: Genetic disruption of the oxytocin receptor affects social interactions in a neutral arena test

Authors: *C. P. VADALA, E. A. AULINO, H. K. CALDWELL;
Kent State Univ., Kent, OH

Abstract: The neuropeptide oxytocin (Oxt) is well known for its neuromodulatory effects on social behaviors. These effects are mediated predominantly by Oxt signaling through the Oxt receptor (Oxtr), and disruption of Oxtr signaling results in impaired expression of social behaviors. For example, transgenic mice with lifelong genetic deletion of the Oxtr, i.e. Oxtr —/— mice, display increased inter-male aggression in a resident-intruder test. However, whether or not this heightened aggression phenotype extends to other social contexts remains unknown. Thus, in this experiment a neutral arena test, which lacks the "home cage advantage" of a resident-intruder test, was utilized. We hypothesized that male Oxtr —/— mice would exhibit heightened aggressive behavior compared to Oxtr wildtype (+/+) mice in this novel/neutral context. To test this hypothesis, adult male Oxtr +/+ or Oxtr —/— mice were placed into a clean cage with an adult stimulus male and the behavior of the experimental animal recorded for five minutes and later scored; this test was repeated across three days. Behaviors scored included nonsocial, prosocial, and aggressive behaviors. On Day 1, Oxtr —/— mice spent significantly more time being nonsocial and engaged in less anogenital contact than Oxtr +/+ mice. On Day 2 and Day 3, no genotypic differences in behavior were observed. These results indicate that disrupted Oxtr signaling impacts the early stages of social interactions in a context-specific manner. This work is relevant as it suggests that Oxtr signaling may be particularly important in territorial displays of aggression, and perhaps to a lesser extent in other types of inter-male interactions. More work must be done to determine the ways in which the Oxt system influences male-typical behaviors.

Disclosures: C.P. Vadala: None. **H.K. Caldwell:** None. **E.A. Aulino:** None.

Digital Abstract Session

P233. Neurobiology of Social Behavior

Program #/Poster #: P233.04

Topic: F.02. Behavioral Neuroendocrinology

Support: RIKEN CBS
JSPS KAKENHI JP16K19011 (F.K.) and 18H02710 (K.K.)
JSPS

Title: Calcitonin receptor neurons in the MPOA regulate affiliative social contact behaviors

Authors: K. FUKUMITSU[1], ***K. O. KURODA**[1], M. KANEKO[1], T. MARUYAMA[1], C. YOSHIHARA[1], A. J. HUANG[3], T. J. MCHUGH[3], Y. TUNEOKA[2], S. ITOHARA[3], M. TANAKA[4];
[1]Lab. Affiliative Social Behavior, [2]Lab. for affiliative social behavior, RIKEN Ctr. for Brain Sci.

- Wako, Saitama, Japan; [3]RIKEN Ctr. for Brain Sci., Wako-shi, Japan; [4]Dept. of animal science, Nippon Vet. and Life Sci. Univ., Musashino, Japan

Abstract: Prosocial animals actively engage in affiliative contacts among conspecifics, and exhibit stress responses upon isolation. The neural mechanisms required for sensing and seeking for social contacts, however, have yet to be elucidated. Here we report that the amylin-calcitonin receptor (Calcr) signaling in the medial preoptic are (MPOA), which is recently found to be essential for parental care, also mediates social contacts among adult female mice. Calcr expressing MPOA neurons were activated by affiliative social reunion, but not by defensive huddling against an environmental threat, suggesting two separate circuits for affiliative vs. self-defensive social contacts. On the other hand, social isolation activates neurons in the medial part of central amygdala (CeM). Targeted knockdown of Calcr gene in the MPOA attenuated contact seeking behaviors during social isolation and reunion. MPOA expression of brain-gut neuropeptide Amylin, the ligand of Calcr, were depleted by physical isolation, and restored by social reunion. Disruption of amylin-Calcr signaling by using an antagonist of Calcr attenuated contact behaviors. These data present a novel and conserved neuromolecular basis of mammalian affiliative behaviors.

Disclosures: K. Fukumitsu: None. **K.O. Kuroda:** None. **M. Kaneko:** None. **T. Maruyama:** None. **C. Yoshihara:** None. **A.J. Huang:** None. **T.J. McHugh:** None. **Y. Tuneoka:** None. **S. Itohara:** None. **M. Tanaka:** None.

Digital Abstract Session

P233. Neurobiology of Social Behavior

Program #/Poster #: P233.05

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH R01MH102456

Title: Involvement of the nucleus accumbens oxytocin system in the regulation of social play behavior of male and female juvenile rats

Authors: *S. M. BOWDEN[1], J. D. A. LEE[1], R. BREDEWOLD[1], A. H. VEENEMA[2]; [1]Psychology, [2]Psychology and Neurosci., Michigan State Univ., East Lansing, MI

Abstract: Juvenile social play is a highly rewarding behavior displayed by many mammalian species. Deficits in social play behavior are a hallmark symptom of children with Autism Spectrum Disorder (ASD). Although the oxytocin (OXT) system is being considered as a potential therapeutic to restore social functioning in ASD, little is known about the involvement of the OXT system in regulating social play. Here we show that infusion of the selective OXT receptor (OXTR) antagonist des-Gly-NH_2,d$(CH_2)_5$[Tyr(Me^2),Thr^4]OVT into the nucleus accumbens (NAc) of juvenile rats significantly decreased social play in both sexes, with males requiring a higher dose of the OXTR antagonist (100ng/ 0.5 µl) than females (10ng/ 0.5 µl). As a first step to understand the underlying mechanisms, we determined whether OXTR-expressing

neurons in the NAc were sex-specifically recruited in response to social play. Using in situ hybridization, we show that the proportion of activated *oxtr*-positive neurons (*oxtr+fos/oxtr* [%]) in the NAc was significantly lower in male and female rats exposed to social play compared to those not exposed to social play. Together, these findings suggest that the OXT system in the NAc facilitates social play in both sexes through inhibition of Oxtr-expressing NAc neurons. To further examine sex differences in the regulation of social play by the NAc-OXT system, we are currently focusing on the ventral pallidum (VP) as a potential downstream target of the NAc-OXT system. Using the retrograde tracer Cholera toxin B, we are determining whether activation of the NAc^{OXTR} to VP pathway is altered in response to social play, and if so, whether this occurs in a sex-specific manner. Overall, these experiments will provide insights into the neural circuitry by which OXT regulates juvenile social play behavior in sex-specific ways, with potential relevance to social play deficits seen in ASD, a disorder with higher prevalence in males.

Disclosures: S.M. Bowden: None. **J.D.A. Lee:** None. **R. Bredewold:** None. **A.H. Veenema:** None.

Digital Abstract Session

P233. Neurobiology of Social Behavior

Program #/Poster #: P233.06

Topic: F.02. Behavioral Neuroendocrinology

Support: Swedish Research Council

Title: Dopamine receptors and social preference in young zebrafish

Authors: P. CRONELL, S. SHAMS, ***L. WESTBERG**;
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Abstract: The family of neurodevelopmental disorders commonly known as autism is characterized by social deficits, and severely affects the life of 1% of the global population. There are no established pharmacological treatment options available. To identify pharmacological tools for reversing social deficits we use the model organism zebrafish (*Danio rerio*), which is a highly social vertebrate relying on visual rather than chemosensory information in their social interactions. Zebrafish reproduce in large numbers and show robust social interaction already at 3-weeks of age, which enables more efficient and higher-throughput testing of sociability compared to other species. This study aims to investigate to what extent dopamine receptors are involved in social preference behavior in young zebrafish. Dopamine plays a prominent role in social reward and has been suggested to be relevant for the social deficits seen in individuals with autism. In this study, we investigate if agonists and antagonists for dopamine D1 and D2 receptors may alter social preference behaviour in three-week-old zebrafish. The behavioural experiments were performed in testing arenas flanked by social (fish stimuli) and non-social compartments (empty). Test fish were treated with different concentrations of

agonists and antagonists for dopamine receptor D1 (agonist, SKF-38393: 0, 1, 2.5, 7.5, and 15 uM; antagonist, SCH-23390: 0, 3, and 30 uM) and D2 (agonist, quinpirole: 0.5, 1, 2.5, 5 and 10 uM; antagonist, haloperidol: 0, 5, 10, 15 uM) for 60 minutes. Time and location of the fish in the arenas were scored and a social preference index was calculated. Generally, fish treated with dopamine receptor antagonists preferred the social stimulus side less than vehicle-treated fish. Moreover, higher concentrations of both agonists and antagonists decreased the distance travelled compared to the vehicle group. In addition, there was no apparent correlation between distance moved and social preference index. Our results suggest that dopamine receptors may regulate, independently of one another, social preference and locomotion in developing zebrafish.

Disclosures: P. Cronell: None. **S. Shams:** None. **L. Westberg:** None.

Digital Abstract Session

P233. Neurobiology of Social Behavior

Program #/Poster #: P233.07

Topic: F.02. Behavioral Neuroendocrinology

Support: Graduate Research and Creative Activity Grant 1549

Title: Domestic Cats with Latent Toxoplasma gondii Infection Show Higher Levels of Affiliative Behavior

Authors: *M. ALYETAMA;
Univ. of Nebraska At Omaha.

Abstract: Toxoplasma gondii alters risk-averse, exploratory and locomotory behaviors in rats to enhance their capture by felids, the only host where T. gondii can reproduce sexually. Among felids, the population size of domestic cats makes it likely that they are the largest environmental contributor of infectious T. gondii oocysts. Hence, parasite distribution could be markedly affected if T. gondii also manipulates cat behavior. We developed a humane, least-invasive paradigm to evaluate this issue in naturally infected pet cats. We asked pet-cat owners to present their cat with either a predator or control scent, and video-recorded their behaviors. We analyzed a subset of cat behaviors that were, based on studies in lab-infected rodents, predicted to be altered by parasite exposure. Time-budget and movement analyses indicate that unlike lab-infected rats, cats who were seropositive for prior T. gondii exposure and seronegative cats showed similar amounts of exploratory/locomotory, calm and fear behaviors, and responded similarly to predator and non-predator scents. However, seropositive cats displayed increased affiliative behavior. These results imply that naturally occurring T. gondii exposure of a definitive host leads to more subtle behavioral modification than that seen in lab-infected intermediate hosts, and, in this experimental paradigm, that prior infection either leads to a persistent increase in affiliative behavior or that felines with increased affiliative behavior are at greater risk of T. gondii infection.

Disclosures:

Digital Abstract Session

P233. Neurobiology of Social Behavior

Program #/Poster #: P233.08

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF Grant DGE-1747503

Title: Intranasal oxytocin increases hippocampal immature neuron density and social approach following delay

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Abstract: Intranasal oxytocin increases hippocampal immature neuron density and social approach following delaySocial avoidance is a common symptom underlying several psychiatric illnesses and neurodevelopmental disorders, and intranasal oxytocin is of potential therapeutic value to mitigating social avoidance due to its ability to promote affiliative behaviors. Moreover, oxytocin is known to regulate adult neurogenesis, which in turn is likely involved in anxiety regulation and social behavior. To examine how chronic intranasal oxytocin influences adult neurogenesis and social approach, we administered intranasal oxytocin to unpaired male and female California mice for seven days, and subsequently assessed their approach to a simulated unfamiliar intruder using an aggressive vocal playback test (No-Delay cohort). A separate cohort of males and females was also administered a seven-day series of intranasal infusions but received the approach test three weeks later (Delay cohort). In both cohorts we assessed the number of doublecortin positive cells in the dorsal and ventral hippocampus as well as the volume of the dentate gyrus. Intranasal oxytocin did not impact the amount of time males or females in the No-Delay cohort spent near the playback stimulus and did not increase the doublecortin-positive cell density in either the dorsal or ventral hippocampus for either sex. However, intranasal oxytocin increased the amount of time both males and females in the Delay cohort spent near the playback stimulus, and also increased the doublecortin-positive cell density in both the dorsal and ventral hippocampus of both sexes. Our results suggest that in California mice extended intranasal oxytocin treatment regulates social approach, but only after an extended delay. Additionally, this change in behavior is associated with a change in immature neuron density in the hippocampus, suggesting that adult neurogenesis may play a role in the oxytocin-mediated regulation of social approach.

Disclosures: P. Monari: None. **C. Marler:** None. **Z. Herro:** None.

Digital Abstract Session

P234. Behavioral Effects

Program #/Poster #: P234.01

Topic: F.05. Neuroimmunology

Title: Mechanisms Associated with Placental Inflammation and Serotonin Production in Response to Maternal High Fat Diet

Authors: *C. HUYNH, A. CEASRINE;
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Abstract: Maternal obesity is a rapidly increasing epidemic in the United States. Maternal high fat diet (mHFD) increases the risk for neuropsychiatric disorder development, such as anxiety and depression, in offspring potentially by promoting a fetal environment of chronic inflammation. With these observations in mind, we aim to analyze neurological mechanisms that contribute to offspring behavior changes in response to mHFD. One neurotransmitter that plays critical biological roles in behavior is serotonin. Serotonin levels can be negatively impacted by inflammation, and diminished levels of serotonin contribute to anxiety-like or depressive-like phenotypes in mice. Importantly, the placenta, a prime interface between the maternal inflammatory state (induced by maternal HFD) and the developing brain, is the source of fetal forebrain serotonin (synthesized from maternally-derived tryptophan). We hypothesize that mHFD-associated inflammation is communicated to the fetus through the placenta and decreases serotonin bioavailability. Preliminary immunohistochemistry data from our lab indicated male-specific inflammation in fetal mHFD placenta. Moreover, male mHFD offspring had significantly less serotonin in placenta, fetal forebrain, and adult prefrontal cortex (PFC). Interestingly, preliminary data demonstrate sex-specific behavior changes in male and female mHFD offspring. Both male and female neonatal mHFD offspring show diminished ultrasonic vocalizations, while adult male offspring show anhedonia (decreased sucrose preference) and juvenile female offspring show decreased social behavior. These data raise questions on sex differences in mechanisms contributing to fetal inflammation and serotonin bioavailability. My aim was to identify changes in inflammation-related and serotonin-related gene expression in mHFD placental macrophages, and assess whether increasing serotonin bioavailability during embryonic development would be sufficient to alleviate behavioral changes in male mHFD offspring. My data suggest that toll-like receptor signaling, particularly through Tlr4, may be contributing to increased inflammatory response in both male and female mHFD placenta. Further, preliminary experiments suggest that maternal tryptophan enrichment (hypothesized to increase embryonic serotonin bioavailability) rescues some male-specific behavior changes. Together, these findings point toward a mechanism through which mHFD-induced placental inflammation influences male and female behavior outcomes, likely through serotonin deficiency in males and through a distinct, yet still unknown, mechanism in females.

Disclosures: C. Huynh: None. **A. Ceasrine:** A. Employment/Salary (full or part-time):; Duke University.

Digital Abstract Session

P234. Behavioral Effects

Program #/Poster #: P234.02

Topic: F.05. Neuroimmunology

Support: CIHR project grant (PJT-162312)

Title: Overexpression of interleukin-12 in the frontal cortex mediates impulsivity and protects against subsequent immune challenge

Authors: *K. M. HRELJA, T. J. HYNES, K. ONG, C. A. WINSTANLEY;
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Abstract: One behavioural change that has been noted following traumatic brain injury (TBI) is an increase in impulsive behaviour. In addition to affecting daily functioning, this puts individuals who have suffered TBI at risk of developing numerous psychiatric disorders. Following TBI, inflammatory responses are initiated resulting in increased levels of pro-inflammatory cytokines and diffuse white matter pathology, which are the neuropathological hallmarks of TBI. It is thought that these changes may underlie the behavioural effects of TBI. One cytokine of particular interest is interleukin-12 (IL-12), which is known to be increased following TBI and is correlated with increases in impulsive behaviour. In the current experiment, 32 male Long Evans rats were used to assess whether the overexpression of IL-12 alone is sufficient to cause behavioural changes detectable using the 5-choice serial reaction time task (5-CSRTT). In short, animals were trained to stability on the cognitive task, which requires them to make a nose poke response in an illuminated aperture. Upon reaching stable performance on the final training stage, rodents underwent stereotaxic surgery where they received bilateral viral infusions of AAV5-IL12-MYC (n=16) or control (GFP; n=16) into the orbitofrontal cortex. After recovery from surgery, rodents resumed daily 5-CSRTT sessions and differences in behaviour pre- and post- viral infusion were measured over the course of one month. After this time, animals were given two injections of lipopolysaccharide (LPS), one week apart, to act as an additional immune challenge. Following sacrifice, viral expression was confirmed and brain tissue was analyzed for TBI-related pathology. Contrary to our initial hypotheses, we found that overexpression of IL-12 reduced impulsive responding on the 5-CSRTT relative to controls, as evidenced by a decrease in premature responding. This is similar to what is seen after exposure to an acute stressor, and may suggest a biphasic effect of IL-12 on cognition. We also found that excess IL-12 offered some protection from LPS-induced sickness, which is consistent with the tolerance effects commonly observed following repeated immune stimulation. This project will lend insight not only into the molecular basis of TBI, but will also shed light on the behavioural effects of IL-12 itself. Furthermore, if successful, it will also indicate which neural correlates future treatments for the behavioural symptoms of TBI can target, as there are currently no existing treatments for these.

Disclosures: **K.M. Hrelja:** None. **T.J. Hynes:** None. **K. Ong:** None. **C.A. Winstanley:** None.

Digital Abstract Session

P235. Mechanisms of Aggressive Behavior

Program #/Poster #: P235.01

Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC RGPIN 2019-04999

Title: Gut microbiome depletion in early development and adulthood alters sociosexual behaviors in male mice

Authors: ***S. SALIA**, Y. MARTIN, L. JACKMAN, F. F. BURKE, L. A. MYLES, F. BAMBICO, A. SWIFT-GALLANT;
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Abstract: Gut microbes influence the brain and behavior via a bidirectional communication between the gut and brain. Gut depletion has shown a sex-dependent effect on behavior, such as anxiety-related behavior in germ-free and antibiotic-treated rodent models. The current study assessed whether gut depletion during early development or adulthood affects sex-typical sociosexual behaviors in male and female C57bl/6 mice. We used broad-spectrum antibiotics (AB) in drinking water to deplete the microbiota in either early development (embryonic day 16 to postnatal day 21) or adulthood (day 60 to 80) while the control group received normal drinking water. We measured preference for male and female soiled bedding in an olfactory preference test, and we quantified territorial aggression on the resident intruder paradigm, where mice were exposed to a castrated male mouse swabbed with the urine of a sexually experienced male in their home cage. Sexual behaviors were measured in response to an estrus-induced stimulus female introduced to their home cage. The olfactory preference test revealed that adult AB-treated males had a significantly decreased preference for female bedding, whereas the early treatment group did not differ from control males. Both early and adult AB-treated males showed a significant decrease in male aggression compared to control males. Male treatment groups did not differ in sexual behavior in response to an estrus-induced female. Female experimental mice displayed female-typical sociosexual behavior regardless of treatment condition. Our findings suggest sociosexual behaviors are permanently altered by antibiotic treatment in early development as well as adulthood in male mice. Given the androgen dependence of these behaviors future work will assess the gut microbiome's interaction with the endocrine system. Authors have no conflicts to declare.

Disclosures: S. Salia: None. **Y. Martin:** None. **L. Jackman:** None. **F.F. Burke:** None. **L.A. Myles:** None. **F. Bambico:** None. **A. Swift-Gallant:** None.

Digital Abstract Session

P235. Mechanisms of Aggressive Behavior

Program #/Poster #: P235.02

Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC Grant RGPIN 05937-2017
Graduate Enhancement of Tri-council Stipends (GETS) program

Title: Environmental enrichment diminishes aggression and alters brain-derived neurotrophic factor gene expression in group-housed male mice

Authors: M. ALDHSHAN, *T. M. MIZUNO;
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Abstract: Mice are the most commonly used experimental animal in biological research and are typically group-housed. Although group housing is important for the welfare of social animals, it can increase within-cage aggression, one of the most serious welfare as well as scientific concerns in animal experiment. Environmental enrichment (EE) is an animal housing technique that aims to improve the welfare of the animal by providing complex sensory, motor and social stimulations that facilitate the expression of species-typical behaviors. EE is associated with improved cognitive function that is linked to increased neurogenesis and enhanced expression of brain-derived neurotrophic factor (BDNF). BDNF is a major neurotrophin in the central nervous system and participates in the regulation of aggression. It is encoded by multiple splice variants that exhibit brain-region specific expression and have distinct functions. It has been shown that mice housed under enriched conditions are less aggressive compared to mice housed under standard laboratory conditions, but the exact mechanism remains unknown. These findings led to the hypothesis that EE reduces aggressive behavior by altering the expression of *Bdnf* mRNA in a variant- and brain region-specific manner. To address this hypothesis, 3-4-week-old male C57BL/6 mice were randomly group housed (5/cage) under standard or EE conditions for 6-8 weeks. EE mice were housed in large cages supplemented with a house, running wheels, igloos, wood logs, a tube maze and nesting materials. Control mice were housed in a regular cage and were only provided with nesting materials. Aggressive interactions within each cage were evaluated by direct visual observations for 1 h 5 days a week throughout the experiment. Biting, chasing and fighting were considered as aggressive behavior. Mice were euthanized, and brain tissues were collected for gene expression analysis at the end of the experiment. The incidence of aggression was significantly reduced in EE mice (17.1%) compared to control mice (51.4%, $P < 0.005$ by chi-squared test, n = 35/group). Levels of *Bdnf* mRNA variant 1 in the prefrontal cortex (PFC) and the hypothalamus were significantly reduced in EE mice compared to control mice ($P < 0.01$ and $P < 0.05$, respectively, by Student's *t*-test, n = 8-9/group), while *Bdnf* mRNA variant 1 levels were significantly increased in the amygdala of EE mice ($P < 0.05$ by Student's *t*-test, n = 6-9/group). EE did not significantly alter levels of *Bdnf* mRNA variants 2c, 4 and 6 in the PFC and the hypothalamus. These findings support the possibility that EE diminishes intermale aggression by altering *Bdnf* mRNA expression in a variant- and brain region-specific manner.

Disclosures: T.M. Mizuno: None. **M. Aldhshan:** None.

Digital Abstract Session

P235. Mechanisms of Aggressive Behavior

Program #/Poster #: P235.03

Topic: F.02. Behavioral Neuroendocrinology

Support: Swedish Research Council 2015-03219
Swedish Research Council 2019-01676
Sahlgrenska University Hospital 723941

Title: The glucagon-like peptide-1 receptor agonist, exendin-4, reduces aggressive behaviors in male mice

Authors: *J. VESTLUND, E. JERLHAG;
Dept. of Pharmacol., Inst. of Neurosci. and Physiology, The Sahlgrenska Acad. at the Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Abstract Aggression is a complex social behavior regulated by various hormones, peptides and neurotransmitters. Available pharmacotherapies for aggressive behaviors have moderate efficacy and substantial side effects. Thus, new pharmacological targets to treat these pathologies are warranted. Recent advances showed that the orexigenic gut-brain peptide ghrelin enhances various social behaviors, including aggressive behaviors in male mice. Glucagon-like peptide-1 (GLP-1) is another gut-brain peptide, which in contrast to ghrelin suppresses food intake. Recent studies in male mice show that a GLP-1 receptor (GLP-1R) agonist, exendin-4 (Ex4), reduces social behaviors such as sexual interaction behaviors. We therefore aimed to establish the effects of activation of the GLP-1R on aggressive behaviors in male mice, and to determine possible downstream mechanism participating in this behavioral outcome. We therefore investigated the effect of acute or repeated systemic injections of Ex4 on aggressive behaviors in male mice exposed to the resident intruder paradigm. We further evaluated the levels of monoamines in brain regions as well as corticosterone and testosterone levels in plasma in these mice. Compared to vehicle repeated, but not acute, Ex4 injections reduced attack behaviors in male mice. The mice treated repeatedly with Ex4 enhanced their prosocial behaviors, whereas Ex4 displayed no effect on non-social behaviors. Further, the serotonin and noradrenaline levels in nucleus accumbens were lower in Ex4 treated mice from the resident intruder test compared to those treated with vehicle. However, there were no differences in the plasma levels of testosterone or corticosterone between the treatment groups. Collectively, this indicates that the effect of clinically available GLP-1R agonists on aggression-related pathologies should be evaluated in a clinical population.

Disclosures: J. Vestlund: None. **E. Jerlhag:** None.

Digital Abstract Session

P235. Mechanisms of Aggressive Behavior

Program #/Poster #: P235.04

Topic: F.02. Behavioral Neuroendocrinology

Support: JSPS overseas fellowship
Uehara postdoctoral fellowship
R01 HD092596
R21MH105774
U19 NS107616
1R01HD092596

Title: Spatially-distinct neurons in the posterior amygdala generate male sexual and aggressive behaviors.

Authors: *T. YAMAGUCHI[1], D. LIN[2];
[1]New York Univ., New York, NY; [2]Neurosci. Inst., New York Univ. Sch. of Med., New York, NY

Abstract: The main pillars of social behaviors involve (1) mating, where males identify sexually-receptive females through olfactory and auditory communication, and (2) aggression, where males fight conspecific male competitors in territory guarding. Decades of study have shown that the hypothalamus governs social behaviors. The medial preoptic nucleus (MPN) works as the main integrative structure for male sexual behaviors, while the ventrolateral part of ventromedial hypothalamus (VMHvl) controls inter-male aggression. However, it remains ambiguous what area directs excitatory control of the hypothalamic activity and generates the initiation signal for social behaviors. The limbic area, including the amygdala, is a strong candidate area for relaying external information into the hypothalamus and controlling its activity. Through neural tracing, neural activity recording and functional manipulations, we found that PA sends "top-down" regulation signals into hypothalamic areas and determines the timing of behavior initiation. Combining retrograde neural tracer and genetic tools, we found direct projections from PA estrogen receptor alpha (Esr1) expressing cells, into MPN and VMHvl. Importantly, PA^{Esr1+} projection cells into MPN and VMHvl are spatially distinct populations and provide excitatory inputs. Using pathway-specific fiber photometry, we found that $PA^{Esr1+MPN}$ projectors were strongly activated during female investigation and sexual behaviors, while $PA^{Esr1+VMHvl}$ cells showed dominant responses during male investigation and inter-male aggression. Pharmacogenetic inhibition of $PA^{Esr1+MPN}$ cells abolished sexual behaviors while inactivation of $PA^{Esr1+VMHvl}$ cells decreased inter-male aggression. Pharmacogenetic activation of $PA^{Esr1+MPN}$ and $PA^{Esr1+VMHvl}$ neurons promoted sexual and aggressive behaviors, respectively. On a conceptual level, this study provides a wiring diagram for understanding how social behaviors are initiated at the circuit level.

Disclosures: T. Yamaguchi: None. **D. Lin:** None.

Digital Abstract Session

P236. Hormones and Cognition: Decision-Making, Memory, Development

Program #/Poster #: P236.01

Topic: F.02. Behavioral Neuroendocrinology

Support: R00 MH111926
F32 MH125448
T32 MH019524

Title: Regulation of value-based decision making by estrogenic hormones

Authors: *C. E. GOLDEN[1], A. MAH[1], L. CHEN[2], Y. LI[3], C. CONSTANTINOPLE[1];
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Abstract: Individuals decide between alternatives based on their perceived subjective value. In humans, this value-based decision-making behavior varies by sex and across the menstrual cycle. However, it is not understood how endogenous sex hormones influence the neural dynamics that underlie decision-making. Estradiol, the principle estrogenic hormone that fluctuates across the reproductive cycle, is a compelling candidate because it is known to activate dopaminergic signaling in the nucleus accumbens, a key center for reward processing in the brain. We are exploring the effects of estradiol on value-based decision-making behavior in female rats as it naturally fluctuates in expression across their reproductive cycle (estrus cycle). To track the estrus cycle, we generated an automated classifier that determines estrus stage from vaginal cytology, which we have validated with enzyme-linked immunosorbent assays of estradiol expression. We have trained rats to perform a novel task wherein they must decide between waiting for an unspecified amount of time for a reward that is being offered or moving on to the next trial. The magnitude of reward being offered varies from trial to trial, and the local reward statistics vary over blocks of trials. In male rats, the decision to wait for the reward that is being offered depends on the magnitude of the rewards that they recently received (i.e., the local reward context). In female rats, the extent to which local reward context influences their willingness to wait for rewards varies across the estrus cycle. We have also found that dopaminergic signaling aligned to value-based decision-making epochs varies over the estrus cycle. A greater understanding of how sex hormones influence brain function could be revealing of the underlying etiology of neuropsychiatric disorders that present differently across the sexes in their incidence, progression, and severity.

Disclosures: C.E. Golden: None. **A. Mah:** None. **C. Constantinople:** None. **L. Chen:** None. **Y. Li:** None.

Digital Abstract Session

P236. Hormones and Cognition: Decision-Making, Memory, Development

Program #/Poster #: P236.02

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant 2R15GM118304-02
NIH Grant R01MH107886
UWM Research Foundation/Bradley Catalyst Grant

Title: Long-term oral administration of a novel estrogen receptor beta agonist enhances memory consolidation and alleviates vasomotor symptoms in a mouse model of menopause

Authors: *A. FLEISCHER[1], J. C. SCHALK[2], E. A. WETZEL[3], A. M. HANSON[4], D. S. SEM[4], W. A. DONALDSON[3], K. M. FRICK[1];
[1]Dept. of Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Dept. of Psychology, Univ. of Nebraska-Lincoln, Lincoln, NE; [3]Dept. of Chem., Marquette Univ., Milwaukee, WI; [4]Dept. Pharmaceut. Sciences, Ctr. for Structure-Based Drug Design and Develop., Concordia Univ. Wisconsin, Mequon, WI

Abstract: Rationale: Menopause is associated with hot flashes, cognitive decline, anxiety, and depression. Estrogen-based therapies reduce these symptoms, but also increase risks of cancer and other health issues due to the activation of the alpha (ERα), but not beta (ERβ), estrogen receptor isoform. Thus, ERβ-selective therapies are promising options to mitigate negative menopausal symptoms without the complications associated with ERα activation. **Objective:** Our goal here was to determine whether long-term treatment with EGX358, a novel highly selective ERβ agonist, could reduce negative symptoms in a mouse model of menopause. **Methods:** Nine week-old female, ovariectomized C57BL/6 mice (n=10/group) were orally gavaged daily for 10 weeks with vehicle (10% DMSO), the highly potent estrogen 17β-estradiol (E_2; 0.2 mg/kg), the ERβ agonist diarylpropionitrile (DPN; 0.05 mg/kg), or EGX358 (0.5 mg/kg). Hot flash-like symptoms were first measured following 14-18 days of treatment by thermal imaging of tail skin temperature (T_{Skin}) after injection of vehicle or senktide, an NK-3 tachykinin receptor agonist that induces heat dissipation from the tail. Long-term treatment effects on hot flash-like symptoms were also determined by measuring T_{Skin} on the final day of behavioral observations, day 63 of treatment. Between days 22-29 days of treatment, anxiety-like behavior was assessed in the open field (OF) and elevated plus maze (EPM), and depression-like behavior was assessed in the tail suspension (TST) and forced swim tests (FST). Finally, between days 40-51 of treatment object recognition and spatial memory were assessed in the object recognition (OR) and object placement (OP) tasks, respectively. **Results:** E_2, DPN, and EGX358 reduced senktide-mediated increases in T_{Skin} and enhanced OP and OR memory relative to vehicle controls. Additionally, 63 days of E_2 treatment reduced T_{Skin} compared to vehicle. Although E_2 increased time in the center of the OF, no other treatment affected behavior in the OF, EPM, TST, or FST compared to vehicle. **Conclusions:** Long-term treatment with the highly selective novel ERβ agonist EGX358 reduced hot flash-like symptoms and improved spatial and object recognition memory consolidation in ovariectomized mice. As such, ERβ activation may be a promising avenue for reducing menopause-related hot flashes and memory dysfunction.

Disclosures: A. Fleischer: None. **J.C. Schalk:** None. **E.A. Wetzel:** None. **A.M. Hanson:** None. **D.S. Sem:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual

property rights/patent holder, excluding diversified mutual funds); Estrigenix Therapeutics, Inc. **W.A. Donaldson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Estrigenix Therapeutics, Inc. **K.M. Frick:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Estrigenix Therapeutics, Inc..

Digital Abstract Session

P236. Hormones and Cognition: Decision-Making, Memory, Development

Program #/Poster #: P236.03

Topic: F.02. Behavioral Neuroendocrinology

Support: F32MH118782
R01MH107886
2R15GM118304-02
Alzheimer's Association SAGA-17-419092

Title: Extracellular matrix metalloproteinase-9 (MMP-9) is required for estradiol enhancement of hippocampal memory consolidation

Authors: *K. S. GROSS, C. M. LINCOLN, M. M. ANDERSON, G. E. GEIGER, K. M. FRICK;
Psychology, Univ. of Wisconsin Milwaukee, Milwaukee, WI

Abstract: The potent estrogen 17β-estradiol (E_2) is a powerful modulator of hippocampal plasticity and memory. Research on molecular mechanisms of hippocampal E_2 signaling has uncovered multiple intracellular pathways that contribute to these effects, but few have questioned the role that extracellular signaling processes may play in E_2-induced plasticity. The extracellular matrix (ECM) is increasingly recognized as a critical synaptic component, and ECM modifying enzymes like matrix metalloproteinase-9 (MMP-9) are required for hippocampal learning and memory. Little is established about the extent to which E_2 regulates MMP-9 in the hippocampus, and the influence this interaction may have on hippocampal memory is unknown. Therefore, we sought to determine whether E_2 increases MMP-9 activity in the hippocampus to promote memory consolidation on object placement and recognition tasks. Female C57BL/6 mice were ovariectomized and cannulated in the dorsal hippocampus (DH) and dorsal third ventricle (ICV). Immediately following object training, mice were infused with vehicle or a non-memory impairing dose of an MMP-9 inhibitor into the DH and vehicle or E_2 into the ICV. Object placement or object recognition memory was then tested 24 or 48 hours later, respectively. We found that MMP-9 inhibition blocked the memory enhancing effects of E_2, suggesting that ECM modification may be a critical factor in estrogenic regulation of memory. Continuing work is examining how E_2 treatment influences MMP-9 expression and activity in the dorsal hippocampus.

Disclosures: **K.S. Gross:** None. **C.M. Lincoln:** None. **M.M. Anderson:** None. **G.E. Geiger:** None. **K.M. Frick:** None.

Digital Abstract Session

P236. Hormones and Cognition: Decision-Making, Memory, Development

Program #/Poster #: P236.04

Topic: F.02. Behavioral Neuroendocrinology

Support: State of Washington dedicated marijuana account (dMAc) grant from the Alcohol and Drug Abuse Research Program (ADARP) grant number 130625–003 to J.F.D.

Title: Cannabis co-opts the ghrelin signaling system to direct feeding behavior.

Authors: ***J. BRUTMAN**[1], E. WHEELER[1], J. DE HOWITT[1], S. GILL[1], S. WATSON[1], S. YU[1], C. DIAZ[1], P. CHOI[1], C. MOHR[1], C.-S. WU[2], Y. SUN[2], D. ROSSI[1], J. DAVIS[1]; [1]Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA; [2]Dept. of Nutr. and Food Sci., Texas A&M Univ., College Station, TX

Abstract: Background: The legalization of cannabis in the United States derived from its medical purposes. In this context, it is well established that *cannabis sativa* exposure promotes appetite, however, the mechanisms that contribute to this response are unresolved. Ghrelin is an appetite stimulatory hormone that promotes diverse aspects of feeding behavior. This study evaluated if ghrelin signaling contributed to the appetite promoting effects of inhaled cannabis. **Methods:** We used a novel vapor delivery system to measure the appetite-stimulatory and reinforcing effects of cannabis in rodents. Specifically, adult male Long Evans rats and mice were passively exposed to a behaviorally relevant dose whole cannabis plant matter (7-10% THC, 0.5% CBD, NIDA). Commercially available ELISA assays were used to measure cannabis induced changes in the appetite promoting hormone ghrelin. In separate studies, L-cysteine and JMV2959 were used to determine if ghrelin secretion or ghrelin-1a receptor (GHSR-1a) signaling contributed to cannabis induced feeding. In addition, we used *in vivo* optical imaging to visualize calcium dynamics in arcuate nucleus (ARC) neurons and slice electrophysiology to record spontaneous inhibitory synaptic events (sIPSCs) within hunger promoting agouti-related peptide (AgRP) neurons following cannabinoid-1 receptor (CB1R) agonism. Finally, we examined cannabis induced feeding following genetic ablation of GHSR-1a within AgRP neurons (AgRP-Cre; GHSR-1a$^{f/f}$). **Results:** Vapor cannabis exposure increased meal frequency, stimulated operant responding for sucrose, and promoted gastric ghrelin secretion. Pharmacologic blockade of gastric ghrelin secretion or central GHSR-1a activity attenuated this response. *In vivo* imaging studies revealed that expectation of palatable food led to increased calcium influx in ARC neurons and that cannabis augmented this process. Mechanistic studies confirmed that activation of the CB1R reduced sIPSCs onto AgRP hunger neurons. Further, genetic ablation of GHSR-1a in AgRP neurons attenuated cannabis induced feeding. **Conclusions:** In summary, vapor cannabis exposure augments anticipatory and consummatory

aspects of feeding behavior. The appetite stimulatory effects of cannabis vapor are regulated by ghrelin and cannabinoid receptor signaling within hypothalamic neurons that promote hunger. These data highlight the ability of cannabis to co-opt endogenous biological signaling pathways that regulate energy balance to support the appetite stimulating effects of inhaled cannabis.

Disclosures: **J. Brutman:** None. **E. Wheeler:** None. **J. De Howitt:** None. **S. Gill:** None. **S. Watson:** None. **S. Yu:** None. **C. Diaz:** None. **P. Choi:** None. **C. Mohr:** None. **C. Wu:** None. **Y. Sun:** None. **D. Rossi:** None. **J. Davis:** None.

Digital Abstract Session

P236. Hormones and Cognition: Decision-Making, Memory, Development

Program #/Poster #: P236.06

Topic: F.02. Behavioral Neuroendocrinology

Support: DFG-STE531/18-3
DFG-STE531/26-1
DFG-STE 531/27-1
DFG-NE911/3-1
DFG-NE911/5-1

Title: Neuropeptide mapping of the cockroach circadian system at the single cell level

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Abstract: Transplantation studies located the circadian clock of the Madera cockroach Rhyparobia maderae to the accessory medulla (AME), ventromedially to the medulla of the brain´s optic lobes. The AME receives photic entrainment from compound eye photoreceptors and orchestrates circadian rhythms in physiology and behavior synchronized to the external 24h light-dark cycles. About 240 neurons innervate the AME which are abundant of partly co-localized neuropeptides. The structural diversity and spatial localization of neuropeptides reflect their influence in an extensive variety of physiological processes controlled by the clock. The best studied neuropeptide of the insect circadian clock is pigment-dispersing factor (PDF) that orchestrates rest-activity cycles. However, the identity and functions of most other neuropeptides of the circadian clock are not known. As prerequisite to a functional analysis of neuropeptides in the cockroach circadian clockwork we started to identify the neuropeptidome of the AME of the Madera cockroach. We performed a transcriptome analysis of the central nervous system to assemble and identify 37 neuropeptide and protein hormone precursors of R. maderae brains. Peptidomics yielded a complete coverage for many of the neuropeptide propeptides. In total, we were able to identify more than 169 mature neuropeptides in the cockroach brain, using Q Exactive Orbitrap and MALDI-TOF mass spectrometry. By applying direct tissue profiling, we identified more than 140 mature neuropeptides in the AME. In a next step we started to identify

neuropeptides from individual neurons of the AME. Employing immunocytochemistry and backfilling with subsequent single cell analysis by mass spectrometry (SCMS) we obtained precise evidence of co-localization of neuropeptides from different neuropeptide genes at the single cell level. The results of our study provide novel and necessary input for subsequent experiments revealing the functional role of neuropeptides in the insect´s circadian clock network.
[Supported by DFG grands STE531/18-3, STE531/26-1, STE 531/27-1 to MS, NE911/3-1 and NE911/5-1 to SN]

Disclosures: S. Neupert: None. **I. Schirmer:** None. **J. Schendzielorz:** None. **A. Werckenthin:** None. **M. Stengl:** None.

Digital Abstract Session

P237. HPG Axis

Program #/Poster #: P237.01

Topic: F.03. Neuroendocrine Processes

Support: National Sciences and Engineering Research Council Grant 211075-190799-2001

Title: Pubertal immune challenge suppresses the hypothalamic-pituitary-gonadal axis in male and female mice

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Abstract: Kisspeptin is a neuropeptide responsible for propagating the hypothalamic-pituitary-gonadal (HPG) axis and initiating puberty. Pubertal exposure to an immune challenge causes enduring sexual behavior dysfunction in males and females, but the mechanism underlying this stress-induced sexual dysfunction remains unknown. Previous findings show that stress exposure can downregulate the HPG axis in adult females. However, it is unclear whether stress induced HPG axis suppression is limited to adult females or also extends to males and to pubertal animal models. The current study was designed to investigate the sex-specific consequences of a pubertal immune challenge on specific components of the HPG axis. Forty 6-week old pubertal male and female mice were treated with saline or with lipopolysaccharide (LPS), a bacterial endotoxin. Expression of hypothalamic kisspeptin ligand (Kiss1) and kisspeptin receptor as well as serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and growth hormone were examined. Pubertal LPS-treatment decreased hypothalamic Kiss1 expression in both males and females, however, only males showed decreases in circulating LH and FSH. These results show that pubertal immune challenge suppresses the HPG axis by inhibiting Kiss1 production and decreasing serum gonadotropin concentrations in pubertal males, but points to a different mechanism in pubertal females.

Disclosures: **K.B. Smith:** None. **E. Murray:** None. **R. Chandrasegaram:** None. **J. Liang:** None. **J. Mallet:** None. **J.D. Blaustein:** None. **N. Ismail:** None. **C. Matar:** None.

Digital Abstract Session

P237. HPG Axis

Program #/Poster #: P237.02

Topic: F.03. Neuroendocrine Processes

Support: SUPPORTED BY THE ÚNKP-20-3 NEW NATIONAL EXCELLENCE PROGRAM OF THE MINISTRY FOR INNOVATION AND TECHNOLOGY FROM THE SOURCE OF THE NATIONAL RESEARCH, DEVELOPMENT AND INNOVATION FUND.

Title: Zearalenone modifies the function of GnRH neurons in prepubertal female mice

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Abstract: Reproduction is controlled by the hypothalamo-pituitary-gonadal (HPG) axis. Environmental endocrine disruptors (EEDs) can accelerate the onset of puberty and cause infertility in adults. Zearalenone (ZEA) is a potent estrogenic metabolite produced by some Fusarium and Gibberella species which can infect grains. A previous study has revealed that this mycotoxin advances puberty onset in young female rats by the premature activation of HPG axis, very likely by increasing the hypothalamic kisspeptin-GPR54 signaling to GnRH neurons (Yang et al 2016.). However, the direct effect of ZEA on GnRH neurons has not been examined yet. In order to elucidate its action in the regulation of reproduction at the level of GnRH neurons, *in vitro* electrophysiological experiments were carried out on GnRH-GFP neurons in acute brain slices from prepubertal (23-29 days) female mice. Whole-cell patch-clamp measurements demonstrated increased frequency (to 138.0±10.78% compared to the control values) of the spontaneous postsynaptic currents (sPSCs) after application of 2nM ZEA. ZEA also activates estrogen receptor beta (ERβ) in GnRH neurons and increases the frequency (to 140.6±13.02% compared to the control) of miniature postsynaptic currents (mPSCs). The ZEA-triggered increase in the frequency of mPSCs was prevented by using the nonselective ER receptor blocker, Faslodex or the specific ERβ receptor antagonist, PHTPP.The study provided clear electrophysiological evidence that ZEA is capable of increasing the frequency of both spontaneous and miniature PSCs of GnRH neurons with the involvement of ERβ in the process. Therefore, the direct targeting of GnRH neurons by this mycotoxin is conceivable.

Disclosures: **V. Csillag:** None. **F. Bálint:** None. **Z. Liposits:** None. **I. Farkas:** None.

Digital Abstract Session

P237. HPG Axis

Program #/Poster #: P237.03

Topic: F.03. Neuroendocrine Processes

Title: Reproductive parameters of female rats treated with Topiramate during childhood

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Abstract: The Developmental Origins of Health and Disease suggest that the exposition to certain factors during critical stages of development, such as childhood and puberty, may influence features on adult individuals. Topiramate (TOP) is an antiepileptic drug approved by the Food and Drug Administration (FDA) for treatment of epilepsy during childhood, one of those critical stages. Female adult Sprague-Dawley rats treated with TOP (100 mg/kg/day) for 28 days displayed a significant reduction in the ovaries mass as well as embryos weight (Khouri, 2005). The aim of the present study was to discover if the treatment with TOP during childhood is capable of affecting the female reproductive system. Female Wistar rats (n=7-13) were treated with TOP (41 mg/kg/day) (TOP groups) or water (CTR groups) by gavage during childhood (from postnatal day (PND) 16 to PND 28) and subsequently evaluated either immediately after the end of treatment (PND 29) – Group 1, and during adult life (PND 90-110) – Group 2. The parameters analyzed on both groups were body weight, reproductive organs' weights, anogenital distance, and histomorfometric analysis of the uterus, whereas the puberty installation (day of vaginal opening and of first estrus occurrence) and estrous cycle evaluation were analyzed only on Group 2. Data were compared by analysis of covariance (ANCOVA), Student's *t* test or Mann-Whitney U. Our results show that the treatment with TOP during childhood caused an increase in the perimetrium thickness of the adult rats' uterus (CTR2: 12.45 μm (11.47 – 13.55); TOP2: 13.70 μm (11.62 – 16.15)*; $p<0.05$, Mann-Whitney U), while the young rats uterus showed no differences. The occurrence of this alteration solely on the adult rats suggests TOP may have induced epigenetic alterations which lead to changes only observed later in life. Further studies are necessary to elucidate the mechanisms behind the changes observed.

Disclosures: J.O. Bilibio: None. **L.I. Borges:** None. **S. Forcato:** None. **D.G. da Silva:** None. **M.P. Franco:** None. **G.S. Ceravolo:** None. **D.C. Gerardin:** None.

Digital Abstract Session

P237. HPG Axis

Program #/Poster #: P237.04

Topic: F.03. Neuroendocrine Processes

Support: National Ataxia Foundation Young Investigator grant to AMM
Cleon C. Arrington Research Initiation Grant Program (RIG-93) grant to AMM
Brains and Behavior Fellowship to AJG
National Science Foundation of Hungary (K128317, PD125393) to EH
Hungarian Brain Research Program (2017-1.2.1-NKP-2017-00002.) to EH
Georgia State University Funds to NF

Title: Ubiquitin-dependent control of the hypothalamic-pituitary-gonadal axis constituents

Authors: *A. J. GEORGE[1], Y. C. HOFFIZ[1], C. B. WARE[1], B. DONG[2], N. FANG[2], E. HRABOVSZKY[3], A. M. MABB[1];
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Abstract: Ubiquitin E3 ligases are enzymes that catalyze the ubiquitination of protein substrates, which can result in their removal. Recessive mutations in the E3 ligase *RNF216/TRIAD3* cause Gordon Holmes syndrome (GHS), with pathologies of hypogonadotropic hypogonadism, cognitive impairment, dysarthria, cerebellar ataxia, and dementia. Individuals diagnosed with GHS have dysfunction at multiple levels of the hypothalamic-pituitary-gonadal (HPG) axis. This includes deficiencies in the release of pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), with the diminished secretion of the gonadal hormones, estradiol and testosterone in males and females. The role of *RNF216/TRIAD3* in the HPG axis and its selective vulnerability in the hypothalamus, specifically gonadotropin-releasing hormone (GnRH) neurons, is unknown. Previously, we found that RNF216/TRIAD3 co-localizes with clathrin-coated pits at endocytic zones in neurons, a region where receptor-mediated endocytosis takes place. Here, we found that both knockdown and overexpression of the *RNF216/TRIAD3* isoform, Triad3A, in cortical neurons disrupts the trafficking of the ubiquitous transferrin receptor, suggesting RNF216/TRIAD3 may broadly control receptor trafficking. To test the role of RNF216/TRIAD3 function in regulating surface receptor expression within the HPG axis, we knocked out *Rnf216/Triad3* via CRISPR-Cas9 in a GnRH immortalized cell line (GT1-7), and measured membrane expression of key HPG axis receptors, GPR54 and GnRHR. We next used the cytosolic Ca^{2+} sensor R-GECO1 to determine if Rnf216/Triad3 KO GT1-7 cells had altered baseline activity. We found that KO cells had a reduction in both frequency and amplitude of Ca^{2+} transients that were coupled to a reduction in GnRH. To determine if these alterations led to changes within the intact HPG axis, we generated a *Rnf216/Triad3* knockout mouse. While female mice were fertile and had normal gonadal weights, they surprisingly exhibited a reduction in the proestrus phase without alterations in baseline FSH and LH levels. Male mice had dramatically reduced testicular weights, a marked reduction in fertility, and a significant increase in FSH. We further found that the density of GnRH neurons was not significantly different in male and female KO mice compared to controls; however, the morphology of these cells was altered in both male and female KO mice. Taken together, our work illuminates how disruptions in *RNF216/TRIAD3* lead to early-onset changes in the HPG axis in males and females and informs translational research for neurological disorders involving HPG axis disruption.

Disclosures: **A.J. George:** None. **Y.C. Hoffiz:** None. **C.B. Ware:** None. **B. Dong:** None. **N. Fang:** None. **E. Hrabovszky:** None. **A.M. Mabb:** None.

Digital Abstract Session

P237. HPG Axis

Program #/Poster #: P237.05

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant K99 HD096120 to A.M.M
NIH Grant R01 HD039916 to M.N.L.

Title: Kisspeptin/neurokinin b/dynorphin (KNDy) neurons exhibit reduced progesterone sensitivity and dynorphin expression in a mouse model of polycystic ovarian syndrome

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Abstract: Polycystic ovarian syndrome (PCOS) is the most common cause of infertility in women of reproductive age worldwide. PCOS patients exhibit an increase in luteinizing hormone (LH) pulse frequency due to impaired central steroid hormone feedback to gonadotropin-releasing hormone (GnRH) neurons, the final output cell in a larger neuronal network controlling fertility. Neurons in the arcuate nucleus (ARC) that co-express kisspeptin, neurokinin B and dynorphin, known as KNDy cells, are postulated to mediate steroid hormone feedback and are strongly implicated as the GnRH/LH pulse generator. We sought to test the hypothesis that reduced steroid hormone sensitivity in KNDy cells may lead to impaired negative feedback in PCOS. To achieve this, we used a mouse model of PCOS generated using prenatal androgen (PNA) treatment that exhibits the reproductive and neuroendocrine symptoms of the syndrome. RNAscope Hiplex (ACD) fluorescent *in situ* hybridization was performed on coronal slide-mounted brain sections from PNA female (n=6) and prenatal vehicle-treated (PNV) male (n=5) and female (n=4) adult mice to simultaneously visualize and quantify gene expression of receptors for androgens, estrogen and progesterone (AR, ESR1, PGR), KNDy neuropeptides (KISS1, TAC2, PDYN), receptors for neurokinin B and dynorphin (TAC3R, OPRK1) and markers of the major neurotransmitters glutamate (SLC17A6, vGluT2) and GABA (SLC32A1, vGaT). RNA transcripts and DAPI labeling of cell nuclei was imaged using confocal microscopy and the average number of RNA transcripts for each gene within cells containing KISS1 (representative of KNDy cells) or VGaT were automatically quantified using Cellprofiler software. Compared to PNV female mice, the number of AR RNA transcripts in KISS1 and VGaT cells were elevated in PNA mice ($p<0.05$) to levels comparable with PNV males, indicating increased testosterone action at these cells. Conversely, in the same KISS1 and VGaT cells, the number of PR transcripts were reduced in PNA female mice compared to PNV females ($p<0.05$), consistent with reduced progesterone sensitivity. KISS1 cells in PNA mice with changes in steroid hormone sensitivity additionally exhibited a reduction in the number of DYN transcripts and a conversely elevated number of OPRK1 transcripts compared to male ($p<0.05$)

and female ($p<0.05$) controls. These data suggest that high testosterone levels in PCOS patients disrupt central progesterone feedback at ARC GABA and KNDY cells and suggest a mechanism by which testosterone reduces progesterone inhibition of dynorphin in KNDy neurons, leading to an increase in GnRH neuron activity and high LH pulse frequency in PCOS.

Disclosures: **A.M. Moore:** None. **L.M. Coolen:** None. **M.N. Lehman:** None.

Digital Abstract Session

P238. Neuroendocrine Anatomy and Physiology

Program #/Poster #: P238.01

Topic: F.03. Neuroendocrine Processes

Support: NSF Grant SBE-1808803
NIMH Grant R21MH115680
Josiah Charles Trent Memorial Foundation Endowment Fund
Duke Institute for Brain Sciences
Duke Lemur Center
Duke University Office of Undergraduate Research Support
Charles Lafitte Foundation for Research in Psychology & Neuroscience

Title: Neural correlates of mating system diversity: Oxytocin and vasopressin receptor distributions in monogamous and nonmonogamous Eulemur

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Abstract: Contemporary theory on the role of oxytocin and vasopressin in mammalian social bonds has been shaped by seminal vole (*Microtus)* research that revealed interspecific variation in neuroendocrine circuitry by mating system. This paradigm has since been extended to explain social bondedness in humans. Nevertheless, there exist substantial challenges to the interpretation and translation of findings in rodents to humans and other mammalian groups. Research on strepsirrhine primates is particularly well-suited to fill this gap, as the *Eulemur* genus contains both socially monogamous (MO) and non-monogamous (NM) species, making it the sole primate analog to voles. This clade thus offers a rare opportunity for comparative nonapeptide research that holds increased evolutionary relevance to humans. Relying on natural mortality in these critically endangered species, we performed oxytocin and arginine vasopressin 1a receptor (OXTR; AVPR1a) autoradiography using competitive binding on 12 *Eulemur* brains (4 MO; 8 NM) from seven closely related species. We first characterized OXTR and AVPR1a distributions in this genus, and then examined variation in receptor distribution as a function of mating system. We found some OXTR and AVPR1a binding patterns reminiscent of olfactory-

guided rodents and others congruent with more visually oriented haplorhines, consistent with strepsirrhines occupying an ‘intermediary’ evolutionary niche between these two groups. As there were no significant sex differences in binding profiles, we combined males and females in each species. Although we found differential receptor expression by mating system in a few nuclei, most areas previously identified as part of a "pair-bonding circuit" in monogamous voles and primates were not similarly distinguished in monogamous *Eulemur*. While illuminating neurobiological bases underlying species diversity, mapping nonapeptide receptors in these nontraditional primates questions existing assumptions about conserved neuroendocrine function and informs proposed evolutionary explanations about the biological bases of monogamy.

Disclosures: **A. Sharma:** None. **N.M. Grebe:** None. **C.M. Drea:** None. **S.M. Freeman:** None. **M.C. Palumbo:** None. **H.B. Patisaul:** None. **K.L. Bales:** None.

Digital Abstract Session

P238. Neuroendocrine Anatomy and Physiology

Program #/Poster #: P238.02

Topic: F.03. Neuroendocrine Processes

Support: CIHR Grant

Title: Ghrelin receptor signalling is not required for glucocorticoid-induced obesity in female mice.

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Abstract: Chronically elevated glucocorticoids (GC) increase food intake, weight gain and adiposity, which can lead to metabolic imbalance. Like GC, chronically elevated ghrelin levels also result in increased food intake, weight gain and adiposity. These data suggest that the effects of chronic GC could be mediated by ghrelin. The purpose of this study was to determine if the ghrelin receptor (GHSR) was required for the metabolic and behavioural effects of increased corticosterone (CORT). To do this, female WT and GHSR KO mice were treated with either CORT in a 1% ethanol (EtOH) solution or 1% EtOH alone in their drinking water for five weeks. Daily measures included food and water intake as well as estrous cycle testing. Behavioural testing was done after four weeks of CORT treatment to determine changes in coping behaviors due to chronic CORT. Metabolic data includes weight gain, glucose tolerance, adiposity, and various hormone levels (ghrelin, LEAP-2, leptin etc.) to establish endocrine profiles. Behaviour testing included the forced swim test, social interaction test, open field test, and sucrose preference test. Results showed that CORT treatment resulted in increased food intake, weight gain and adiposity, with no differences observed between GHSR KO and WT mice. CORT treatment also impaired glucose clearance independent of genotype. Both GHSR KO and WT CORT-treated mice experienced disruptions of the reproductive system in the form of

suppression of regular cycling. Behaviourally, CORT treatment resulted in increased locomotion and time in the perimeter of the open field arena in WT CORT-treated mice, and increased time in the center of the arena in GHSR KO-CORT treated mice, potentially reflecting changes in coping behaviors in GHSR KO CORT-treated mice compared to WT CORT-treated mice. CORT treatment also resulted in decreased sucrose preference independent of genotype. Overall, these data demonstrate that the metabolic effects of CORT are largely independent of GHSR signalling in female mice.

Disclosures: Z. Silver: None. **S. Abbott-Tate:** None. **L. Hyland:** None. **F. Sherratt:** None. **B. Woodside:** None. **A. Abizaid:** None.

Digital Abstract Session

P238. Neuroendocrine Anatomy and Physiology

Program #/Poster #: P238.03

Topic: F.03. Neuroendocrine Processes

Support: OSU-CHS intramural award (KSC)
OCAST HR18-089 (KSC)

Title: Effects of ovariectomy on body weight, metabolic hormones and neuroreceptors in rats

Authors: *K. BURCH, K. CURTIS, K. MCCRACKEN, R. DAVIS, D. BUCK;
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Abstract: Obesity effects over 40% of American adults, and particularly concerning is the greater incidence of obesity in women, especially after menopause. This specific area of health issues has not been greatly studied. We model post-menopausal weight gain in ovariectomized (OVX) rats and recently reported that post-OVX weight gain is accompanied by changes in neuroimmune signaling in central nervous system (CNS) regions that respond to metabolic hormones including leptin, ghrelin and insulin. In this study, 90 day old female Sprague-Dawley rats were divided into groups of OVX (n=24) or sham OVX (aka control, n=24). Rats were housed individually in plexiglass cages on a 12:12 dark:light cycle and given ad libitum access to standard laboratory chow and water throughout the experiment. After acclimation to the colony room, rats were bilaterally OVX or sham OVX and body weights were recorded daily for 5 days postoperatively. One subgroup of rats (OVX: n=8, sham: n=8), was sacrificed on day 5. Remaining subgroups of rats were weighed weekly thereafter and sacrificed at 33 (OVX: n=8, sham: n=8) or 54 (OVX: n=8, sham: n=8) days post-operatively. At termination, body weight was determined and brains and plasma were collected. Brain punches were obtained from the arcuate nucleus (ARC), dorsal vagal complex (DVC), and paraventricular nucleus (PVN) and then homogenized. ELISA kits were used to measure leptin, ghrelin and insulin receptor expression in these brain regions, in addition to circulating insulin, ghrelin and leptin levels. Body weight increased rapidly and progressively in OVX rats but not in sham OVX rats. Plasma leptin levels increased over time, especially in OVX rats, while plasma ghrelin and insulin levels

were increased more transiently. Insulin, ghrelin and leptin receptor levels in the ARC did not differ between OVX and sham OVX rats after an initial increase in leptin receptor levels. In the DVC, insulin receptor levels decreased over time in OVX rats and were significantly less than those in sham OVX rats by day 54; however, neither ghrelin or leptin receptor levels differed. In the PVN, all receptor levels were reduced in OVX rats by day 54, with the leptin receptor levels also less at day 33. These results do not demonstrate a clear relationship among body weight gain, circulating hormone levels, and hormone receptors in OVX rats. Nonetheless, an inverse relationship between circulating leptin and leptin receptor levels that occur with body weight gain in OVX rats was evident in the PVN. Moreover, the PVN showed the largest change in hormone receptor levels, suggesting it is an area of interest to understand hormone effects in the CNS and their role in body weight gain.

Disclosures: **K. Burch:** None. **R. Davis:** None. **K. Curtis:** None. **K. McCracken:** None. **D. Buck:** None.

Digital Abstract Session

P238. Neuroendocrine Anatomy and Physiology

Program #/Poster #: P238.04

Topic: F.03. Neuroendocrine Processes

Support: NIMH Grant: R01 MH086828
Training Program in Integrative Membrane Biology: T32GM008181-30

Title: Evidence for hippocampal contributions to neuroendocrine regulation

Authors: ***A. B. COLE**[1], S. M. THOMPSON[2];
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Abstract: The neuroendocrine stress response broadly and rapidly alters a number of physiologic processes in order to respond to an acute threat. Because of the powerful and wide-ranging effects of glucocorticoids, the hypothalamic-pituitary-adrenal (HPA) axis exerts strict control over glucocorticoid production to avoid the deleterious effects seen when levels become either too high, too low, or follow an abnormal time course. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is one of the most consistent biological findings in patients with major depression. The hippocampus has long been implicated as a critical regulator of the HPA axis, although the mechanisms and circuits are not known. The primary neuronal effector cells for the production of glucocorticoids are corticotropin-releasing factor positive (CRF+) neurons in the paraventricular nucleus of the hypothalamus (PVN). Lesions of the hippocampus or its projections to the PVN have been shown to result in an increase in HPA axis activation. The major output of the hippocampus is excitatory, whereas the physiology seems to indicate an inhibitory contribution to PVN function. One possible explanation is that intermediary brain regions are important in this regulation by switching the hippocampal output from excitatory to

inhibitory. The GABAergic inhibitory cells of the bed nucleus of the stria terminalis (BNST) are a likely candidate. In order to test this hypothesis, we used a combination of electrophysiology and optogenetics to isolate hippocampal contributions to PVN regulation. Cre-dependent expression of fluorophores allowed for cell-type specific patch-clamp recordings of GAD2+ inhibitory neurons in the BNST and CRF+ neurons in the PVN. Anatomical tracing in male and female mouse brain indicates that there are substantial axonal terminals projecting from the ventral hippocampus and subiculum to both the BNST and PVN. Optical stimulation of these hippocampal terminals resulted in both excitatory and inhibitory evoked responses in these two brain regions. In GAD2+ BNST cells, the vast majority ofa majority of cells (7/8) displayed monosynaptic excitation, followed by as well as delayed inhibition. In CRF+ PVN cells, although monosynaptic excitation was occasionally observed, cells displaying only inhibitory responses predominated (4/5). These results are consistent with a model in which hippocampal output drives disynaptic inhibition of CRF+ PVN cells via GABAergic cells in the BNST, thereby providing a circuit for hippocampal dependent negative feedback of the HPA axis. Future work will test the neuroendocrine and behavioral consequences of modulating these synapses *in vivo*.

Disclosures: A.B. Cole: None. **S.M. Thompson:** None.

Digital Abstract Session

P238. Neuroendocrine Anatomy and Physiology

Program #/Poster #: P238.05

Topic: F.03. Neuroendocrine Processes

Support: VA Merit Review Grant 1 I01 BX003757

Title: Alcoholism: cortisol and the course of neuroendocrine healing

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Abstract: Background: A lifetime alcohol dependence (AD) diagnosis applies to 20%-50% of patients in public and university general hospitals. Subtle early brain healing processes may involve changes in the neuro-endocrine stress system that can affect both cognition and decisions about treatment options. Method: To test whether neuro-endocrine healing occurs in frequent, heavy drinkers, this study 1) compared AD test subjects (n=16) with light drinking control subjects (n=15) at baseline and then 2) assessed the test subjects prospectively at three, and six months of supervised, disulfiram assisted abstinence. The subjects provided diurnal salivary cortisol samples on waking, waking +30 minutes, noon and 4 PM for each of the follow-up time points. Significance required alpha = 0.05. Results: Baseline test group cortisol means were significantly higher than control levels ($p < 0.04$ to <0.003) with a notable absence of the morning cortisol response. The Three Month average diurnal curve suggested an exaggerated

morning response ($p< 0.038$), while the Six Month average curve approximated the baseline control values ($p>0.05$). Conclusions: These data indicate that neuro-endocrine healing may take up to six months to reach its full potential. This suggests the possibility that 1) neuro-endocrine effects may impair complex cognition and decision making early in the healing course and 2) neuro-endocrine healing late in the course may account for the often unrealistic sense of well-being that occasions alcohol relapse. Focused treatment may serve to expedite healing and add to the likelihood of sustained remission.

Disclosures: T. Beresford: None. **P.J. Ronan:** None.

Digital Abstract Session

P239. Sexual Differentiation

Program #/Poster #: P239.01

Topic: F.03. Neuroendocrine Processes

Support: NSF GRFP awarded to LRC
GSU Brains & Behavior seed grant awarded to NGF

Title: Dna methylation of estrogen receptor alpha and its role in specifying sex differences in cell phenotype within the arcuate nucleus

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Abstract: Most mammals display sex-specific behaviors, such as mating, aggression and parental care, that are controlled by sexually dimorphic groups of neurons defined by their gene expression (*i.e.*, neurochemical phenotype). Within the arcuate nucleus of the hypothalamus (ARC), for example, females have more neurons that express estrogen receptor alpha (ERα;) and kisspeptin than do males. Little is known regarding how these sex differences develop, but our research suggests that epigenetic mechanisms play a role. We previously showed that expression of the epigenetic enzymes that place and remove DNA methyl marks is highest during the first week of life in the hypothalamus, and differs by sex (Cisternas et al., 2020). In this study, we manipulated DNA methylation or de-methylation during the perinatal critical period for sexual differentiation and examined its effects on sex differences in the ARC several weeks later. In the first study, a global DNA methylation inhibitor (zebularine) or 10% DMSO in saline (vehicle) was injected into the ventricles of male and female pups (n = 9-13 per group) on the first two days of life; animals were sacrificed at weaning and ERα; and kisspeptin labeling examined. Neonatal zebularine treatment abolished the sex difference in ERα; in the ARC by decreasing labeling in females to male-like levels. Kisspeptin labeling was also reduced in females after zebularine treatment, but a sex difference remained. To test the role of de-methylation in specification of ERα; cell type, we used intracerebroventricular injections of targeting or non-

targeting siRNA to knock-down expression of *Tet2* and *Tet3* in neonates (n = 6-9 per group). At weaning, ERα; labeling in the ARC of males was feminized (i.e., increased) to female-like levels. Thus, both DNA methylation and demethylation early in life shape sex differences in cell type in the hypothalamus. We are currently using pyrosequencing to map DNA methylation levels in the *Esr1* promoter region in male and female mice on the day of birth (when there are no sex differences in ERα; labeling) and during the juvenile period (after sex difference have appeared). Our findings shed light on the molecular mechanism(s) that establish sex differences in neurochemical phenotype in the brain.

Disclosures: L.R. Cortes: None. **C.D. Cisternas:** None. **D. Mason:** None. **I.N.K.V. Cabahug:** None. **A. Castillo-Ruiz:** None. **N.G. Forger:** None.

Digital Abstract Session

P239. Sexual Differentiation

Program #/Poster #: P239.02

Topic: F.03. Neuroendocrine Processes

Support: NIH OD026560
NIH HL114474
NIH DK120342

Title: "Four core genotypes" rats: comparing XX and XY rats with the same type of gonads to detect sex chromosome effects that cause sex differences in physiology and disease

Authors: *A. P. ARNOLD[1], X. CHEN[1], M. GRYZBOWSKI[3], L. LAZCARES[3], A. TAKIZAWA[3], L. VERGNES[2], C. B. WIESE[4], H. SKALETSKY[5], D. C. PAGE[5], K. REUE[2], M. R. DWINELL[3], A. M. GEURTS[3];
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Abstract: The mouse Four Core Genotypes (FCG) model has successfully allowed comparison of XX and XY mice with the same type of gonads, to determine if sex differences in mouse traits are caused by cell-autonomous effects of X and Y genes ("sex chromosome effects", SCEs). For example, among 30 brain regions shown by MRI to have different volumes in FCG mice, almost 45% showed differences caused SCEs (XX vs. XY), whereas others showed differences caused by gonadal hormones (Corre et al., 2016, PMID 25445841). We sought to produce an FCG-like model in rats, because many sex differences are studied in rat brain and behavior (and other traits), but measuring SCEs has not been possible in rats. A major issue is that rats have 10 or more *Sry* genes, with unknown functions beyond testis determination. We knocked out the testis-determining function on the Y chromosome using CRISPR, to produce XY^{Δ} gonadal female rats that breed easily, producing and XY^{Δ} and XX rats, both with functional ovaries, in the same litters. These rats are compared to measure the differential effects of XX vs. XY sex

chromosomes (SCEs) in rats with ovaries. Secondly, we integrated a BAC transgene (*Sry*-tg) encoding *Sry4A*, *Sry3C*, and *Sry1*, onto an autosome to cause testis development in XX rats lacking any other Y genes, showing for the first time that these 3 *Sry* genes are sufficient as a group to cause testis differentiation. XY(*Sry*-tg+) males, mated to WT XX females, produce XX(*Sry*-tg+) and XY(*Sry*-tg+) rats with testes, allowing comparison of XX and XY rats with testes. XX(*Sry*-tg+) rats have testes lacking sperm, as expected from studies in other mammals. XX and XY rats have similar anogenital distance in the first postnatal week if they have the same type of gonad, suggesting XX and XY levels of testosterone prenatally are comparable within each gonadal sex. Body weight at 7 weeks was higher in rats with testes than ovaries, as previously reported, but there was also greater body weight in XY than XX rats, an SCE. Adult XX and XY rats differed in fat storage in gonadal fat pads, but fat accumulation in subcutaneous fat pads was different in rats with ovaries vs. testes. Thus, we have created a novel rat model that allows easy breeding of XX and XY rats with the same type of gonads, which can be compared to investigate SCEs that cause sex differences in any rat phenotype.

Disclosures: **A.P. Arnold:** None. **X. Chen:** None. **M. Gryzbowski:** None. **L. Lazcares:** None. **A. Takizawa:** None. **L. Vergnes:** None. **H. Skaletsky:** None. **D.C. Page:** None. **K. Reue:** None. **M.R. Dwinell:** None. **A.M. Geurts:** None.

Digital Abstract Session

P240. Mechanisms Contributing To Stress Susceptibility, Emotionality and Antidepressant Treatment

Program #/Poster #: P240.01

Topic: G.05. Mood Disorders

Support: CIHR Project Grant PJT#165852
CAMH Discovery Fund
Campbell Family Mental Health Research Institute of CAMH

Title: Reduced Anterior Cingulate Cortex Volume Induced by Chronic Stress Correlates with Increased Behavioral Emotionality and Decreased Synaptic Puncta Density

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Abstract: Chronic stress is an influential risk factor for major depressive disorder (MDD) and chronic-stress based animal models have established that repeated stress exposure results in depressive-like behaviors. Corticolimbic brain regions such as the anterior cingulate cortex

(ACC), amygdala (AMY), nucleus accumbens (NAc) and hippocampus (HPC) have identified behavioral, cellular and molecular deficits following chronic stress exposure. In this study, magnetic resonance imaging (MRI) was used to assess brain region specific structural alterations associated with mice exposed to 2 and 5 weeks of chronic restraint stress (CRS). These structural changes were linked to behavioral and synaptic alterations. Brains were then scanned using ex-vivo MRI and synaptic changes in key brain regions showing volumetric changes were assessed using immunohistochemistry and puncta analysis for PSD95 (postsynaptic density protein 95) and VGLUT1 (vesicular glutamate transporter 1). We confirmed that CRS mice display anhedonia- and anxiety-like behaviors. Macrostructural changes in total brain volume showed that CRS mice exhibited reduced brain volume proportional to changes in depressive-like behavior ($r= -0.490$; $p=0.002$). A region-specific *a priori* analysis focusing on structural changes in key corticolimbic brain regions, identified a significant decrease in volume of the ACC in mice exposed to 2 and 5 weeks of CRS ($p<0.05$). This decrease in volume negatively correlated with depressive-like behavior ($r=-0.50$; $p=0.002$). Although no structural changes of the AMY, NAc and HPC were identified as a result of CRS, there was a significant negative correlation between volume of the AMY, and NAc with depressive-like behavior. Structural covariance network (SCN) analysis identified a progressive decrease in degree of the ACC and increase in the AMY following 2 and 5 weeks of CRS exposure. Microstructural changes in synaptic puncta density of PSD95 and VGLUT1 was not significantly altered in the ACC following CRS. However, ACC volume positively correlated with PSD95 puncta density ($r=0.35$, $p<0.05$), and negatively correlated with depressive-like behavior ($r=-0.36$, $p<0.05$). Analysis of the AMY identified significant increases in VGLUT1 puncta density that significantly correlated with depressive-like behavior ($r=0.404$, $p<0.05$) but not amygdala volume. Our results demonstrate that chronic stress effects on ACC volume and synaptic density are linked to the expression of depressive-like deficits. Our findings highlight key structural and morphological alterations in the ACC relevant to stress-related illness including mood and anxiety disorders.

Disclosures: K.A. Misquitta: None. **A. Miles:** None. **T.D. Prevot:** None. **J.K. Knoch:** None. **C. Fee:** None. **D.F. Newton:** None. **J. Ellegood:** None. **J.P. Lerch:** None. **E. Sibille:** None. **Y.S. Nikolova:** None. **M. Banasr:** None.

Digital Abstract Session

P240. Mechanisms Contributing To Stress Susceptibility, Emotionality and Antidepressant Treatment

Program #/Poster #: P240.02

Topic: G.05. Mood Disorders

Support: CAMH Discovery Feed Fund
Brain & Behavior Research Foundation (NARSAD)
Campbell Family Mental Health Research Institute of CAMH

Title: Chronic Stress Alters Complexity of Astrocyte Morphology In Mouse Prefrontal Cortex

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Abstract: There is growing evidence that suggests astroglia anomalies play a key role in the pathogenesis of mood disorders including major depressive disorder (MDD). Studies consistently report reduction of astroglia density and astroglial marker expression in the prefrontal cortex (PFC) of patients with MDD. Similar reductions have also been reported in mouse model of chronic stress. In rodents these reductions are also associated with alterations of neuronal morphology and atrophy. However, it is yet to be confirmed if chronic stress exposure also alters astroglia morphology and whether this is linked to the behavioral deficits associated with chronic stress. We used mice expressing GFP (green fluorescent protein) expressing mice under the GFAP (glial fibrillary acid protein) promotor were subjected to 7, 21 or 35 days of chronic restraint stress (CRS). Behavioral deficits induced by CRS were measured (coat state assessment, sucrose intake and PhenoTyper tests) at each time point and summarized as a Z-emotionality score. We used Sholl analysis to investigate changes PFC GFP- or GFAP-positive cells morphology following CRS. Specifically, we adapted the standard Sholl analysis to obtain a more comprehensive quantification of astrocyte complexity using a complexity index (CI). We confirmed that chronic stress increases in anxiety-like behavior at 7, 21 and 35 days of CRS and anhedonia-like behavior at 35 days. While standard Sholl analysis was not able to detect effects of CRS, the adapted analysis revealed a significant reduction in intersection number for distal radius steps following 21 and 35 days of CRS in both GFAP+ and GFP+ cells. GFP+ cells also showed an increase in proximal processes associated with stress. When considering overall morphology using the CI, we found that GFP+ cell morphology complexity was significantly reduced following 21 and 35 days of CRS. This CI of PFC GFP-cells significantly negatively correlated with anxiety-like ($r=-0.503$, $p<0.01$) but not anhedonia-like behavior ($r= 0.023$, $p>0.05$). Additionally, for both GFAP+ and GFP+ cells CI were trending towards a significant negative correlation with Z-Emotionality ($r=-0.330$, $p=0.065$; $r=-0.381$, $p=0.079$). Chronic stress exposure induces a progressive atrophy of cortical astroglia cells which may contribute to maladaptive neuroplastic changes associated with stress-induced behavioral deficits and stress-related disorders.

Disclosures: S.A. Codeluppi: None. **D. Chatterjee:** None. **T.D. Prevot:** None. **K. Misquitta:** None. **M. Banasr:** None. **E. Sibille:** None.

Digital Abstract Session

P240. Mechanisms Contributing To Stress Susceptibility, Emotionality and Antidepressant Treatment

Program #/Poster #: P240.03

Topic: G.05. Mood Disorders

Support: NIH Grant R01MH118237

Title: Effect of social defeat on the activity of medial amygdala subnuclei

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Abstract: Depression is among the most prevalent psychiatric disorders, and social stressors are a common factor in depression. The medial amygdala (MeA) regulates social behaviors and responses to social cues. There is evidence for reduced MeA volume and connectivity in depression, suggesting that altered MeA activity may regulate social symptoms of depression. Repeated social defeat stress induces long term changes consistent with depression and results in increased MeA immediate early gene expression. However, despite its strong connection with social behavior, the role of the MeA in depression and sensitivity to social stress is understudied. The goal of this study was to determine the effect of social defeat on MeA activity in rats. We hypothesized that repeated social defeat would increase the spontaneous firing rate of neurons in the MeA. 40 adult male Sprague Dawley rats underwent five days of social defeat with a retired breeder Long Evans rat using the resident-intruder paradigm or control procedures. Control rats were placed in a transport cage for the same amount of time. Within 10 days of social defeat or control procedures, we performed *in vivo* extracellular single-unit electrophysiology in anesthetized rats and recorded the firing rate of spontaneously active neurons in the posterodorsal (MeApd) and posteroventral (MeApv) nuclei of the MeA. There was no significant difference in overall MeA firing rate between social defeat and control groups, however we found evidence of an interaction of MeA subnucleus and social defeat on firing rate, such that socially defeated rats had a higher firing rate in the MeApd and lower firing rate in the MeApv (Control-MeApd: 1.1 ± 0.4/16 neurons, Stress-MeApd 2.2 ± 0.5/24 neurons, Control-MeApv 1.2 ± 0.5/17 neurons, Stress-MeApv 0.7 ± 0.2/14 neurons, data expressed as mean ± SEM). There was also a trend towards a positive association of the number of times the rat was attacked per day and the average firing rate of the MeA ($R^2 = 0.33$, n = 8 rats), suggesting a dose-dependent effect of the intensity of the stress on MeA firing. These results may indicate that the MeA is sensitive to prolonged social stressors and that social defeat may differentially affect the activity of MeApd and MeApv subnuclei.

Disclosures: A.C. Ritger: None. **M.K. Loh:** None. **N.C. Ferrara:** None. **J.A. Rosenkranz:** None.

Digital Abstract Session

P240. Mechanisms Contributing To Stress Susceptibility, Emotionality and Antidepressant Treatment

Program #/Poster #: P240.04

Topic: G.05. Mood Disorders

Support: NIMH grants MH093897 and MH105910

Title: Impact of stress and antidepressants on excitatory/inhibitory balance: Excitatory/inhibitory imbalance promotes stress susceptibility

Authors: *S. POTHULA, R.-J. LIU, A.-N. SLIBY, C. H. DUMAN, R. J. DILEONE, R. S. DUMAN;
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Abstract: Mounting evidence suggests an impaired excitatory/inhibitory (E/I) balance in major depressive disorder (MDD) with functional deficits in both glutamatergic and inhibitory transmission. Chronic psychosocial stressors are susceptibility factors for stress-induced behavioral and synaptic deficits in prefrontal cortex (PFC), a key region implicated in MDD. Further, antidepressants are known to reverse these deficits. However, the impact and underlying mechanisms of stress- and antidepressant-induced changes in glutamatergic and GABAergic systems, and whether E/I functional imbalance enhances susceptibility to stress still remains elusive. Here, we used patch clamp electrophysiology, biochemical and behavioral experiments using chronic unpredictable stress (CUS) paradigm to evaluate the functional impact of stress on glutamatergic and GABAergic transmission as well as effect of rapid antidepressants on expression of excitatory/inhibitory synaptic proteins in PFC. Our results demonstrate that CUS reduces both excitatory and inhibitory spontaneous postsynaptic currents in layer V pyramidal neurons which are reversed with the rapid antidepressant rapastinel. Also, we found that rapid antidepressants like ketamine and rapastinel increase both excitatory and inhibitory synaptic proteins in PFC, suggesting that antidepressants enhance plasticity in both excitatory and inhibitory systems. Further, we generated Sst-cre$^{GluN2Bfl/fl}$ mice by conditional deletion of NMDAR-GluN2B subunits specifically from Sst-interneurons, which mimics stress-induced reduction in GABAergic input to excitatory neurons, and evaluated if these mice are susceptible to stress. Our results confirm both a reduction in inhibitory input to layer V pyramidal neurons and behavioral deficits in Sst-cre$^{GluN2Bfl/fl}$, but not WT, mice after sub-chronic stressors, demonstrating that E/I imbalance promotes susceptibility to stress.

Disclosures: S. Pothula: None. **R. Liu:** None. **A. Sliby:** None. **C.H. Duman:** None. **R.J. DiLeone:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIMH (MH093897). **R.S. Duman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; MH093897 and MH105910; NIMH.

Digital Abstract Session

P240. Mechanisms Contributing To Stress Susceptibility, Emotionality and Antidepressant Treatment

Program #/Poster #: P240.05

Topic: G.05. Mood Disorders

Support: 5R01MH111918
5T32MH015174

Title: Deconstructing the neural circuitry of stress susceptibility and resiliency in mice

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Abstract: Depression ranks amongst the most disruptive disorders worldwide. Treatment after diagnosis can successful but prevention before depression onset holds an even greater potential to reduce suffering and treatment cost. Depression often arises following a stressful event but not all individuals are susceptible. Even in the presence of severe stressors, some display natural stress-resilience. Therefore, to inform prevention we must first understand of what mediates stress-susceptibility and resiliency.

It is well established that stress alters neural circuitry in susceptible rodents but it is unknown if neural circuity is different in susceptible or resilient rodents prior to stress. Our research aims to uncover and understand the individual variability in circuits of affect. We hypothesize that divergent stress-responses result from exacerbation of individual variability in functional and structural connectivity that exists prior to stress-exposure.

A commonly used depression model is chronic social defeat stress (CSDS). CSDS is an etologically relevant and highly validated paradigm to study depressive-like behavior in rodents. We developed an acute social defeat stress (ASDS) model as a tool to probe for preexisting differences in stress responses. Importantly, we expanded this paradigm to include females. ASDS produces behavioral responses that are predictors of later resilience or susceptibility in stress-naïve animals. To uncover if preexisting differences exist in the brains of susceptible and resilient animals, the immediate early gene cFos was analyzed following ASDS. Preliminary data uncovered divergent connectivity between the control, susceptible, and resilient mice: the basolateral amygdala (BLA) emerges as a critical node, while the prelimbic area (PL) to BLA emerges as a critical pathway selectively active in susceptible mice.

To further determine if depressive-like behavior in individuals that are susceptible to CSDS are due to preexisting differences in connectivity that can be seen in ASDS and that this susceptibility circuit becomes uniquely strengthened during stress-activation, we are utilizing the TRAP2/Ai14 mice. The acute response as the probe for preexisting differences will be visualized by trapped dTomato in neurons activated by an initial stress and the chronic response as the probe for long-term outcome will be visualized by cFos. These experiments will establish a framework for the investigation of preexisting variability in neural circuits leading to divergent stress responses.

Disclosures: K.R. Anderson: None. **D. Dumitriu:** None.

Digital Abstract Session

P240. Mechanisms Contributing To Stress Susceptibility, Emotionality and Antidepressant Treatment

Program #/Poster #: P240.06

Topic: G.05. Mood Disorders

Support: NIMH Grant P50MH096890
NIMH Grant R01MH051399
NIH Grant R01MH051399
NIH Grant DA042100
NIH Grant T32MH087004
Hope for Depression Research Foundation (HDRF)

Title: Sperm long noncoding RNAs are associated with paternal transmission of stress phenotypes

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Abstract: Depression risk has long been known to be highly influenced by both genetic and environmental factors. More recently, it has been proposed that epigenetic mechanisms may also contribute, representing a third basis of risk. Paternal stress can induce long-lasting changes in germ cells potentially propagating heritable changes across generations. To date, no studies have investigated differences in transmission patterns between stress-resilient and susceptible animals. We tested the hypothesis that transcriptional alterations in sperm during chronic social defeat stress (CSDS) transmit increased susceptibility to stress phenotypes to the next generation. We demonstrate differences in offspring from stressed fathers that depend upon paternal category (resilient vs susceptible) and offspring sex. Importantly, artificial insemination reveals that sperm mediates some of the behavioral phenotypes seen in offspring. Using RNA-sequencing we report substantial and distinct changes in the transcriptomic profiles of sperm following CSDS in susceptible vs resilient fathers, with alterations in long non-coding RNAs (lncRNAs) predominating especially in susceptibility. Using co-expression network analysis, we identify key transcriptional modules and a susceptible-specific lncRNA hub gene, Gm27211. Together, these studies advance our understanding of transgenerational epigenetic transmission of behavioral experience.
Funding sources: National Institution of Mental Health (P50MH096890 and R01MH051399); National Institutes of Health (R01MH051399, DA042100, and T32MH087004); Hope for Depression Research Foundation

Disclosures: **A.M. Cunningham:** None. **D.M. Walker:** None. **A. Ramakrishnan:** None. **M.A. Doyle:** None. **R.C. Bagot:** None. **H.M. Cates:** None. **C.J. Peña:** None. **O. Issler:** None. **C. Lardner:** None. **C. Browne:** None. **S.J. Russo:** None. **L. Shen:** None. **E.J. Nestler:** None.

Digital Abstract Session

P240. Mechanisms Contributing To Stress Susceptibility, Emotionality and Antidepressant Treatment

Program #/Poster #: P240.07

Topic: G.05. Mood Disorders

Support: Brain and Behavior Research Foundation (Grant No. 570769) to EAH

Title: Targeted neuroepigenetic editing regulates stress in a sex-specific manner

Authors: ***A. S. SASE**[1], B. A. SANTHUMAYOR[1], S. I. LOMBROSO[1], K. S. CZARNECKI[1], M. N. DITTRICH[1], E. A. HELLER[2];
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Abstract: Major depressive and posttraumatic stress disorder (MDD and PTSD) share stress as an etiological contributor. The vast majority of preclinical studies to elucidate gene regulatory underpinnings of these disorders are limited to male rodents. Yet, the human epidemiological and neurobiological studies report female-specific stress responsivity. Over the past several decades, it has become clear that gene expression changes conferred by epigenetic modifications underlie mood regulation and resilience to environmental stressors. This has led to a growing interest in the sex-specific molecular and epigenetic mechanisms underlying these diseases. Cyclin-dependent kinase 5 (Cdk5) is known to regulate such behaviors and the magnitude of these behaviors. Although there is much evidence on the function of the Cdk5 protein, very little is known about the sex-specific epigenetic regulation of *Cdk5* expression in stress-related disorders. Recently we discovered that targeted *Cdk5* histone acetylation is sufficient to reduce fear memory only in female, while *Cdk5* deacetylation decreased fear memory only in male mice. We hypothesized that histone modification(s) at the *Cdk5* promoter is sufficient to regulate its expression and influence sexually-dimorphic stress response in MDD and PTSD. We used chronic unpredictable mild stress (CUMS) to study stress response in mice, as it is a robust translational paradigm used successfully to elucidate molecular mechanisms of MDD and PTSD. Followed by multiple behavioral tests to measure anxiety, anhedonia, and depression-like behavior. We then analyzed *Cdk5* mRNA expression and histone modifications by chromatin immunoprecipitation at *Cdk5* promoter in male and female mice. Next, we used CRISPR mediated epigenetic editing at the *Cdk5* promoter paired with quantification of the stress response, expression of *Cdk5*, and enrichment of histone modifications at the *Cdk5* promoter. We found sex-specific stress responses to 14, 21 and 28 days of CUS. We also found that male, but not female, mice activated *Cdk5* expression in response to 14 days of CUS and that this

activation was specific to the nucleus accumbens. CRISPR mediated *Cdk5* activation was sufficient to induce stress response after 7 days in both male and female mice in the absence of stressors. This stress response persisted after 14 days of CRISPR mediated *Cdk5* activation. In conclusion, our work provides a model of stress evoked sex-specific chromatin remodeling at the C*dk5* promoter and reveals *Cdk5* transcriptional regulation's causal relevance to the stress response.

Disclosures: A.S. Sase: None. **B.A. Santhumayor:** None. **S.I. Lombroso:** None. **K.S. Czarnecki:** None. **M.N. Dittrich:** None. **E.A. Heller:** None.

Digital Abstract Session

P240. Mechanisms Contributing To Stress Susceptibility, Emotionality and Antidepressant Treatment

Program #/Poster #: P240.08

Topic: G.05. Mood Disorders

Support: NIMH
HDRF

Title: Blood microRNAs as a biomarker for stress susceptibility or resiliency and treatment response in major depressive disorder

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Abstract: Major depressive disorder (MDD) is an episodic form of mental illness that is characterized by mood disturbances, anhedonia, and alterations in cognitive function. There is an urgent need for objective biomarkers for diagnosing depression, assigning treatment, and assessment of treatment response. MicroRNAs (miRNAs) are small noncoding RNA molecules, which can be detected in body fluids and have emerged as potential biomarkers of disease conditions, including depression. These molecules act as potent epigenetic post-transcriptional regulators of gene expression. Interestingly, miRNAs can be detected in the circulation and evidence suggests correlation between specific circulating miRNAs levels and several disease states. The present study explored the potential use of miRNAs as biomarkers for MDD and for prediction and assessment of treatment response. We profiled the expression levels of approximately 600 circulating blood miRNAs from mice that was collected before and after exposure to chronic social defeat stress (CSDS), as well as after either repeated imipramine or single-dose ketamine treatment. We observed robust differences in blood miRNA signatures between resilient and susceptible mice after an incubation period but not immediately after exposure to stress. Furthermore, treatment with ketamine, but not imipramine, re-established

baseline miRNA expression levels in mice that responded to the drug, but not in non-responders. Analysis of candidate miRNAs in human blood samples validated a subset identified in mice as candidate biomarkers to aid depression diagnosis and predict ketamine treatment response. Lastly, we demonstrate that systemic manipulation of one of these validated miRNA targets is sufficient to reduce the depression-related phenotype in susceptible mice after stress, without an appreciable effect in resilient or control mice. Taken together, this study enhances our understanding of epigenetic changes in response to stress and identifies candidate miRNAs that warrant further investigation as biomarkers for depression treatment response.

Disclosures: **Y.Y. Van Der Zee:** None. **O. Issler:** None. **C.K. Lardner:** None. **D.M. Walker:** None. **A. Torres-Berrío:** None. **A. Ramakrishnan:** None. **E.M. Parise:** None. **A.M. Cunningham:** None. **B. Rutten:** None. **J.W. Murrough:** None. **E.J. Nestler:** None.

Digital Abstract Session

P240. Mechanisms Contributing To Stress Susceptibility, Emotionality and Antidepressant Treatment

Program #/Poster #: P240.09

Topic: G.05. Mood Disorders

Support: NIH grant R01MH086828 (SMT)

Title: 5ht2a receptors are not required for the anti-anhedonic and electrophysiological actions of psilocybin in chronically stressed mice: implications for antidepressant treatment

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Abstract: Major depression is a devastating psychiatric illness affecting millions of individuals worldwide. Current first-line pharmacologic treatments, the selective serotonin reuptake inhibitors, are only about 60% effective and take 4-6 weeks before clinical effects are reported. Fast-acting and more effective treatments are sorely needed. Recent studies have shown that psilocybin has rapid and enduring antidepressant effects in human depression. It is believed that the serotonin 2A (5HT-2A) receptor is required for this antidepressant action because they are known to mediate the mind-altering effects of psilocybin. However, the mechanism of action has not been tested. We tested the hypothesis that psilocybin has rapid-acting antidepressant effects in a stress-based mouse model of anhedonia and tested the role of 5HT-2ARs pharmacologically. Chronic multimodal stress in male mice caused anhedonic responses in the sucrose preference and female urine sniff tests. Anhedonic mice were pretreated with saline or ketanserin, a 5HT2A/C antagonist, 60 minutes prior to receiving psilocybin or saline (4 groups, 2 cohorts). Hedonic behavior was reassessed 24-48 hours after treatment. Preferences for sucrose and female urine recovered to prestress values in psilocybin treated mice, regardless of pretreatment, but not in vehicle controls. Additionally, electrophysiological measurements of the stress-

sensitive hippocampal TA-CA1 synapse after the final hedonic assessment revealed that psilocybin-treated mice had higher AMPA:NMDA ratio, indicative of greater synaptic strength, whether pretreated with ketanserin or saline. We ensured the efficacy of ketanserin by measuring head-twitches and psilocybin-induced decrease in hippocampal low-frequency oscillations, both of which were inhibited by ketanserin pretreatment. We have demonstrated that psilocybin has rapid-acting antidepressant-like effects in a stress-based mouse model of anhedonia, facilitating study of psilocybin's mechanism of antidepressant action. We conclude that the anti-anhedonic effects of psilocybin are independent of the 5HT-2A receptors, suggesting that altered perception may not be required for psilocybin's antidepressant effects. We further suggest that a 5-HT2AR-independent restoration of synaptic strength in cortico-mesolimbic reward circuits may contribute to its antidepressant action. The possibility of combining psychedelic compounds and a 5-HT2AR antagonist offers a potential means to increase their acceptance and clinical utility, although human studies are required.

Disclosures: **N. Hesselgrave:** None. **T. Troppoli:** None. **A.B. Wulff:** None. **A. Cole:** None. **S.M. Thompson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent pending.

Digital Abstract Session

P240. Mechanisms Contributing To Stress Susceptibility, Emotionality and Antidepressant Treatment

Program #/Poster #: P240.11

Topic: G.05. Mood Disorders

Support: Hope for Depression Research Foundation
NIH (R01MH104261)
ONR (N00014-12-1-0366)
NIDA (U01DA043098)

Title: Implicating the Hippocampal Glycome in the Regulation of Emotional Behavior

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Abstract: The Heparan Sulfate Proteoglycan (HSPG) family is a major component of the glycome and key players in the brain extracellular matrix, mediating neuronal migration, synaptic maturation and the activity of multiple growth factors and morphogens. However, their roles in temperament and emotional reactivity remains unexplored. In this study we use a genetic rat model of temperamental tendencies to address the role of the glycome in emotionality. The genetically bred High Responder (bHR) and Low Responder (bLR) rat lines are models for externalizing and internalizing mood disorders, and provide an insight into how the environment

and genetic predisposition for emotionality can intersect. Recent meta-analysis showed that expression of HSPG genes differs between bHR and bLR hippocampus. In situ hybridization was used to assess the anatomical specificity of this expression difference for two HSPGs, Syndecan-4 (Sdc4) and Glypican-1 (Gpc1), and whether P1 FGF2, adolescent EE (P35-P60) and/or social defeat stress (P60-P64) impacted their expression. Bilateral heparinase-1,3 administered to the hippocampus of adult rats (P75) determined whether neural HSPGs directly influence behaviour, and cell culture was used to directly investigate how heparinase-1,3 impacts cellular signalling. All animals used in these studies were male, to match the social defeat stress animals.Endogenous Gpc1 and Sdc4 are differentially expressed in the hippocampus and NAcc of animals with genetic differences in emotionality. These HSPGs also respond differentially to environmental manipulations, suggesting that they may play a role in affective behaviors. Gpc1 is responsive to P1 FGF2, adolescent EE and social defeat stress while Sdc4 is responsive only to P1 FGF2, suggesting that the brain glycome should not be considered as a single, uniform structure. Dissolving hippocampal HSPGs in vivo exacerbates anxiety-like behavior in the more vulnerable bLR line, and in vitro dissolution of HSPG sidechains decreases cellular responsiveness to FGF2. Whether the increased anxiety in vivo is due to the decreased effectiveness of endogenous FGF2 remains to be determined.Our work shows that breeding for temperament alters the hippocampal glycome, likely leading to differences in growth factor and morphogen signaling efficacy in this brain region. We also show that the hippocampal glycome is responsive to environmental and chemical interventions. This is the first demonstration of HSPGs both regulating emotional behaviours. The HSPG system offers a promising therapeutic target to fine-tune growth factor and morphogen signaling in mood disorders and other neurological conditions.

Disclosures: **A.M. O'Connor:** None. **L.A. Dokas:** None. **T. Pardo-Garcia:** None. **E.K. Hebda-Bauer:** None. **P. Maras:** None. **K.E. Prater:** None. **M.H. Hagenauer:** None. **S.J. Watson:** None. **H. Akil:** None.

Digital Abstract Session

P241. Stress and Neuroimmunology

Program #/Poster #: P241.01

Topic: F.04. Stress and the Brain

Support: Indian Council of Medical Research, Government of India

Title: Basolateral amygdalar inactivation promotes resilience to chronic immobilization stress through microglial modulation in the prefrontal cortex

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Abstract: Stress is a risk factor in the etiology of affective disorders and cognitive deficits. Both, stress-induced affective symptoms and cognitive deficits are associated with aberrant changes in microglial structure and function. Additionally, stress differentially modulates the functioning of prefrontal cortex (PFC) and basolateral amygdala (BLA). Chronic stress causes hyperactivation of BLA leading to enhanced functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Contrastingly, PFC undergoes degeneration following stress, which leads to impaired negative feedback to the HPA axis. The BLA and PFC have intense reciprocal connections, which could modulate stress outcomes. Recently, we showed that the silencing of BLA promotes resilience to stress via modulation of corticosterone levels, glucocorticoid receptors, and volumes of PFC; however, effects of BLA silencing on stress-induced alterations in affective behaviors, cognitive performance, and associated changes in cortical microglia cells are relatively unknown. Thus, we hypothesized that BLA inactivation during chronic-immobilization-stress (CIS) might preclude affective symptoms, impaired social interaction, cognitive deficits, and associated microglial anomalies in the PFC. To address this, adult male Wistar rats were subjected to BLA inactivation by infusion of lidocaine during each session of CIS for 10 consecutive days. Then, rats were subjected to assessment of depressive-, anxiety-like behaviors, social interaction, and cognitive test. Thereafter, rats were sacrificed and Iba-1^{+} microglial cells were localized using immunohistochemistry. Microglial expression was stereologically quantified, and their processes were reconstructed in prelimbic and anterior cingulate subregions of PFC. We demonstrate that inactivation of BLA precludes CIS-induced depressive- and anxiety-like behaviors, impaired social interactions, and cognitive deficits. Interestingly, BLA inactivation-associated cognitive remission to depression and anxiety was accompanied by the prevention of microgliosis in the prelimbic and anterior cingulate cortex. Further, BLA inactivation was sufficient to preclude CIS-induced hyper-ramification of microglial cells in the PFC. Our results indicate that BLA inactivation might prevent heightened immune-mediated microglial response in the PFC, which in-turn could promote resilience to chronic stress. We propose that combating BLA hyperactivity during stress might be an effective therapeutic approach to limit microglial/immune-associated dysfunctions in the PFC.

Disclosures: S.J. Tripathi: None. **S. Chakraborty:** None. **B.S. Shankaranarayana Rao:** None.

Digital Abstract Session

P241. Stress and Neuroimmunology

Program #/Poster #: P241.02

Topic: F.04. Stress and the Brain

Title: Cumulative effects of early life adversity on prefrontal cortex microglia morphology and perineuronal net integrity: A role for sex-dependent neuroimmune priming

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Abstract: Early life is rife with sensitive periods during which aberrant experience can have lasting effects on plasticity and behavioral development. Microglia, the main immunocompetent cells in the brain, play an important role in synaptic development and can become chronically sensitized following an initial insult, priming them for over-activation. Here, we aimed to determine whether early life adversity increases vulnerability to a secondary hit later in development in a sex-dependent manner. First, male and female rat pups underwent maternal separation (MS) for 4 hours per day from postnatal day (P) 2-20 (or control rearing) and were exposed to the endotoxin lipopolysaccharide (LPS) in juvenility or adolescence. We observed that both MS and LPS administration affected morphological characteristics of Iba1+ microglia in the prefrontal cortex (PFC) of juvenile males and females, with MS exposure leading to a greater increase in soma size following LPS in females. Evidence also suggests that microglia influence extracellular development and degradation. Formation of perineuronal nets (PNNs) - specialized extracellular structures that preferentially enwrap fast-spiking parvalbumin (PV)-expressing interneurons - is essential for proper neurodevelopment. It is unknown, however, whether multiple adverse experiences during sensitive developmental periods has lasting effects on PNN and PV expression and whether this differs by sex. In a separate cohort, pups were exposed to MS (or control rearing), followed by juvenile social isolation (SI) from P21-35 (or standard pair-housing). Anxiety-like behavior was measured in early adulthood using the elevated zero maze. In adulthood, MS paired with juvenile SI resulted in decreased structural integrity of PNNs surrounding PV cells and reduced PV cell count, in females only. This potentiated effect of SI on MS-exposed females altered the relationship between PV+ PNN intensity and anxiety-like behavior. To determine whether the observed sex-dependent effects may be influenced by neuroimmune priming during MS, we conducted qRT-PCR at P9 to quantify levels of gene expression of high mobility group box 1 (HMGB1), a damage-associated molecular pattern that is upregulated during sensitization to stress. We present evidence that multiple hits of adversity influence neuroimmune and neurostructural development in a sex-dependent manner that may impact later-life behavioral outcomes. Moreover, the current work helps lay the foundation for mechanistic investigation of sex-specific changes in plasticity, which will further our understanding of neuropsychiatric sequelae and potential preventative therapies.

Disclosures: K.R. Gildawie: None. **L.M. Ryll:** None. **R. Orso:** None. **J.C. Hexter:** None. **S. Peterzell:** None. **A.A. Valentine:** None. **H.C. Brenhouse:** None.

Digital Abstract Session

P241. Stress and Neuroimmunology

Program #/Poster #: P241.03

Topic: F.04. Stress and the Brain

Support: NIH - 1R01MH123545-01
NIH - 1F32MH123051-01

Title: Microglial P2RY12 mediates stress-induced synapse loss in the prefrontal cortex and associated cognitive-behavioral deficits.

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Abstract: Recent studies in our lab demonstrate that chronic unpredictable stress (CUS) drives microglia-mediated neuronal remodeling, which contributes to synapse loss in the prefrontal cortex (PFC) and associated cognitive and behavioral deficits. Despite this, it remains unclear what mechanisms regulate neuron-microglia interactions in chronic stress. Other work indicates that neuronal activity-dependent purinergic signaling directs microglial processes via the purinergic receptor, P2RY12, which is exclusively expressed by microglia in the brain. Thus, microglial P2RY12 may play an important role in regulating stress effects on synaptic structure and function in the PFC. To investigate this, we used genetic (*P2ry12-/-*) or pharmacological (clopidogrel, 50 mg/kg, i.p. daily) approaches to block P2RY12 signaling in the context of CUS. Various behavioral, cytometric, and molecular endpoints were analyzed. Our results showed that both P2RY12-deletion and treatment with clopidogrel prevented CUS-induced increases in forced swim test immobility and attenuated discrimination deficits in temporal object recognition. In separate studies, immunohistology and flow cytometry revealed that genetic ablation of P2RY12 or clopidogrel significantly reduced P2RY12 expression in frontal cortex microglia. Further analyses showed that P2RY12-deletion or treatment with clopidogrel altered other markers (CX3CR1 and CD115) on frontal cortex microglia, independent of CUS exposure. Confocal imaging in Thy1-GFP(M) mice showed that clopidogrel blocked CUS-induced increases in the number of neuronal inclusions per microglia and prevented dendritic spine loss in the medial PFC, suggesting that P2RY12 regulates microglia-mediated neuronal remodeling. Together, these findings indicate that microglial P2RY12 mediates stress-induced dendritic remodeling in the medial PFC and subsequent behavioral deficits.

Disclosures: J.L. Bollinger: None. **D.T. Dadosky:** None. **I.L. Rainer:** None. **S.C. Woodburn:** None. **E.S. Wohleb:** None.

Digital Abstract Session

P241. Stress and Neuroimmunology

Program #/Poster #: P241.04

Topic: F.04. Stress and the Brain

Support: MH049698
MH107487

Title: Molecular neurobiology of enrichment loss: multi-omics analysis reveals a role of microglia

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Abstract: Background: Psychological loss impacts nearly all of our lives, yet little is known about what happens in the brain during this experience. Loss occurs when one is deprived of something perceived as important, such as a valued relationship, home, or job, and its symptomology resembles atypical depression. Given the common occurrence of loss-precipitating events, there is an urgent need to uncover the mechanisms underlying loss and identify potential therapeutic targets. Our laboratory simulates loss in rats by removing environmental enrichment, producing unique behavioral and physiological phenotypes that resemble loss in humans. Here we probe the molecular neurobiology of enrichment loss with a multidimensional approach that spans big data techniques, brain regions, and sex. Methods: Male and female rats were divided into 3 groups: environmentally enriched (EE), enrichment removed (ER), and control (CON). EE and ER animals were housed in groups of 10 in large, multi-level cages with toys. ER animals were removed from enriched housing after 4 weeks and moved to single-housing. Two weeks after removal, brains were collected. Bilateral micropunches were taken of regions implicated in loss by c-fos expression, including the infralimbic prefrontal cortex, basolateral amygdala (BLA), and medial amygdala. These regions were run on parallel RNAseq, shotgun proteomics, and kinomics platforms. A series of bioinformatics analyses were then used to derive molecular signatures of loss that span RNA, protein, and kinase activity levels. Results: Immune signaling, MAPK activity, and cell-matrix communication were consistently dysregulated in the BLA of ER males. Many of the genes driving these pathways were predominantly expressed in microglia, and these results were supported across RNA, protein, and kinase levels. Given that the BLA is a positive mediator of stress, impaired microglial regulation of neurons in this region could permit increased anxiogenic signaling, contributing to ER phenotypes. We have also identified signaling hubs that represent intriguing candidates for ameliorating ER phenotypes. Further analyses will continue to expand these signatures of enrichment loss across brain regions and sex. Conclusion: Taken together, these results allow us to start to understand what is happening in a rat's brain during enrichment loss, offering insight into the mechanisms that could underlie psychological loss in humans. They also point to potential therapeutic targets that may offer relief to people experiencing loss and have broader applications in depression.

Disclosures: M.A. Smail: None. **B.L. Smith:** None. **R. Shukla:** None. **J.K. Reigle:** None. **K. Alganem:** None. **J.P. Herman:** None. **R.E. McCullumsmith:** None.

Digital Abstract Session

P241. Stress and Neuroimmunology

Program #/Poster #: P241.05

Topic: F.04. Stress and the Brain

Support: VA Merit Award 1I01BX004712

Title: Calcineurin Inhibition Attenuates Stress-Induced Neuroinflammation

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Abstract: We have shown that immunosuppressants (ICV) that act through the inhibition of calcineurin (CLN) reduce alcohol intake in mice. Calcineurin is an abundant phosphatase in brain and plays a key role in the transcription of neuroinflammatory and stress signaling molecules. Ethanol use induces both neuroinflammatory responses as well as many stress-induced molecules associated with withdrawal effects such as corticotropin releasing factor (CRF). We have shown that stress-induced activation of CRF in the central nucleus of the amygdala (CeA) is attenuated by cyclosporine A (CsA). Stress also induces neuroinflammatory responses. Here we sought to determine the effects of CsA on stress-induced neuroinflammatory markers in brain. Rats (n=8) were given either CsA (30 mg/kg, I.P.) or vehicle, exposed to 30 minutes of restraint stress and returned to their home cage for 90 minutes. Brains were rapidly dissected and frozen in isopentane on dry ice. The CeA and PVN were microdissected from 300 um frozen sections and qRT-PCR was performed. Overall, Cyclosporine inhibited the expression of a wide range of neuroinflammatory markers in these regions including cytokines such as IL-2, IL-1β, IL-6, TNFα; markers of glial activation: CD45 and Iba-1; chemokine and chemoattractant molecules such as CCR2 and CCL2; as well as other inflammatory signaling molecules such as COX-2. Some of the largest effects were seen on IL-1 β and IL-6 expression in both CeA and PVN. While CsA inhibited the expression of CD45 and iba1 in the CeA, in the PVN these effects were striking. This suggests that stress rapidly induces glial activation in these regions which is inhibited by CsA. Together these data suggest that rapid, stress-induced neuroinflammatory signaling is attenuated by inhibition of CLN activity. This has implications for the treatment of multiple disorders in which stress is an etiological or maintenance factor such as PTSD and AUD.

Disclosures: P.J. Ronan: None. **T. Mehta:** None. **T.P. Beresford:** None.

Digital Abstract Session

P241. Stress and Neuroimmunology

Program #/Poster #: P241.06

Topic: F.04. Stress and the Brain

Support: NIMH Grant MH113892
NARSAD Young Investigator Award 26809

Title: Neuroinflammation in the Locus Coeruleus of Female Rats Enhances Anxiety-like Behaviors Induced by Social Stress

Authors: *S. J. BOUKNIGHT[1], B. S. POPE[2], G. MARTINEZ-MUNIZ[1], E. HARRINGTON[1], L. AUGENBLICK[1], S. K. WOOD[1];
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Abstract: Stress-mediated anxiety is a prevalent psychosocial phenomenon that is associated with neuroinflammation. It is well known that women are more susceptible to developing anxiety-related disorders, thus may be more vulnerable to neuroinflammatory effects, as well. As a defining feature of neuroinflammation, cytokine overproduction occurs during stress and may regulate associated anxiety-like behavior. This study focused on the locus coeruleus (LC) due to its dense noradrenergic neuron population and significant role in behavioral and physiological responses to stress. We previously reported that microglia depletion in the LC reduces anxiety-like burying in females exposed to social stress in the form of witnessing a social defeat encounter between two males. To further support the role of microglia in anxiogenesis, this study seeks to examine how activation of microglia in the LC via the endotoxin lipopolysaccharide (LPS) affects witness stress-evoked burying behavior. Young adult female Sprague-Dawley rats with a bilateral LC cannula received a local injection of LPS (1µL; 0µg, 1µg, or 3µg) into each LC (n=6 per group) over a 60-second period. Forty-five minutes post-injection, rats were briefly handled and returned to their home cage to be subjected to "control stress" and video recorded for 15 minutes. Immediately following the control period, each rat was exposed to witness stress for 15 minutes (video recorded), then euthanized for blood and brain collection. All videos were analyzed by two researchers blinded to subject treatment with inter-rater reliability within 10 percent. ELISAs were used to analyze levels of plasma corticosterone (CORT) and plasma interleukin (IL)-1β in duplicate. Intra-LC LPS injection produced no effect during control stress. However, there was a dose dependent increase in LPS-induced burying, with rats that received 3µg of LPS displaying the most anxiety-like burying and the quickest burying latency after the start of witness stress. Additionally, rats that received 3µg of LPS exhibited significantly elevated levels of CORT and tended to have higher levels of IL-1β in the plasma, both likely resulting from the LPS-induced neuroinflammation that primed the release of norepinephrine and elicited a greater anxiety-like response. Taken together, these data suggest that neuroinflammation driven by microglial activation in the LC exacerbates the anxiety-like behavior induced by social stress in female rats.

Disclosures: S.J. Bouknight: None. **B.S. Pope:** None. **G. Martinez-Muniz:** None. **E. Harrington:** None. **L. Augenblick:** None. **S.K. Wood:** None.

Digital Abstract Session

P241. Stress and Neuroimmunology

Program #/Poster #: P241.07

Topic: F.04. Stress and the Brain

Support: NIH grant GM133510
NIH grant DK090823

Title: Effects of estrogen on host gut microbial population and their metabolites in rats with chronic restraint stress

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Abstract: The brain-gut axis is a bidirectional communication system between the central nervous system and the gastrointestinal tract with diverse population of microorganisms, whose metabolites change constantly and feedback to the brain. Stress activating the hypothalamus-pituitary-adrenal (HPA) axis is known as an important factor for both mental and physical health. Both rodents and humans display sex-specific stress responses dependent on estrogen status. In this study, gut microbial population and their metabolites were characterized in rats with and without estrogen under control or stress conditions. Female Sprague Dawley rats received either sham operation thus having intact ovaries and normal estrogen levels (Sham) or ovariectomy (OVX) thus lack of endogenous estrogen. OVX rats received either oil (OVX+Oil) or estradiol (OVX+E2) replacement. Rats underwent either daily one-hour restraint stress or no-stress for 13 continuous days. Gut and trunk blood was collected on the last day. Plasma corticosterone (CORT) levels in tail blood were measured using ELISA. Stress significantly increased CORT levels of OVX+Oil rats during the entire stress period. In contrast, stress increased CORT levels of Sham and OVX+E2 rats at the beginning of the stress period, but such increase disappeared at the end of the stress period. Integrated 16S rDNA sequencing and LC-MS/MS based metabolic profiling were utilized to discover any significant differences in gut microbial genus and microbial metabolites. Beta diversity of microbial community was characterized by principal coordinates analysis (PCoA) that indicated the stress rats tended to form a cluster distinctly separated from the no-stress rats in both sham and OVX+E2 groups, but not OVX+Oil group. The microbial metabolic analysis revealed a similar attribute, with separation in major metabolic pathways between stress and no-stress rats of Sham rats and OVX+E2 rats, but not OVX+Oil rats. Therefore, when estrogen level was reduced as seen in OVX+Oil rats, the HPA axis stress response persisted, indicated by increased CORT levels throughout the stress period. However, a diminished stress response involving gut microbiota was observed, indicated by overlapping patterns of microbial community and similar microbial metabolite profiles between no-stress and stress OVX+Oil rats. Normal levels of estrogen seen in Sham and OVX+E2 groups relieved stress response involving the HPA axis but persisted the response involving gut microbiota. We conclude that estrogen may benefit brain-gut homeostasis, at least partially, via relieving prolonged stimulation of the HPA axis but maintaining microbial sensitivity to stress.

Disclosures: M. Xu: None. **H. Shi:** None. **K. Krolick:** None. **J. Zhu:** None.

Digital Abstract Session

P241. Stress and Neuroimmunology

Program #/Poster #: P241.08

Topic: F.04. Stress and the Brain

Support: NSERC RGPIN-05570-2014

Title: The effect of gut dysbiosis and probiotics on stress-induced neuroinflammation in pubertal male and female CD1 mice

Authors: *P. ESPOSITO, M. KEARNS, K. SMITH, N. ISMAIL;
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Abstract: Puberty is a critical period of development marked by sexual maturation and central nervous system (CNS) remodeling and reorganizing. These physiological and neurological pubertal changes render the CNS particularly sensitive to stressors. The gut microbiome plays a critical role in the development of the immune system and neuro-immune signaling. Exposure to the bacterial endotoxin lipopolysaccharide (LPS) during puberty induces changes in microbial composition that can result in permanent deficits in brain functioning and immune regulation in adulthood. Probiotic supplementation has been shown to influence immunomodulation and have positive effects on immune responsivity. However, it is unknown whether probiotic supplementation could reverse the effects of microbial dysbiosis on LPS-induced neuroinflammation. Therefore, the objective of the current exploratory research project was to examine whether the adverse effects of pubertal microbial dysbiosis on LPS-induced immune response can be mitigated by probiotic supplementation in male and female mice. A total of 120 (72 male and 48 female) mice were used in this experiment. At 5 weeks of age, male and female CD1 mice received either broad-spectrum antibiotics or sterile water through gavage, twice a day, for seven days. During the same period, mice received either a probiotic supplement or placebo in their drinking water. At 6 weeks of age (pubertal sensitive period), mice received either an intraperitoneal injection (i.p.) of LPS or saline. Eight hours following the injection, mice were euthanized and brains were collected. The hypothalamus and pre-frontal cortex (PFC) were extracted using micropunches and processed with a real-time quantitative PCR (RT-qPCR) to examine central cytokine mRNA expression (i.e. IL-1β, TNF- α, and IL-6). It is expected that antibiotic treatment will increase LPS-induced central cytokine mRNA expression in the PFC and hypothalamus. Additionally, probiotic supplementation is expected to mitigate these effects. The results from this experiment will further our understanding of how pubertal gut dysbiosis influences neuro-immune signaling following LPS exposure, and whether probiotics can be used to mitigate these effects.

Disclosures: P. Esposito: None. **M. Kearns:** None. **K. Smith:** None. **N. Ismail:** None.

Digital Abstract Session

P241. Stress and Neuroimmunology

Program #/Poster #: P241.09

Topic: F.04. Stress and the Brain

Support: Ontario Brain Institute

NSERC

Title: Factors influencing microbiota, brain and behaviour

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Abstract: Microbiota-brain communication influences behaviour and brain function. Evidence from both rodent studies and clinical studies demonstrate an association between microbiota and anxiety. Genetic and environmental factors contribute to the composition and function of the gut microbiota, it is important to determine the influence of these factors on host systems in order to better determine how microbiome-brain signaling pathways impact behaviour. Common mouse strains show distinct phenotypes related to anxiety-like behaviour. Growing evidence shows that differences in anxiety-related behaviours in mice are associated with altered gut microbiomes. Previously, our lab examined the microbiome in healthy male and female mice (C57Bl/6, Balb/C, FVB, CD1) and identified bacterial taxa that were influenced by strain and sex. Further, 77 taxa were associated with anxiety-like behaviour, including 10 taxa that were associated with anxiety-like behaviour across all strains tested. The current study extended this work to consider housing conditions and source/supplier on microbiota composition. First, inbred male and female (C57BL/6, Balb/C, FVB) and outbred (CD1) mice were bred in house or pregnant dams were ordered from outside supplier and fecal microbiota composition analyzed at postnatal day 24 (P24) by amplifying 16S rRNA gene and sequencing using the Illumina MiSeq platform. Data was analyzed using DADA2, and additional analysis conducted using R software. Second, C57BL/6 and FVB mice were ordered from an outside suppler at P22 and housed with the same strain or in a mixed strain condition. Fecal microbiota composition was analyzed at P23, P27, P30, and P37. Notably, at P37, differential abundance of 18 taxa (ASVs) was observed between C57Bl/6 and FVB housed with the same strain, yet only 1 taxa distinguished C57Bl/6 and FVB mice housed in the mixed strain condition. Further, the identified housing-related taxa were distinct from the anxiety-like behaviour taxa previously identified.

Disclosures: E. Tolsdorf: None. **B. Kwiecien-Delaney:** None. **J.A. Foster:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Ontario Brain Institute, NSERC. F. Consulting Fees (e.g., advisory boards); Novozymes A/S, NoRothmans, Benson & Hedges Inc..

Digital Abstract Session

P242. Effects of Stress On Cellular Function and Behavior

Program #/Poster #: P242.01

Topic: F.04. Stress and the Brain

Support: NSERC DG
CIHR-CRC

Title: Peripheral reelin has fast-acting antidepressant-like effects evaluated in the repeated-corticosterone paradigm of chronic stress

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Abstract: Chronic stress often precedes the onset of depression and can be modeled preclinically by subjecting rats to several weeks of daily corticosterone (CORT) injections. CORT-treated rats exhibit a depressive-like phenotype that is paralleled by decreases in hippocampal reelin, an extracellular matrix protein that regulates neuronal plasticity in the adult brain. We recently reported that intrahippocampal infusions of reelin had fast-acting antidepressant-like actions, rescuing CORT-induced despair-like behavior in the forced swim test (FST), neurogenesis, and imbalances in GABAergic and glutamatergic systems. In the present study, we aimed to evaluate whether peripheral intravenous administration of reelin would have a similar antidepressant-like effect. Rats received either daily vehicle or CORT injections for 21 days along with either vehicle or 3µg of reelin administered every 10 days over the CORT-injection period, or a single injection of reelin (either 3µg, 5µg, 7µg, or 9µg) on day 21. They were then subjected to the FST followed by post-mortem analyses of the number of reelin-, GABAAβ2/3-, and GluA1-positive cells. Peripheral reelin (repeated and singular infusions) rescued CORT-induced depressive-like behavior in the FST and decreases in reelin expression. As well, CORT-induced decreases in the number of GABAAβ2/3- and GluA1-positive cells were reversed by reelin. There were no apparent sex differences in response to reelin treatment. These novel findings show that peripheral reelin has antidepressant-like effects associated with the restoration of neurochemical deficits in the hippocampus. Although additional mechanistic and pharmacokinetic studies are necessary, our data open the possibility of developing reelin peptides with antidepressant activity.

Disclosures: J.T. Allen: None. **R. Romay-Tallon:** None. **J. Johnston:** None. **M.A. Mitchell:** None. **K.J. Brymer:** None. **G. Pinna:** None. **L.E. Kalynchuk:** None. **H.J. Caruncho:** None.

Digital Abstract Session

P242. Effects of Stress On Cellular Function and Behavior

Program #/Poster #: P242.02

Topic: F.04. Stress and the Brain

Support: NIEHS ES028202
NICHD HD097093
NIMH MH 104184
NIMH MH 108286

Title: Cellular mechanisms of allostasis involving mitochondria alter extracellular vesicle cargo to coordinate enduring effects of stress

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Abstract: Lifetime experiences including stress, trauma, and infection are associated with lasting effects that alter reproductive and embryo developmental outcomes. However, the cellular mechanisms contributing to these lasting post-exposure effects are not clear. For males, somatic cell reprogramming that involves the reproductive tract and germ cell maturation is an especially intriguing model to examine allostatic mechanisms. Sperm within the epididymis receive maturation signals, including growth factors and extracellular vesicles, from epididymal epithelial cells (EECs). In mice, we have previously shown that the content of these signals is altered following a chronic period of stress and that these changes are causal in post-conception neurodevelopmental outcomes. However, how EECs store past stress experiences at the molecular level is unclear. The glucocorticoid receptor (GR) is a key regulator of the stress response and is a known cellular target for orchestration of allostasis. To examine the hypothesis that stress-dependent cellular programming involves GR, we used EEC targeted reduction of GR and male stress to look at the passage of signals to sperm and offspring phenotype. Reduction of EEC GR in fact prevented the passage of post-stress signals and normalized the offspring brain development and physiological phenotype. Transcriptomically, genes differentially expressed between wildtype and GR^{Het} EEC accounted for two clusters of co-regulated genes related to metabolic processes and mitochondrial transport, and chromatin-modifying processes as defined by functional annotation analysis. Using an EEC cell culture model of stress and Seahorse XFe technology to assess the GR capacity to program basal mitochondrial function, prior stress hormone treatment profoundly decreased basal respiration. Transmission electron microscopy demonstrating condensed mitochondria after stress hormone treatment further confirmed the enduring impact of stress on mitochondrial state. Together these studies demonstrate GR's role in programming vital functions including baseline energy demand and production to regulate a sustained change in the cell's *basal* state, or homeostatic set point, in response to stress. These studies demonstrate GR's vital importance at the intersection between epigenetic and metabolic consequences of stress and explain how paternal stress or trauma experience can produce important changes in offspring development long after the experience occurred.

Disclosures: **N.R. Kanyuch:** None. **J. Chan:** None. **C. Morgan:** None. **C. Mannella:** None. **N. Yadava:** None. **T.L. Bale:** None.

Digital Abstract Session

P242. Effects of Stress On Cellular Function and Behavior

Program #/Poster #: P242.03

Topic: F.04. Stress and the Brain

Support: NIEHS ES028202
NICHD HD097093
NIMH MH104184
NIMH MH108286

Title: Cell-type specific labeling of epididymal extracellular vesicles with CD63-HA: listening in on the somatic to germ cell communication of life's lessons

Authors: *C. P. MORGAN[1], R. MARX-RATTNER[1], B. ALTAS[1], J. C. CHAN[2], M. KANE[3], T. L. BALE[1];
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Abstract: Extracellular vesicles (EVs) are a unique mode of intercellular communication capable of incredible specificity in transmitting signals involved in cellular function. One example of this is the essential role EVs secreted by epithelial cells lining the lumen of the reproductive tract play in the post-spermatogenic maturation of sperm. EVs released by epididymal epithelial cells (EECs) transport bioactive cargoes including small non-coding RNA (sncRNA), proteins and lipids that are required for sperm to develop essential properties, including the ability to swim and fuse with the ovum. We recently demonstrated that this fundamental process also plays a causal role in the somatic-to-germline transmission of information regarding paternal stress experience capable of altering fetal development in a preclinical mouse model. In this model, paternal preconception stress produces lasting histone and transcriptomic alterations in mouse EECs *in vivo*, with corresponding persistent changes in the miRNA, proteomic, and lipid composition of EVs secreted by immortalized DC2 EECs treated with corticosterone (cort) *in vitro*. Demonstrating causality, we showed that sperm incubated with EVs collected from these cort-treated DC2 EECs produce offspring with the same significant changes in neurodevelopment and adult stress reactivity observed in offspring of stressed males. While we would like to extend these results using EEC EVs collected *in vivo*, EV populations within the epididymal lumen originate from diverse cell types and are highly heterogeneous. To address this challenge, we have developed a transgenic construct that would allow for cell-type specific labeling of EVs via Cre-induced hemagglutinin (HA) tagging of the endogenous CD63 protein. CD63 is a tetraspanin protein enriched in EVs generated by diverse cell types in tissues throughout the body. Concurrent with our efforts to generate a transgenic mouse model, we are validating this construct in a stably transduced immortalized EEC cell line. We demonstrate that this transgene responds appropriately to Cre expression, resulting in HA-tagged EVs that do not differ from those produced by parallel cell lines naïve to Cre at the level of quantity produced, size distribution, and molecular composition. These studies present novel

and mechanistic insight into the somatic to germline transmission of environmental signals to maturing germ cells involved in altering fetal development. In addition, a CD63-HA transgenic mouse would be a valuable tool with applications in the study of EV function across biomedical fields.

Disclosures: C.P. Morgan: None. **R. Marx-Rattner:** None. **B. Altas:** None. **J.C. Chan:** None. **M. Kane:** None. **T.L. Bale:** None.

Digital Abstract Session

P243. Early-Life Stress Effects On Brain Function

Program #/Poster #: P243.01

Topic: F.04. Stress and the Brain

Title: Early life adversity leads to enhanced fear-potentiated startle in female mice

Authors: *C. DEMAESTRI, K. G. BATH;
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Abstract: Stress-related disorders such as generalized anxiety disorders, post-traumatic stress disorders, and panic disorders affect over a third of the population. Experiencing early life adversity (ELA) increases our lifetime risk of developing these disorders, and females show increased prevalence and severity of symptoms. Patients with stress-related disorders, and those who have experienced ELA, such as resource scarcity, exhibit increased fear-potentiated startle and increased acoustic startle responses with females having the potential for greater risk. Here, we test the impact of ELA on the evolutionarily conserved startle response and threat anticipation in a mouse model. Using the limited bedding model of ELA, we've found that, after tone-shock conditioning, mice who experience ELA exhibit enhanced startle to an intense white noise, both when presented in the presence and absence of the conditioned tone. This effect was only observed in females. Furthermore, we explored potential mechanistic changes in startle circuitry by testing for disturbances in corticotrophin releasing hormone expression patterns in the bed nucleus of the stria terminals and central amygdala. Disruption in the ability to accurately predict threat exclusively when it is present is critical for mental health and may lay the groundwork for sex-specific vulnerability to psychopathology. Understanding the mechanism by which disrupted threat-anticipation and enhanced startle occurs, and sex differences in risk following ELA, will be key in understanding behavioral disturbances in stress-related disorders and sex-specific vulnerabilities.

Disclosures: C. Demaestri: None. **K.G. Bath:** None.

Digital Abstract Session

P243. Early-Life Stress Effects On Brain Function

Program #/Poster #: P243.02

Topic: F.04. Stress and the Brain

Support: NMIH RO1 M115914
NMIH 5R01 M115049

Title: Early life adversity reveals sex-specific outcomes in rebound feeding in adolescent mice

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Abstract: Early life adversity (ELA) increases the risk for psychiatric disorders later in life, including anxiety, depression, and eating disorders. Eating disorders disproportionately affect young women and lead to a host of negative consequences, including metabolic disorder, adult obesity, and [maybe include one more]. ELA during critical developmental windows can alter the functioning of systems associated with stress and feeding, which may underlie an increased risk of developing disordered feeding patterns in adolescence. Using a resource-based mouse model of ELA, we tested the effects of ELA on adolescent feeding behavior after a period of starvation. To assess for changes in stress- and feeding-related systems, we measured plasma levels of leptin and corticosterone and hypothalamic mRNA expression of leptin receptor. We found that experiencing ELA resulted in hyperphagic "binge-like" feeding behavior on high-fat food in males, while both control and ELA-reared females showed an overall increase in "binge-like" behavior. Behavioral patterns were accompanied by higher basal corticosterone levels in males and increased leptin levels across groups. These results indicate that ELA has sex-specific effects on behaviors that increase the risk for developing eating disorders in adolescence that may be mediated through changes in the development of stress and and feeding-related systems. Understanding of the sex-specific effects of ELA on feeding behavior in adolescence as well as the mechanisms underlying this relationship is essential for treatment and prevention of pathological disordered eating.

Disclosures: M. Critz: None. **C. Demaestri:** None. **K.G. Bath:** None.

Digital Abstract Session

P243. Early-Life Stress Effects On Brain Function

Program #/Poster #: P243.03

Topic: F.04. Stress and the Brain

Support: NIEHS ES028202
NICHD HD097093
NIMH MH104184
MH108286

Title: Maternal Preconception Stress Produces Female Offspring-Specific Changes in Hypothalamic Extracellular Matrix Neurodevelopment

Authors: *K. MONTGOMERY, Y. CISSE, T. L. BALE;
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Abstract: Lifetime maternal exposure to chronic stress and trauma represents a significant risk factor for the development of neuropsychiatric disorders in their offspring; however, the exact mechanisms by which preconception experiences are transmitted to the next generation and influence neurodevelopment are not well understood. Our previous work established a novel mouse model of maternal preconception stress (MPS) where female, but not male, offspring of MPS dams have heightened stress reactivity in adulthood, a hallmark of many neuropsychiatric disorders. We hypothesized that MPS imparts sex-specific changes to the developing paraventricular nucleus of the hypothalamus (PVN), the master regulator of the hypothalamic-pituitary-adrenal (HPA) axis, that result in the observed elevated stress phenotype. To test this, we used our MPS model and performed PVN Sim-1 neuron-specific RNA sequencing in male and female postnatal day 21 offspring. Female offspring of MPS dams had over 2200 differentially expressed genes (DEGs) compared to female offspring from control dams. In contrast, male MPS offspring had only 63 DEGs compared to control males. Functional analysis of female DEGs revealed significant enrichment of genes involved in production and remodeling of the extracellular matrix. The brain's extracellular matrix undergoes considerable change during development, transitioning from a juvenile state to the adult state beginning in the second postnatal week. This transition coincides with the closure of plasticity windows in specific brain regions, though the majority of this work has been conducted solely in males and in non-hypothalamic brain areas. Dysregulation of either the developmental rate and/or composition of the extracellular matrix could impart lasting effects on the formation of PVN circuitry, including the organization of the HPA response, that persist into adulthood. We next determined that MPS enacts lasting effects on offspring PVN neurons by conducting RNA sequencing on adult MPS offspring. Female offspring again had the highest enrichment of DEGs related to the extracellular matrix. Taken together, these results suggest that MPS imparts enduring sex-specific changes to the formation and maintenance of neuronal extracellular matrix in the PVN. Ongoing studies will determine how dysregulation of extracellular matrix formation in MPS female offspring results from increased neuronal activity and accelerated developmental maturation rate. Results from these studies will provide insight into the significant contributions of maternal preconception experiences on female neurodevelopment and lifetime disease risk and resilience.

Disclosures: K. Montgomery: None. **Y. Cisse:** None. **T.L. Bale:** None.

Digital Abstract Session

P244. Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Program #/Poster #: P244.01

Topic: F.04. Stress and the Brain

Support: NSERC Discovery Grant RGPIN-2019-04837
Discovery Accelerator Supplement RGDAS-2019-00033
Canada Foundation for Innovation Grant 32631

NSERC CGSM

Title: Lipopolysaccharide induces local glucocorticoid production in the mouse brain during early development

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Abstract: Glucocorticoids (GCs) are steroid hormones and critical modulators of nervous system development and function. Stressors activate the hypothalamic-pituitary-adrenal (HPA) axis and lead to the secretion of GCs from the adrenal glands into the blood. GCs are also locally produced in extra-adrenal organs, such as the brain. During early development (postnatal day (PND) 2 to 12), mice have very low circulating GC levels at baseline and small increases in response to stressors, this is termed the stress hyporesponsive period (SHRP). Local GC production in the brain may be of particular importance during the SHRP, to allow GC signaling in specific brain regions without general effects throughout the body. However, the effects of an immunological stressor during the SHRP on local GC levels within specific brain regions remain unknown. To study these effects, we assessed systemic and brain GC levels in neonatal (PND5) C57BL/6J mice 4 hours after administration of lipopolysaccharide (LPS) (50 µg/kg i.p.) or vehicle control (n=12/group). We examined blood and microdissected brain regions (prefrontal cortex, hippocampus, hypothalamus, and amygdala) using the Palkovits punch technique. Using a within-subject design, GCs (corticosterone and 11-dehydrocorticosterone) were measured via liquid chromatography tandem mass spectrometry (LC-MS/MS), and transcripts of key steroidogenic enzymes (*Hsd11b1*, *Hsd11b2*) were measured via RT-qPCR. LPS administration induced greater increases in corticosterone in every brain region but the prefrontal cortex, compared to blood. In contrast, for 11-dehydrocorticosterone, LPS treatment induced greater decreases in every region but the prefrontal cortex, compared to blood. Transcripts of key steroidogenic enzymes are present in all brain regions examined. Taken together, these results suggest that LPS rapidly stimulates local corticosterone production in the neonatal mouse brain and provide insight on the effects of bacterial infections on the developing nervous system.

Disclosures: H. Bajaj: None. **M.X. Li:** None. **J.E. Hamden:** None. **M. Salehzadeh:** None. **C. Ma:** None. **K.K. Soma:** None.

Digital Abstract Session

P244. Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Program #/Poster #: P244.02

Topic: F.04. Stress and the Brain

Support: DAFCYT-2003IDPTNNN0020

Title: Serotonin transporter in the ventral hippocampus of prenatally stressed rats

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Abstract: Previously, we have shown that prenatal stress causes changes in serotoninergic system function, with low ventral hippocampus serotonin release after behavioral forced swim tests in adulthood. In the current study, changes in serotonin transporter (SERT) in the ventral hippocampus of prenatally stressed rats after sucrose intake and after forced swim tests, was evaluated. Three months old, prenatally stressed male rats were used for the study. Sucrose intake was assessed, identifying anhedonic and non-anhedonic rats. Serotonin extracellular concentration were assessed by microdialysis, after sucrose intake test. Ventral Hippocampus were dissected and SERT evaluated by Western Blot. Other males were submitted to the forced swim test. Serotonin release was assessed, and at the end of the behavioral test, ventral hippocampi were dissected and SERT was evaluated in control and prenatally stressed, anhedonic and non-anhedonic rats. The results show an increase in SERT content in the ventral hippocampus of control rats after the forced swim test. In anhedonic and non-anhedonic rats, however, SERT did not change neither after sucrose teste, nor after the forced swim test. These data indicate that higher immobility in forced swim test in prenatally stressed males are related to lower serotonin release, but not to SERT content.

Disclosures: F.J. Jiménez-Vasquez: None. **D. Mendez:** None. **M. Rubio:** None. **C. Rubio:** None. **S. Orozco-Suarez:** None. **S. Retana-Márquez:** None.

Digital Abstract Session

P244. Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Program #/Poster #: P244.03

Topic: F.04. Stress and the Brain

Support: NIMH RO1MH096093
Harvey Family Endowment

Title: Live imaging of brain network dynamics in an acute threat response by MEMRI: Effects of early life adversity on noradrenergic system anatomy

Authors: *T. W. USELMAN[1], R. E. JACOBS[2], E. L. BEARER[1,3];
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Abstract: Monoaminergic signaling in cortical-limbic circuits regulate both normal physiology and responses to stress. Indeed, monoaminergic reuptake transporters, located on the pre-synaptic terminals of distal projections, are common pharmacological targets for mental

disorders, like anxiety and depression. Early life adversity (ELA), which has an interaction with monoaminergic systems, increases vulnerability to mental disorders later in life. Despite this, we are yet to understand how ELA alters brain-wide patterns of neural activity. Here, we combine Manganese-enhanced MRI (MEMRI) with behavioral tracking and immunohistochemistry to investigate the effects of ELA on the modular neuroarchitecture and behavioral response to acute threat. ELA was administered from P2-P9 by depriving dams of adequate bedding. Mice (10 weeks, n=24) +/- ELA were subject to paired MEMRI and behavior longitudinally. Intraperitoneal injections of paramagnetic Mn(II) (0.3 mmol/kg) highlights brain activity in awake behaving mice. Behavior was recorded during Mn(II) uptake to assess avoidance and motility before, during and nine days after acute exposure to predator odor (TMT, 2,3,5-Trimethyl-3-thiazoine). Histochemical analysis was performed on serial brain sections from mice sacrificed and perfused at the conclusion of paired behavior-imaging experiments. MEMRI images were skull-stripped, spatially registered, and intensity normalized. To measure the degree of activity and relationships between brain regions we performed statistical parametric mapping (SPM), segmentation of 90 brain regions, cross-correlation analysis, and Louvain community detection. Predator stress increased avoidance behavior (percent of time in light) for both groups ($p<0.05$, Tukey-Adjusted), while only inter-subject variance was observed to be different between +/- ELA groups. Results suggest basal neural activity of ELA mice resembles that of acute fear in normally reared mice, with increased activity in the basal forebrain and hindbrain. Additionally, ELA disorganized network structure, increased modularity of basal brain networks and altered the dynamic response to acute threat. Staining for the norepinephrine transporter revealed lesser numbers of distal termini, suggesting alterations in neural activity could be due to an effect of ELA on the development of the noradrenergic system. Together our data find that ELA disrupts the arborization of noradrenergic projections, alters the coordination of basal neural activity and of neural activity in response to acute threat.

Disclosures: **T.W. Uselman:** None. **R.E. Jacobs:** None. **E.L. Bearer:** None.

Digital Abstract Session

P245. Early-Life Stress: Molecular Mechanisms and Cellular Effects

Program #/Poster #: P245.01

Topic: F.04. Stress and the Brain

Support: NIH Grant R00 MH115096 (Peña)
NJ ACTS Pilot Program Propel Awards (Peña)

Title: Priming of chromatin by monomethyltransferase Setd7 overexpression in the VTA mimics early life stress

Authors: *J.-A. RODIER, A. S. CHEN, C. J. PEÑA;
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Abstract: In humans, adverse childhood experiences increase the risk for psychiatric disorders such as depression. There is evidence that early life stress sensitizes individuals to future experience of stress. To study how epigenetic mechanisms might mediate maladaptive behavioral outcomes following early-life stress (ELS), and their impact on neurodevelopment, we use a stress model in mice. We recently found that in the ventral tegmental area (VTA), the proportions of chromatin modifications associated with a more open or permissive state increases. Here, we verified by Western blot that ELS in male mice increases monomethylation of histone 3 lysine 4 (H3K4me1), an epigenetic modification that has been associated with transcriptional priming in across biological kingdoms. Reanalysis of published RNA-seq data in VTA also revealed a trend for increased mRNA levels of *Setd7*, a mono-methyltransferase specific to H3K4 (t-test, p=0.1012, n=4, males only, trend). We hypothesize this open chromatin state, marked by H3K4me1, may enable more reactive transcription in response to additional stress across the lifespan. To causally test this hypothesis, we manipulated the epigenome *in vivo*: we developed custom viral vector tools to over-express the H3K4-specific monomethyltransferase *Setd7* during an ELS-specific time-sensitive period. We performed stereotaxic surgery to target viral over-expression of *Setd7* in the VTA, a deep brain structure, of male and female pups at 14 days old. We validated that our vector increases SETD7 protein (6.0-fold increase in SETD7 mice compared to endogenous levels in GFP-injected mice, $p<0.001$, n=12) and H3K4me1 (1.35-fold increase, $p = 0.0014$, n=6 GFP and n=11 for SETD7) in the VTA, similar to early-life stress. We then measured depression- and anxiety-like behavior, in both male and female mice, before and after adult chronic social defeat stress. We found an interaction between juvenile *Setd7* overexpression and experience of adult stress on social avoidance behavior. This provides causal evidence linking early life stress to epigenetic modifications in reward circuitry that directly mediate lifelong stress sensitivity.

Disclosures: J. Rodier: None. **A.S. Chen:** None. **C.J. Peña:** None.

Digital Abstract Session

P245. Early-Life Stress: Molecular Mechanisms and Cellular Effects

Program #/Poster #: P245.02

Topic: F.04. Stress and the Brain

Support: NIEHS ES028202
NICHD HD097093
NIMH MH104184
NIMH MH108286

Title: Extracellular vesicles as potentiators of stress signals to alter placental and fetal development

Authors: *H. C. ZIERDEN[1], K. E. MORRISON[2], B. M. NUGENT[3], T. L. BALE[4];
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Abstract: Maternal stress is linked to a variety of adverse fetal and pregnancy outcomes. In our well-established mouse model of early prenatal stress (EPS), we observe impaired cognitive function, alterations in metabolic programming, and heightened stress sensitivity, especially in male offspring. We hypothesize that stress may contribute to fetal neurodevelopment via changes in extracellular vesicle (EV) signaling. EVs are cell-derived particles that participate in intercellular communication by transporting signaling factors between cells and tissues. We first addressed the role of stress in EV signaling by isolating circulating EVs from donor dams in control and EPS-exposed groups. We performed small RNA-seq to analyze the content of these EVs. Next, we injected labeled EVs into naïve dams, and used an *in vivo* imaging system (IVIS) to detect EV trafficking in maternal and fetal compartments. A separate cohort, also receiving EV injections, was used to detect transcriptomic changes in placental and fetal tissues. EVs were administered to a cohort of naïve dams, and offspring were monitored throughout adulthood for growth and hypothalamic-pituitary-adrenal (HPA) stress axis reactivity. To identify the role of EVs in male-specific consequences of EPS, EVs were isolated from control and EPS-exposed neonates, labelled, and injected into age-matched naïve neonates. A cohort of naïve neonates was sacrificed following EV injection to investigate the distribution of EVs throughout the body via IVIS. In a separate cohort, we monitored offspring throughout adulthood for growth and hypothalamic-pituitary-adrenal (HPA) stress axis reactivity. We observed that EVs from control and EPS-exposed neonates were differentially trafficked into the neonate brain in both EPS- and sex-specific ways. EVs derived from EPS-exposed males promoted long-term changes in male body weight and HPA stress axis sensitivity, while female EPS EVs had no long-term programmatic effects on naïve females. Together, these studies provide insight into the role EVs play in promoting stress signals in both maternal and fetal circulation, their interaction at the level of the placenta, and the impact of prenatal stress on important signaling dynamics between maternal and fetal compartments during gestation.

Disclosures: H.C. Zierden: None. **K.E. Morrison:** None. **B.M. Nugent:** None. **T.L. Bale:** None.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.01

Topic: F.04. Stress and the Brain

Support: Hartwick College Faculty Research Grant
Hartwick College Startup Funds

Title: Maternal Stress Increases Anxiety-Like Behavior of Rodent Infant Offspring.

Authors: *H. M. RIVERA, C. COLLINS, J. CAGE, J. GERSTENBERGER, A. HASKELL; Hartwick Col., Oneonta, NY

Abstract: Epidemiological studies and animal studies reveal that adverse events early-on in the life of the mother can have a long-term impact on the health of their children. While data exists on the effect of maternal stress on adult offspring, currently, there is a lack of information in juveniles. Because laboratory studies of mothers can isolate maternal stress as a factor, they are powerful tools in investigating these effects. The goal of the present study was to begin to examine the role of maternal stress on anxiety-like behavior during the infant developmental stage. Rat dams were assigned to one of two groups: the maternal stress group (n = 5, dams underwent variable psychological stress during the last 7 days of gestation) or the maternal control group (n = 4, dams underwent no stress). Dam and offspring body weights were measured at the infant stage (i.e. postnatal day 22). Anxiety-like behavior were determined in offspring with the elevated-plus maze behavioral assay (postnatal day 22). One female and one male offspring were used per day for offspring body weight and behavior analysis to prevent cohort effects. Group differences in dam body weights, offspring body weights, and offspring elevated-plus maze behavior were calculated with unpaired t-tests. During the infant stage, our results showed that stressed dams displayed a significant decrease in their body weights relative to non-stressed dams ($t(7) = 3.34$, $p = 0.01$). In contrast, offspring from stressed dams and non-stressed dams did not exhibit group differences in body weight ($t(16) = 0.13$, $p = 0.90$). When looking at offspring anxiety-like behavior in the elevated-plus maze, offspring from stressed dams displayed no group differences in the time spent on the closed arm relative to the non-stressed group ($t(16) = 0.74$, $p = 0.47$). However, offspring from stressed dams revealed a significantly lower amount of entries in the open arms relative to offspring from non-stressed dams ($t(16) = 2.49$, $p = 0.03$). Together, these findings indicate that maternal stress alone increases offspring anxiety-like behavior in infant offspring. These studies presented here confirm that we started developing an animal model of adverse maternal stress effects on infant development. Building on our unique experience with this animal model, our research group will continue to assess maternal stress across all stages of development.

Disclosures: H.M. Rivera: None. **C. Collins:** None. **J. Cage:** None. **J. Gerstenberger:** None. **A. Haskell:** None.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.02

Topic: F.04. Stress and the Brain

Support: NIH Grant RO1 DA041529
Second Century Initiative Neurogenomics Fellowship

Title: Sex-specific impact of perigestational opioid exposure on juvenile play behavior in rats

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Abstract: Juvenile play is a natural and essential part of rodent social development and is often disrupted in animal models of neuropsychiatric or developmental disorders. It has been previously reported that acute exposure to opioids, including morphine, in juvenile rats has been shown to increase juvenile play in a nucleus accumbens mu-opioid receptor-dependent manner. However, the consequences of developmental exposure to opioids on juvenile play are unknown. Using a novel assay of perigestational opioid exposure that consists of pregestational, gestational, and postnatal exposure to morphine, we tested the hypothesis that chronic exposure to opioids during critical periods of development would disrupt juvenile play due to alterations in reward signaling. Juvenile play behavior was measured in control or perigestationally morphine exposed male and female rats at P25, P35, and P45. Classical features of juvenile play were measured, including social play, time not in contact, pins, and nape attacks. Preliminary data shows that morphine-exposed females participated in less social play, with a corresponding increase in time spent alone. Morphine-exposed females also initiated fewer pins and nape attacks. Together, this data suggests that females perigestationally exposed to morphine are less motivated to participate in social play, potentially due to an alteration in the reward circuitry due to developmental exposure to morphine. We are currently analyzing mu-opioid receptor expression throughout the reward circuit to elucidate a potential mechanism for this behavioral change.

Disclosures: H.J. Harder: None. **C.T. Searles:** None. **L.A. Hanus:** None. **M.G. Gomez:** None. **A.J. Austin:** None. **A.Z. Murphy:** None.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.03

Topic: F.04. Stress and the Brain

Support: NIH grants AA026601 (SH)
NIH grants AA026421 (RS)

Title: Prenatal ethanol exposure leads to increased anxiety-like behavior in male and female rats

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Abstract: Prenatal ethanol exposure leads to increased anxiety-like behavior in male and female rats

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Background. Prenatal ethanol exposure (PE) leads to a variety of psychological conditions. Individuals with Fetal Alcohol Spectrum Disorders (FASD) often show severe anxiety and fear, in which the serotonergic neurons located in the dorsal raphe nucleus (DRN) are implicated. But it is unclear how PE alters serotonergic neurotransmission in the DRN, leading to increased anxiety. A valid rodent model could help unravel the underlying neural mechanisms. The purpose of the present study was to establish a rat model to study anxiety-like behavior in rats with PE. **Materials and Methods.** Pregnant Sprague Dawley rats were gavaged twice/day with 0 or 3 g/kg/treatment ethanol (15% w/v) during gestational days 8-20, mimicking second-trimester heavy PE in humans. Their adult offspring underwent three different tests of anxiety-like behavior: elevated plus maze, elevated zero maze, and open field tests. Some male rats also underwent an acute restraint (2 hour) procedure before the open field test. All the tests were conducted in dimly-lit rooms after rats were habituated for 15 min. Animal behavior was tracked using the ANY-maze software. **Results.** In the elevated plus maze test, PE male rats spent less time on the open arms than controls. In the elevated zero maze test, PE led to a decrease in number of entries into the open quadrants in male rats as well as a decrease in time and distance travelled on the open quadrants in female rats, as different manifestations of increased anxiety-like behavior in PE rats. In the open field, PE female but not male rats spent less time in the center area than controls; the same results, however, were observed in PE males after acute restraint stress. **Conclusion.** The results from all three tests show that PE leads to an increase in anxiety-like behavior in both male and female rats. The increase appears more marked in PE females than in PE males. In addition, PE male rats show increased anxiety in response to acute stress.

Keywords (up to 3): anxiety, acute restraint stress, prenatal ethanol exposureThe work was supported by NIH grants AA026601 (SH) and AA026421 (RS).

Disclosures: V. Micov: None. **F. Kwarteng:** None. **C. Martin:** None. **A. Lei:** None. **R. Wang:** None. **K. Hausknecht:** None. **M. Turk:** None. **S. Oubraim:** None. **S. Haj-Dahmane:** None. **R. Shen:** A. Employment/Salary (full or part-time):; Student employment.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.04

Topic: F.04. Stress and the Brain

Support: 5R01MH117459-03

Title: The estrous cycle modulates early life adversity effects on defensive behavior, activity levels and ventral hippocampal theta rhythm in female mice.

Authors: *B. J. LAHAM, S. MURTHY, M. CLAPPIER, M. HANANI, S. BOYER, E. GOULD;
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Abstract: Early life adversity (ELA) predisposes people to develop neuropsychiatric conditions, including anxiety disorders and attention deficit hyperactivity disorder. Using maternal separation with early weaning, a mouse model of ELA, we previously found increased defensive behavior and activity levels in adult males but no significant effects on these behaviors in adult females (Murthy et al., 2019). These findings were unexpected because the link between ELA and neuropsychiatric disorders is even stronger in women than men. Estrogen has been shown to modulate some stress responses (Eid et al., 2020), so we sought to investigate whether naturally occurring fluctuations in ovarian steroids across the estrous cycle might interact with ELA-induced behavioral changes. When tested during the estrus phase of the estrous cycle, when levels of estrogen are relatively high, adult females previously subjected to ELA exhibited no change in behavior compared to control reared mice in estrus. By contrast, when tested during diestrus, adult ELA females showed a behavioral phenotype similar to adult ELA males: increased defensive behavior and increased activity levels compared to controls. Previous work done in male mice has shown that theta oscillations in the ventral hippocampus are causally linked to defensive behavior (Padilla-Coreano et al., 2019) and that ELA-induced increases in defensive behavior and activity levels are accompanied by increased theta power in this brain region (Murthy et al., 2019). Thus, we sought to explore whether ELA-induced changes in behavior during diestrus are associated with increased theta rhythm by carrying out wireless electrophysiological recordings in awake behaving female mice during diestrus and estrus. We found that during diestrus, but not estrus, ELA female mice exhibit significantly greater theta power in the ventral hippocampus compared to controls. We also found that, compared to controls, diestrus female mice subjected to ELA have smaller perineuronal nets surrounding parvalbumin-positive interneurons, a cell population known to contribute to rhythmic firing. Taken together, these findings show that ELA has similar effects on behavior and ventral hippocampal function in diestrus females as it does in males, but that higher levels of estrogen during estrus may transiently protect against these effects. The extent to which changes in perineuronal nets occur across the estrous cycle and contribute to fluctuations in ELA-induced increases in theta oscillations, defensive behavior and activity levels, remains to be determined.

Disclosures: B.J. Laham: None. **S. Murthy:** None. **M. Clappier:** None. **M. Hanani:** None. **S. Boyer:** None. **E. Gould:** None.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.05

Topic: F.04. Stress and the Brain

Title: Therapeutic effects on working memory and anxiety of early physical exercise in CD-1 adult mice from gestational undernourished dams

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Abstract: Gestational undernutrition (GU) has severe long-term consequences on neuronal organization, which underly cognitive and emotional processes like working memory and anxiety, that alters the well-being and development of individuals. The aim of this work was to analyze the therapeutic effects on working memory and anxiety of early physical exercise in CD1 adult mice with undernourished mothers during gestation. For the underfed groups, female CD-1 mice received different percentages of a balanced diet (50%, 70% and 100%) during the three weeks gestation. At postnatal day 4 (PD4), pups were randomly distributed in different dams and separated on three groups: exercise group (EG), without movement group (WMG) and without exposition group (WEG). Exposure to physical exercise begins at PD15 and ends at P56, contemplating five days of adaptation. Rehearsals are from Monday to Friday for 1 hour, resting on weekends. Weaning was at PD21 and from then pups were given an ad lib diet. At PD60 subjects were evaluated with the Morris Water Maze (MWM) for four days, while at PD64 groups were tested in the open field (OF). Results showed significant differences in weight from PD18 until PD60 between EG and WEG groups ($p<0.0001$) without sex differences. By other hand on MWM, no significant differences were observed on any evaluation day, neither in latency nor in swimming velocity ($p>0.05$). In contrast, in the OF, significance was identified anxiety indicators, being WEG which had less mobility and speed ($p<0.01$), traveled less distance ($p<0.0001$) and spent more time in the periphery ($p<0.001$), compared to the other two groups, while EG showed higher scores. In conclusion, physical exercise in the early stages does not showed an effect on working memory, but in anxiety indicators in animals exposed to undernutrition during prenatal stages. Based on this, longer exposure to exercise could be considered for evaluation, also other behavioral tests could be used to understand the mechanisms that may be affected by GU, as well as its possible therapeutic alternatives.

Disclosures: M. Ortiz: None. **N.A. Moy-López:** None. **M.F. Pinto-González:** None. **R. Pedraza-Medina:** None. **J. Guzmán-Muñiz:** None.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.06

Topic: F.04. Stress and the Brain

Title: Different models of early life adversity differentially alter development of infant ultrasonic vocalizations, adolescent social behavior, and prefrontal cortex cytokine expression across development

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Abstract: Early life adversity impacts the development of later-life social function. Vulnerability to specific psychiatric outcomes are influenced by the type of adversity and biological sex, necessitating the investigation of sex-specific effects of different forms of adversity. Social dysfunction, a shared symptom among several psychiatric conditions including depression and schizophrenia, is associated with aberrant development of the prefrontal cortex (PFC), a late-maturing region susceptible to early environmental insults. Neuroimmune mechanisms involving pro- and anti-inflammatory cytokines are integral to neuronal developmental processes contributing to synaptic plasticity. However, the underlying behavioral factors of different types of adversity that may be mediating later-life social deficits and neural dysfunction are still unclear. We used two rodent models of early life adversity—maternal separation (MS) (P2-P20) and limited bedding (LB) (P2-P14)—to determine the effects of altered maternal care on development in male and female offspring. In neonatal rats, ultrasonic vocalizations (USVs) are a species-typical care-eliciting behavior and communication tool. We modeled the developmental growth curves of USV types based on their sonographic structures on postnatal days (P) 5, 10, 15, and 21 and quantified the predictability of call type sequences using an entropy rate calculation. Then, adolescent (P35) rats underwent a dyadic social interaction test to determine the effects of rearing condition on social approach and play behaviors and whether these are mediated by juvenile USVs. In separate cohorts, adolescent (P35) and young adult (P60) mRNA expression of pro-inflammatory (IL-1β, IL-6, and TNF-α) and anti-inflammatory (IL-10) cytokines in the PFC were quantified via RT-qPCR to determine whether social aberrations were associated with persistent neuroimmune dysfunction. Results indicate that age-related changes in USV emissions and social behavior depend on rearing condition and sex. LB delayed the USV development exclusively in males, while MS accelerated the curve only in females. Social behavior was also impacted differentially in males and females depending on rearing condition. This indicates that dysfunctional mother-pup interactions as measured by adaptive USVs may mediate later-life social dysfunction and cytokine expression across development. These findings highlight specific aspects of the early life environment most critical for neural and behavioral development following adversity.

Disclosures: L. Granata: None. **H.C. Brenhouse:** None.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.07

Topic: F.04. Stress and the Brain

Title: Dreadd mediated activation of parvalbumin cells during early post natal development: effect on mood related behaviours

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Abstract: DREADD mediated activation of Parvalbumin cells during early post natal development: effect on mood related behaviours
AUTHORS: TOSHALI BANERJEE, SONALI S SALVI, STHITAPRANJYA PATI, PRAACHI TIWARI, DARSHANA KAPRIVIDITA A. VAIDYA
Early life stresses (ELS) have been implicated in adult onset of psychiatric disorders such as anxiety, depression, schizophrenia, etc. The Early life window is crucial for the functional development of the nervous system. Experiences during this stage sets the tone for the functional integration of various neurotransmitter and neuromodulator systems (such as Serotonin, endocannabinoids, etc.). Several lines of evidence, across preclinical animal models and clinical post-mortem studies on subjects that have undergone ELS, indicate that an altered cortical Gq signalling, downstream of the Serotonin receptor $5HT_{2A}$ and other Gq coupled receptors such as mGlu2, drives the onset of anxiety like behaviours in adulthood. The same has been corroborated in pharmacological models such as Post-natal fluoxetine treatment and Chemogenetic upregulation of Gq signalling in forebrain excitatory neurons(Pati et al, 2020). While majority of the $5HT_{2A}$ receptors are present on principle excitatory neurons of the cortex, it is also expressed in the inhibitory Parvalbumin interneurons. Interestingly, perturbations in Parvalbumin interneurons signalling via $5HT_{2A}$ has been implicated in various disorders such as Schizophrenia. Hence, we were interested in exploring the effects of an early life perturbation of Gq signalling, exclusively in the Parvalbumin interneurons. In this study, we chemogenetically drive Gq signalling in Parvalbumin cells using hM3Dq-DREADD in the postnatal window P2-P14 and carry out a battery of behaviour experiments to study its effect on anxiety and despair like behaviours in adulthood. We do not observe a difference in development of reflex behaviours during post natal development. We observe a mild, task specific anxiolysis with broad postnatal increase in Gq signalling in Parvalbumin cells on measures such as Open field test and Elevated plus maze test. We do not see this effect in age matched genotype negative controls, hence validating our observation. We also assay for despair like behaviours and do not observe any significant difference. This study could indicate potential differences in cell type based regulation during development and opens up room for investigating circuit specificity for such cell type based regulation.
.

Disclosures: T. Banerjee: None. **S.S. Salvi:** None. **S. Pati:** None. **P. Tiwari:** None. **D. Kapri:** None. **V.A. Vaidya:** None.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.08

Topic: F.04. Stress and the Brain

Support: Kocaeli University Research Department Grant

Title: The effects of lps-induced preterm labor on learning and memory in adult WAG / Rij rats

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Abstract: The effects of lps-induced preterm labor on learning and memory in adult WAG / Rij ratsOBJECTIVE: The risk of preterm birth increases in mothers diagnosed with gram-negative bacterial infection during pregnancy. This situation constitutes important risk factors in the formation of important neurological disorders such as epilepsy, white matter damage in the brain and cerebral palsy in premature babies. Lipopolysaccharide (LPS) is a commonly used agent to create a preterm delivery model in rats. In our study, it was planned to investigate the effects of LPS induced preterm model on neurobehavioral performance, learning and memory in the adult WAG/Rij rats. The aim of this study is to evaluate the effects of preterm birth on learning and memory in a pathological genetic background such as absence seizure activity.METHODS AND METHODS: It was planned to be Group1(naive) n=8, Group2(SF) n=8, Group3(LPS) n=8.SF and/or Lipopolysaccharide(200µg/kg) were injected on the 15th and 16th day of pregnancy. At postnatal six month later, effects on locomotor activity, passive avoidance and morris-water-tank tests were investigated.RESULTS: When locomotor activity was evaluated, there was no significant change in total activity between the groups. In the passive avoidance test, the retention time in the LPS induced group was shortened compared to the naive and SF control groups($p<0.05$). In the Morris-water-tank test, in LPS induced group, there was a decrease in the correct squatting time according to the naive and SF control groups($p<0.05$).CONCLUSIONS: Idiopathic generalized epilepsy has a genetic basis, including absence epilepsy. WAG/Rij rats can be regarded as a valid genetic animal model of absence epilepsy with comorbidity of depression. Behavioral studies indicate that WAG/Rij rats exhibit depression-like symptomps. In WAG/Rij rats with genetically pathological background, premature birth induced by injection of LPS caused disruption in learning and memory tests in the adult period of the offspring. Further studies are needed to investigate the accompanying neurochemical and systemic changes in order to explain changes in learning and memory behavior.**Keywords:** Absence epilepsy, Passive avoidance, Water maze test

Disclosures: A. Nuraydin: None. **D. Sahin:** None. **S. Karadenizli:** None. **T. Sahin:** None. **N. Ates:** None.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.09

Topic: F.04. Stress and the Brain

Support: DFG KI 588/14-1, KI 588/14-2
DFG DA 1151/5-1, DA 1151/5-2

DFG KR 3822/5-1, KR 3822/7-2
DFG NE 2254/1-2
DFG JA 1890/7-1, JA 1890/7-2

Title: The DLPFC as a neural correlate of resilience to major depressive disorder

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Abstract: The DLPFC as a neural correlate of resilience to major depressive disorder
Background. The risk factors familial risk and childhood maltreatment increase the risk for major depressive disorder (MDD). When both risks are present in an individual, they are considered to be at high risk for MDD. However, while some may develop MDD, some are resilient and maintain mental health. The brain morphometric correlates of this resilience are still unknown. To pinpoint this complex adaptation process on a brain morphometric level, interaction analysis of risk and diagnosis are needed.
Methods. We analyzed four groups of participants (N=804) with distinct risk (low-risk, i.e absence of CM and familial risk vs. high-risk, i.e. presence of both risks) and diagnosis (healthy vs. MDD) profiles in a 2x2 design, analyzing brain structural data (3T MRI) by means of voxel-based morphometry (VBM; CAT12 toolbox). Using (pre)frontal region of interest (ROI) analysis, we analyzed the interaction effect of risk and diagnosis.
Results. Resilient subjects (i.e. healthy, high risk participants) showed significantly higher volume in the left dorsolateral prefrontal cortex (DLFPC), compared to all other groups, (k=199, x/y/z= -34/48/12, T= 4.06, p=.047, family-wise error (FWE) corrected). We did not detect significant results for the bilateral superior frontal gyri, frontal poles, pars orbitalis of the inferior frontal gyri, and the right middle frontal gyrus ($p > .05$)
Conclusions. Higher volume in the left DLPFC might be an adaptive process in high risk subjects. The DLPFC is involved in emotional and cognitive processes, and higher volume in this area might aid such high-risk individuals in maintaining mental health. An increased volume in this region might therefore constitute a neural correlate of resilience to MDD in high risk.

Disclosures: **K. Brosch:** None. **F. Stein:** None. **T. Meller:** None. **S. Schmitt:** None. **D. Yuksel:** None. **K.G. Ringwald:** None. **J. Pfarr:** None. **L. Waltemate:** None. **H. Lemke:** None. **N. Opel:** None. **S. Meinert:** None. **K. Dohm:** None. **D. Grotegerd:** None. **J. Goltermann:** None. **J. Repple:** None. **A. Winter:** None. **A. Jansen:** None. **U. Dannlowski:** None. **I. Nenadić:** None. **T. Kircher:** Other; Tilo Kircher received unrestricted educational grants from Servier, Janssen, Recordati, Aristo, Otsuka, neuraxpharm. **A. Krug:** None.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.10

Topic: F.04. Stress and the Brain

Title: Long Term Effects of Early Life Morphine Exposure on Development, Affect and Cognition

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Abstract: Both in-utero and postnatal opioid exposure can lead to neonatal opioid withdrawalsyndrome (NOWS). While the incidence of NOWS has dramatically increased in the last 15years, its potential long-term effects are not well understood. Our study sought to determine ifneonatal opioid exposure and withdrawal in a rodent model leads to changes in development,affect and cognitive performance in adolescence and adulthood. C57/BL6 mice were mated togenerate litters assigned to saline or morphine treatment. All pups in a given litter received thesame treatment. 57 animals were tested from 8 injected litters (3 saline-treated (N=28) and 5morphine-treated (N=29)). Male and female pups were injected bi-daily with morphine or saline(10mg/kg) from PND 1-14 (equivalent to the third trimester of a human pregnancy). Pups wererandomly assigned to different cohorts for behavioral analyses in neonatal (PND 13-15),adolescent (PND 42-44),and adult stages(PND 105-112). Morphine-treated animals showedreduced body weight relative to saline treatment independent of sex ($P < .005$). These weightdiscrepancies persisted to adolescence, $P = <.01$, but not into adulthood. In addition,morphine-treated pups showed deficits in the forelimb grasp ($P=<.01$) and surface righting tests($P=<.01$) on PND7. During adolescence, morphine-treated mice exhibited anxiety-like behaviorby spending less time in the center during the Open Field Test ($P= <.05$). A similar effect wasfound in adulthood, as morphine-treated mice spent more time in the corners ($p < 0.05$), andtrended towards more border time ($P=.08$). In the adult Novel Object Recognition Test,morphine-treated mice showed greater exploration time and novel object exploration time ($p <0.05$). However, there were no differences observed in the recognition index, suggesting thisdifference could be explained by a hyper-active phenotype in morphine-treated mice. Inconclusion, our study suggests that early opioid exposure leads to several anxiety-like behaviorsin adolescence and adulthood comparable to that of a hyperactive, chronic-stress phenotype. Inaddition, early opioid exposure delays the emergence of important developmental milestones.Lastly, early opioid exposure blunts weight gain during PND1-PND14, and this weightdiscrepancy persists into adolescence but recovers before adulthood. Future directions mayconsider how developmental delays and behavioral differences in morphine-treated animalsaffect other behaviors such as memory tasks, mating and maternal behavior, further explore sexdifferences, and study the neural correlates underlying these behavioral differences.

Disclosures:

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.11

Topic: F.04. Stress and the Brain

Support: 1707355
1852259
1400868
1545803
P20GM103475
1736019
1826729

Title: Adaptive strategies of honey bee colonies to hurricane associated loss of resources: A case study with the Gentle Africanized Honey Bees (gAHB) in Puerto Rico

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Abstract: In September 2017, Hurricane Maria hit Puerto Rico and was associated with the loss of approximately 80% of managed honey bee colonies mainly due to the winds and the posterior lack of floral resources. As part of an ongoing project, we monitored activity on a colony of Gentle Africanized bees (gAHB) with videos of the hive entrance, photos from all frames, and various sensors measuring internal parameters. This effort allowed us to capture the period before and after Hurricane Maria and provided a unique opportunity to gain insight into how surviving honey bee colonies responded to this climate event. We were also able to investigate whether sugar syrup and protein patties administration were sufficient to support survival, reproduction, and physiological control of the internal colony environment. Our findings indicate that despite the presence of high levels of honey from our syrup administration, and a small but stable amount of pollen stored, there was a dramatic decrease in brood shortly after the hurricane (October). Intriguingly analysis revealed that brood had already recovered from the October drop to levels comparable to those before the Hurricane, before protein patties supplementation, we observed a slight brood increase six days after the start of the pollen substitute. We interpret brood decrease as being caused by cannibalism since this behavior was generally observed in the apiary during this time period. Using our novel methodology to automatically detect pollen entry showed that by the 2nd month after the hurricane (November), pollen entry was recovered to pre-hurricane levels and coincided with the recovery of brood levels. We speculate that the lack of fresh pollen entry to the colony after the hurricane served as a signal to nurses to cannibalize young larvae.

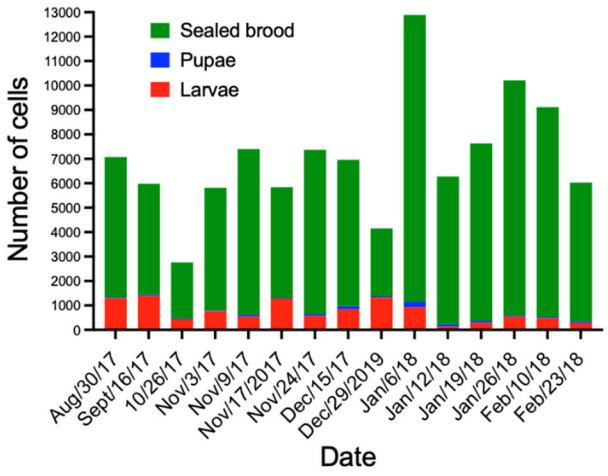

Figure 1. Number of cells of all frames of sealed brood and unsealed brood in each day the photos were taken.

Disclosures: **S.L. Cruz Rodriguez:** None. **I. Claudio- Ford:** None. **I.F. Rodriguez:** None. **E. Florez Gómez:** A. Employment/Salary (full or part-time):; Polytechnic University of Puerto Rico. **J. Alemán Rios:** None. **E. Perez Claudio:** None. **E. Caraballo Sanchez:** None. **I. Collado Hernandez:** None. **R. Merget:** A. Employment/Salary (full or part-time):; university of Puerto Rico. **T. Giray:** A. Employment/Salary (full or part-time):; university of Puerto Rico. **J. Agosto-Rivera:** A. Employment/Salary (full or part-time):; university of Puerto Rico. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; principal Investigator.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.12

Topic: F.04. Stress and the Brain

Support: NICHD F32HD10130
NIEHS ES028202
NICHD HD097093
NIMH MH104184
NIMH MH108286

Title: Racial disparities in stress or health? Modeling consequences of lifetime stress on maternal health and offspring development

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Abstract: Despite major advancements in maternal-fetal health, African-American women are three times more likely to die in childbirth or postpartum than their non-Hispanic white counterparts, independent of education, medical care, and income. Racial discrimination, a pervasive lifetime stressor unmitigated by socioeconomic status, precipitates a state of chronic stress and allostatic load. Lifetime discrimination experience (LDE) is indeed associated with blunted cortisol rhythms and stress responses, as well as an increased risk of chronic and inflammatory diseases in African-American women. Consistent with these data, we report that LDE is positively correlated with circulating cell-free mitochondrial DNA (ccf-mtDNA) in African-American women. Ccf-mtDNA reflects cellular stress, a common etiology of chronic and affective disorders, and serves as a novel peripheral biomarker of disease severity. Maternal lifetime stress is a strong predictor of adverse perinatal outcomes and offspring neuropsychiatric disease risk. However, little is known about the mechanisms by which pre-conception stress history affects offspring neurodevelopment. We developed a novel mouse model of maternal preconception stress (MPS), where female, but not male, offspring of MPS dams show elevated adult stress reactivity[BT1] . Female MPS offspring exposed to stress show an exaggerated stress response, suggesting an additive generational effect of preconception stress. To identify mechanisms by which MPS alters offspring development, we investigated sex-specific changes in the mid-gestation placental and fetal brain transcriptome. As the site of signaling and nutrient exchange, the placenta is a major determinant of fetal development. Placental adaptations modulate the ability of the placenta to support fetal growth in response to an adverse *in utero* environment. Male placentas of MPS dams had a robust transcriptional response, downregulating genes involved in immune function and upregulating genes involved in DNA damage response and repair, and mitochondrial translation. However, placentas of female MPS offspring exhibited minimal changes in gene expression. To investigate the neurodevelopmental consequences of MPS, we identified downregulation of genes involved in synaptic development and steroid hormone secretion in female MPS offspring brains relative to controls during a key window in hypothalamic development.

Disclosures: **Y.M. Cisse:** None. **S. Carter:** None. **T. Jovanovic:** None. **T.L. Bale:** None.

Digital Abstract Session

P246. Early-Life Stress: Effects On Anxiety, Social Function, and Depression

Program #/Poster #: P246.13

Topic: F.04. Stress and the Brain

Support: R01MH122742
T32GM008688
T32NS105602

Title: Chronic corticosterone exposure blunts psilocybin anxiolysis in mice

Authors: *N. JONES[1], Z. ZAHID[2], S. GRADY[2], Z. SULTAN[2], Z. ZHENG[1], M. BANKS[2], C. WENTHUR[1];
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Abstract: Research into psychedelics as psychiatric therapeutics has increased after demonstrating therapeutic efficacy in small scale clinical studies. For example, psilocybin, a 5-HT2AR agonist, has shown positive results when administered to individuals with major depressive disorder or end-of-life anxiety. Interestingly, despite these long-term anxiolytic effects, psilocybin is often acutely anxiogenic in humans, especially at elevated doses. Despite the potential relevance of these acute stress responses to the clinical effects of psilocybin, few studies have assessed the impact of drug-induced stress hormone release on the behavioral effects of psychedelics. In these studies, C57BL/6J mice were chronically exposed to either vehicle or corticosterone (80 ug/mL) in their drinking water, injected with IP psilocybin (3mg/kg), and assessed in the novelty suppressed feeding test, open field test, or sucrose preference test. As validation for the model, psilocybin acutely induced corticosterone-release ($p < 0.05$) compared to saline, but this psilocybin-induced release was significantly blunted in corticosterone-exposed mice ($p < 0.01$), such that there was no difference from saline. Additionally, corticosterone-treated mice spent significantly less time in the center of the open field apparatus ($p<0.01$) and had a lower sucrose preference ($p<0.001$) than vehicle-treated animals. In the novelty suppressed feeding test, psilocybin alone reduced the latency to feed ($p < 0.05$) compared to saline, but this effect was lost in the context of corticosterone pretreatment. In the open field test, an interaction between psilocybin and corticosterone exposure was identified ($p < 0.05$), with vehicle plus psilocybin increasing the time in center, but corticosterone plus psilocybin decreasing the time in center. In the sucrose preference test, corticosterone-exposure resulted in a significant induction of anhedonic effects for psilocybin ($p<0.05$). These results demonstrate that corticosterone pretreatment is associated with an acutely blunted corticosterone release following psilocybin administration and expression of a less anxiolytic and more anhedonic behavioral profile as compared to animals with an intact stress- hormone response to psilocybin.

Disclosures: **N. Jones:** None. **Z. Zahid:** None. **S. Grady:** None. **Z. Sultan:** None. **Z. Zheng:** None. **M. Banks:** None. **C. Wenthur:** None.

Digital Abstract Session

P247. Early-Life Stress: Adolescence

Program #/Poster #: P247.01

Topic: F.04. Stress and the Brain

Support: NIH grant R01MH123686

Title: Sex-dependent long-term effects of adolescent stress on the posterior parietal cortex

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Abstract: Adolescence is a time of intense cortical development and a period of heightened sensitivity to insult. To determine how sex affects the short- and long-term outcomes of adolescent stress, we subjected 30-day old male and female mice to repeated multiple concurrent stressors (RMS). In the posterior parietal cortex (PPC), RMS caused the elimination of excitatory synapses in deeper layers while inhibitory synapse density was predominantly diminished in superficial layers. These short-term effects coincided with reduced visuo-spatial working memory and were similar in both sexes. The loss of excitatory synapses and impaired working memory persisted in males past a 30-day recovery period. In contrast, we observed a remarkable recovery of excitatory transmission and behavioral performance in females. Inhibitory synapse density recovered in both sexes. We have also observed late onset anxiety- and depression-type behaviors in RMS exposed females that were largely absent in males. Overall, our results indicate that there are marked sex differences in the long-term effects of adolescent stress on cortical synapses and behavior.

Disclosures: M. Fariborzi: None. **S.B. Park:** None. **A. Ozgur:** None. **G. Lur:** None.

Digital Abstract Session

P247. Early-Life Stress: Adolescence

Program #/Poster #: P247.02

Topic: F.04. Stress and the Brain

Support: P30 ES020957
R01-DA-042057
Betty Neitzel Foundation (Department of Psychology).

Title: Neurochemical and behavioral responses to an environmental exposure model of combined benzene, toluene, ethylbenzene, and xylene (BTEX) in Swiss-Webster Mice

Authors: *C. J. DAVIDSON[1], N. SADIK[4], S. A. PERRINE[4], J. H. HANNIGAN[1,5,2,3], S. E. BOWEN[1,2];
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Abstract: Title: Neurochemical and Behavioral Responses to an Environmental Exposure Model of Combined Benzene, Toluene, Ethylbenzene, and Xylene (BTEX) in Swiss-Webster Mice
AIM: Benzene, toluene, ethylbenzene, and xylene (BTEX) are common volatile solvents each present in low levels in the environment, particularly in urban environments. However, relatively little is known about the long-term neurobiological and behavioral consequences of exposure to the BTEX combination. The aim of this study was to explore behavioral and neurochemical

changes associated with BTEX using a novel environmental exposure model. **METHODS:** Adolescent male Swiss-Webster mice (N=32; n = 8/group) were exposed on postnatal days (PND) 28-46 to one of three combinations of BTEX vapor representing 10x (ENV1) or 100x (ENV2) estimates of urban environmental levels or a comparison group exposed to occupational levels of exposure (OCC). A group exposed only to air was a control. Mice were exposed for 1.5 h/session, 2 sessions/day, 5 days/week, for 3 weeks. Locomotor activity (distance traveled) was measured during each exposure session and the two were averaged to represent a daily measure. Brains were collected and frozen immediately following the last BTEX exposure on PND 46. Brains were sliced at 2-mm thickness and tissue punches (1-1.5mm dia.) were collected from medial prefrontal cortex (mPFC) and nucleus accumbens (NAc). These tissues were then analyzed by high-performance liquid chromatography to quantify levels of monoamines (dopamine DA, serotonin -5-HT, and norepinephrine -NE) and their metabolites (DOPAC, HVA, 3MT and 5-HIAA). **RESULTS:** Repeated BTEX exposure altered the pattern of locomotor activity across each exposure day ($p < .01$) with differences observed between groups ($p < .001$) (control < ENV1 < ENV2 < OCC). Regionally specific effects on monoamine and metabolite concentrations were found between the mPFC and NAc. Specifically, the OCC group had significantly higher levels of NE within the mPFC ($p < .05$), but not within the NAc ($p = .232$). No other significant regional differences were observed between exposures. **CONCLUSION:** These results represent a first exploration of environmental patterns of BTEX exposure on neurobehavioral functioning in an animal model. The shifts in locomotor patterns and changes in neurotransmitter levels suggests that our environmental BTEX model can produce adverse long-term effects. Future studies should include both locomotor activity and neurochemical analyses as they were shown to be sensitive measures of the repeated effects of BTEX. This model should prove useful in future investigations of BTEX concentrations and exposure durations.

Disclosures: **C.J. Davidson:** A. Employment/Salary (full or part-time):; R01-DA-042057. **S.A. Perrine:** None. **S.E. Bowen:** None. **N. Sadik:** None. **J.H. Hannigan:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; CURES grant (P30 ES020957), Betty Neitzel Foundation (Department of Psychology).

Digital Abstract Session

P247. Early-Life Stress: Adolescence

Program #/Poster #: P247.03

Topic: F.04. Stress and the Brain

Support: NSERC Discovery Grant (211075-190799-2001)

Title: Environmental enrichment increases brain derived neurotrophic factor and post-synaptic density 95 after lipopolysaccharide treatment in pubertal male and female mice

Authors: *M. S. MURACK, K. B. SMITH, J. DAVIDSON, A. KADAMANI, S. AL SHARANI, C. MESSIER, N. ISMAIL;
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Abstract: Puberty is a critical period of development marked by cortical reorganization and increased synaptogenesis. Healthy cortical reorganization and synaptic growth require sufficient environmental stimuli and minimal exposure to chronic stressors during pubertal development. Stimuli deprivation and/or exposure to stress significantly impacts cortical reorganization and reduces the expression of proteins that promote neuronal survival (BDNF) and measure synaptogenesis (PSD-95). However, previous investigations of pubertal cortical development use minimalized “standard” mouse housing conditions that rarely reflect the naturalistic environment. Environmentally enriched (EE) cages include improved social-, physical-, and cognitive stimulation that better simulate the naturalistic environment. We hypothesized that enriched and deprived environmental stimuli will significantly increase and decrease, respectively, the expression of BDNF and PSD-95 following stress exposure during pubertal development. Three-week-old male and female CD-1 mice (n=10) were housed for three weeks in EE, standard or socially deprived housing conditions. Pubertal mice were treated with lipopolysaccharide (LPS) or saline eight hours prior to tissue collection. EE mice displayed greater sickness behaviors than mice in other housing conditions. EE mice also displayed greater medial prefrontal and hippocampal BDNF and PSD-95 expression than standard housing and deprived housing mice. Only LPS-treated mice housed in EE cages displayed resilience to BDNF and PSD-95 reduction in the hippocampal CA3 and dentate gyrus. Interestingly, LPS-treated mice deprived of standard housing showed significant increases in BDNF and PSD-95 expression throughout the medial prefrontal cortex and hippocampus. Pubertal expression of neuroplasticity-promoting proteins is particularly susceptible to the introduction and removal of environmental stimuli and acute stress in CD-1 mouse models. Further investigations into the effects of stress on pubertal cortical reorganization should consider the inclusion of more ecologically valid housing conditions.

Disclosures: M.S. Murack: None. **K.B. Smith:** None. **J. Davidson:** None. **A. Kadamani:** None. **S. Al Sharani:** None. **C. Messier:** None. **N. Ismail:** None.

Digital Abstract Session

P247. Early-Life Stress: Adolescence

Program #/Poster #: P247.04

Topic: F.04. Stress and the Brain

Title: The Long-Term Embedding of Traumatic Early-Life Experiences Within the HPA-axis: A p-curve Meta-Analysis

Authors: *N. HOSSEINI-KAMKAR, J. MORTON;
Psychology, Western Univ., London, ON, Canada

Abstract: Introduction: Traumatic early-life experiences including child maltreatment, sexual abuse, and neglect have been linked to negative health outcomes later in life. One mechanism by which life-experiences may result in lasting changes in the brain is via exerting a long-term influence on the calibration of the hypothalamic-pituitary-adrenal (HPA) system. The *differential susceptibility to context* model presents a U-shaped function to describe the relationship between adversity and biological reactivity, predicting heightened reactivity under both protected and traumatic environmental conditions (Boyce & Ellis, 2005). Empirically, while trauma has consistently been shown to predict HPA dysregulation, the direction of the association remains unclear. Some studies show that trauma is associated with cortisol hyper-reactivity, while others show that trauma is associated with cortisol hypo-reactivity. *P*-curve analyses are ideally suited to resolving apparent contradictions of this kind (Simonsohn et al., 2014a). **Methods:** Here, we used *p*-curve analyses to review evidence concerning the association between traumatic experiences and HPA-reactivity to a psychosocial stressor. Publications that reported blunted HPA-reactivity were included in the "cortisol hypo-reactivity" *p*-curve analysis, while publications that reported heightened HPA-reactivity were included in the "cortisol hyper-reactivity" *p*-curve analysis. **Results: Cortisol hyper-reactivity *p*-curve:** The *p*-curve for the 21 results in the cortisol hyper-reactivity analysis was not significantly right-skewed. A set of results contains evidential value if either the half *p*-curve (*p*-values below .025) is significantly right-skewed at $p < .05$, or if both the half *p*-curve and full *p*-curve are significantly right-skewed at $p < .10$. The *p*-curve for this set of results was not significantly right-skewed (half: $z = -1.5$, $p = .067$; full: $z = -1.14$, $p = .127$). Neither condition was met in this case, therefore, the *p*-curve did **not** indicate evidential value. **Cortisol hypo-reactivity *p*-curve:** The *p*-curve for the 41 results in the cortisol hypo-reactivity analysis was significantly right-skewed (half: $z = -5.3$, $p < .0001$; full: $z = -6.29$, $p < .0001$). In this case, both conditions are met, and the *p*-curve demonstrates evidential value. **Conclusion:** The *p*-curve analysis of the HPA hypo-reactivity literature demonstrates evidential value; in contrast, the *p*-curve analysis of the HPA hyper-reactivity literature was inconclusive. Taken together, these results provide greater support for the literature reporting an association between traumatic life events and blunted HPA-reactivity to stress.

Disclosures: N. Hosseini-Kamkar: None. **J. Morton:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.01

Topic: F.04. Stress and the Brain

Support: Swiss National Science Foundation No. 152614 and 176206
Swiss National Science Foundation NCCR Synapsy grant Nos. 158776 and 185897
Eurotech Postdoc Programme

Title: Mitochondrial dynamics in the nucleus accumbens control anxiety and depression-like behaviors through regulation of medium spinal neurons structure and function

Authors: E. GEBARA[1], O. ZANOLETTI[1], S. GHOSAL[1], J. GROSSE[1], B. SCHNEIDER[1], G. KNOTT[1], S. ASTORI[1], ***C. SANDI**[2];
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Abstract: Mitochondria play central roles in psychiatric disorders, but the underlying molecular players that regulate neuronal structure and function are scarcely known. In this study, we assess the involvement of molecules involved in mitochondrial dynamics in medium spiny neurons (MSNs) from the nucleus accumbens (NAc) in rats selected for their high or low anxiety levels. We find that high anxious animals show increased depression-like behaviors in the forced swim and saccharin preference tests, along with lower expression of the mitochondrial GTPase Mitofusin 2 (Mfn2) in the NAc at the protein (Western blot) and mRNA (qRT-PCR, RNAscope) levels. They also show alterations in mitochondria (i.e., respiration, volume, interactions with the endoplasmic reticulum) and MSNs (i.e., dendritic complexity, spine density and typology, excitatory inputs). Viral AAV9-syn1-MFN2 overexpression in the NAc reverses all these behavioral, mitochondrial and neuronal phenotypes. Our findings demonstrate a causal role for accumbal Mfn2 on the regulation of anxiety and depression-like behaviors through actions on mitochondrial and MSN structure and function. Our work highlights the therapeutic potential of targetting Mfn2 to treat anxiety and mood disorders.

Disclosures: C. Sandi: None. **E. Gebara:** None. **O. Zanoletti:** None. **S. Ghosal:** None. **J. Grosse:** None. **B. Schneider:** None. **G. Knott:** None. **S. Astori:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.02

Topic: F.04. Stress and the Brain

Support: Hope for Depression Research Foundation

Title: Genomic signatures of corticosterone in dorsal and ventral hippocampus of males and females

Authors: ***S. G. CARADONNA**[1], H. KHALIL[2], N. R. EINHORN[1], F. S. LEE[3], H. AKIL[2], B. S. MCEWEN[1], J. MARROCCO[1];
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Abstract: Dorsal and ventral hippocampus are functionally distinct brain structures due to their unique anatomical connectivity and their role in distinct biological functions. More importantly, within the same brain areas, females and males may trigger distinct sets of genes in response to similar stimuli. We aimed to study the whole-genome transcripts of the dorsal (dHPC) and

ventral hippocampus (vHPC) in mice maintained on chronic low-dose (25mg/l) oral corticosterone (CORT), a mouse model that shows disruption of the hypothalamic-pituitary-adrenal axis associated with a blunted stress response. We used male and female mice that were wild-type (WT) or heterozygous for the brain-derived neurotrophic factor gene variant Val66Met (BDNF Val66Met) which increases susceptibility to stress. Differentially expressed genes (DEGs) of both vHPC and dHPC were generated using RNA-sequencing on an Illumina NextSeq 500 using 75-bp single-end reads. We found that CORT induced a greater number of DEGs in the vHPC than in the dHPC across experimental groups. After chronic CORT, WT males showed 184 DEGs in the vHPC and 119 DEGs in the dHPC. This difference was more prominent in BDNF Val66Met males compared to WT males because they showed 493 DEGs in the vHPC and 178 DEGs in the dHPC. Overall CORT induced a lower number of DEGs in females than in males. Curiously, WT females showed more DEGs in the dHPC than in the vHPC when treated with CORT. However, CORT had only a mild effect on DEGs in BDNF Val66Met females compared to WT females, especially in the dHPC. When investigating the common CORT-induced DEGs between males and females, the majority of DEGs were regulated exclusively within either sex across regions and genotypes. This suggests that chronic CORT modulates the genomic landscape of the dHPC and vHPC in a sex-, genotype-, and region-specific fashion. CORT-regulated DEGs of both the dHPC and vHPC were processed and grouped according to their GO terms, which showed unique gene sets in males, e.g., metabolism and cell signaling, and females, where CORT mainly induced immediate early genes. These findings show that discrete genomic signatures in the hippocampus associated with specific biological responses to CORT help to disentangle the separate biological role of the dHPC and vHPC as a function of sex and genotype.

Disclosures: **S.G. Caradonna:** None. **H. Khalil:** None. **N.R. Einhorn:** None. **F.S. Lee:** None. **H. Akil:** None. **B.S. McEwen:** None. **J. Marrocco:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.03

Topic: F.04. Stress and the Brain

Support: FONDECYT 119-0899
ENL0118-01

Title: Immediately and long-term effects of stress on spatial memmory and microRNAomics in male dorsal hippocampus

Authors: ***F. I. AGUAYO**[1], W. A. CORRALES[1], G. DÍAZ-VÉLIZ[2], F. A. OLAVE[1], J. SILVA[1], L. ROMÁN-ALBASINI[1], F. SIGCHO[1], C. NAVARRETE[1], J. A. CIDLOWSKI[3], J. L. FIEDLER[1];
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Abstract: Neuroplasticity is a process that allows short to long-term brain remodeling in response to experiences and changing environment. This includes changes in synaptic remodeling and functional modification of neural circuitries. Acute and chronic stimuli (physical or emotional) trigger the activation of the stress system in which the acute stress allows organism to adapt; while chronic stress lead to a maladaptive response. Different animal models of stress show altered hippocampal-dependent behaviors; nonetheless, little is known about the molecular mechanisms involved.
Considering that miRNAs play a key role in gene expression regulation, we evaluated the miRNA profile expression in dorsal hippocampus and the object location task in acute (2.5 h of restraint), acute recovery (24 h after stress) and chronic stress (2,5 h of restraint for 14 days). We showed that hierarchical clustering of miRNA expression profile shows a similarity between acute and chronic stress. Consistently, acute and chronic stressed animals showed a decrease preference to explore an object in a novel position. Interestingly, acute recovery group displayed not only a similar miRNA profile, but also a similar preference for novel location both compared to controls. The enrichment analysis predicts functional relevance of miRNA roles in modulation of signaling pathways related to cell survival, protein metabolism, inflammation, during the transition between acute and chronic stress.

Disclosures: F.I. Aguayo: None. **W.A. Corrales:** None. **G. Díaz-Véliz:** None. **J. Silva:** None. **F.A. Olave:** None. **L. Román-Albasini:** None. **J.A. Cidlowski:** None. **J.L. Fiedler:** None. **F. Sigcho:** None. **C. Navarrete:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.04

Topic: F.04. Stress and the Brain

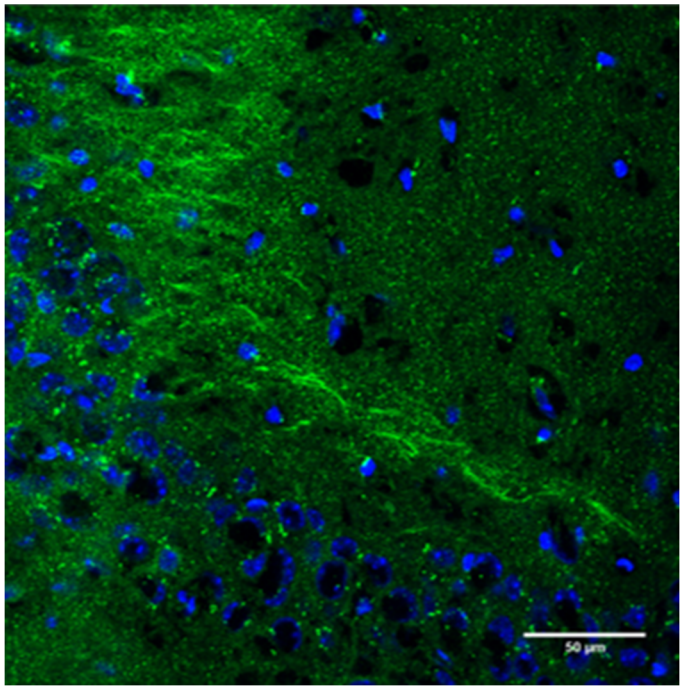
Support: Tubitak Grant 118S558

Title: Chronic stress activates p20 subunit of active caspase 1 in dendrites in CA2

Authors: *A. BAHADIR-VAROL[1], E. ESEN[1], G. YALCIN-CAKMAKLI[1], H. KARATAS[1], M. YEMISCI OZKAN[1], I. YALCIN-CHRISTMANN[2], T. DALKARA[1], B. ELIBOL[1], E. EREN-KOÇAK[1];
[1]Hacettepe Univ., Ankara, Turkey; [2]CNRS INCI, Strasbourg Cedex, France

Abstract: Introduction: Psychological stressor is shown to activate inflammasome complex, which activates caspase-1. We investigated active caspase-1 distribution in hippocampal sub-regions in mice following exposure to chronic stress as a model of depression.Material Method: C57BL6 male mice were exposed to repeated predator , tail suspension and restraint stresses for 35 days (n = 7). Control group was left undisturbed except for the behavioral experiments (n =

4). Hippocampal sections, 35 µm thick, were immunostained with antibodies against p10 and p20 subunits of active caspase-1 and imaged under confocal microscope.
Results and Conclusion: Labeling with antibody against the p20 subunit reveals staining in the apical dendrites of stratum radiatum in proximal parts of CA2 region only in the chronic stress group. Similar staining was not observed with anti-caspase-1 antibody against p10 subunit. In cell somas, on the other hand caspase 1 labeling was observed with both antibodies in both chronic stress and control groups. Our findings indicate that p20 subunit of caspase 1 may have a yet unknown function in dendrites. Given the role of synaptic plasticity in the neurobiology of depression, functional significance of p20 subunit of active caspase1 in regulating synaptic plasticity requires further investigation.



Disclosures: **M. Yemisci Ozkan:** None. **I. Yalcin-Christmann:** None. **T. Dalkara:** None. **A. Bahadir-varol:** None. **E. Esen:** None. **G. Yalcin-cakmakli:** None. **B. Elibol:** None. **H. Karatas:** None. **E. Eren-koçak:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.05

Topic: F.04. Stress and the Brain

Support: NIDA Grant 1R15 DA044500-01A1

Title: A crosstalk between Transient Receptor Potential Vanilloid 1 and Cannabinoid Receptor 1 within the limbic system regulates anxiety and depression-like behaviors triggered by stress in rats

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Abstract: Clinical studies provide strong evidence that stress is an environmental risk factor that can trigger the onset of several neuropsychiatric disorders such as anxiety and depression in humans. Pre-clinical evidence suggests that the endocannabinoid and endovanilloid system within the brain are important neuronal substrates involved in emotional responses to stress. Specifically, studies have proposed that the Transient Receptor Potential Vanilloid 1 (TRPV1) a member of the Transient Receptor Potential (TRP) superfamily, within the brain regulate anxiety and depression behaviors through its interactions with the cannabinoid receptor 1 (CB1R). However, little is known about the cellular mechanisms that regulate these receptors' interactions across the brain and its impact in neuropsychiatric disorders. We investigated the role of TRVP1R and CB1R within several brain regions including the medial prefrontal cortex as part of the dopamine mesocorticolimbic system, the hippocampus and the amygdala in anxiety and depression like-behaviors using rats as animal model. To modulate depression like-behavior, male Sprague Dawley rats were experienced a pretest session for 15 min, followed 24 h later by a 6 min test session in order to examine the effects of blockade of FAHH and its association with the olvanil in depression-like behaviors. One group of animals (acute exposure) received a single dose of URB597 [0; 0.1 ;0.2mg/kg/ip] 2 hours before testing in the FST for 6 min. The other group were treated for 4 days (repeated exposure) at the same doses. In order to examine the effects of the association of blockade of FAHH and the olvanil in depression like-behaviors, animals were treated for 4 days (repeated exposure) with URB597 [0; 0.1 ;0.2mg/kg/ip] and Olvanil [0; 0.3; 0.5 mg/kg/ip] only the last day (day 4^{th}) 2 hours before testing in the FST for 6 min to evaluate time spend in floating and swimming. However, to evaluate anxiety behavior, rats will be exposed to a repeat exposure for 4 days with either vehicle or URB597 at dose [0; 0.1mg;0.2mg/kg/ip] and Olvanil at dose [0; 0.3; 0.5 mg/kg/ip] only the last day (day 4^{th}) 90 min before exposed to 30 min acute restraint stress followed by 15 min light dark box test in order to measure the anxiety response. Our results suggest that the repeated exposure of URB597 significantly decreased depression like-behavior and tend to increase depression like-behavior when URB597 is associated with the olvanil. Ongoing biochemical analysis of the changes in expression of TRPV1 and CB1 receptors following treatment with URB597 alone and when it is associated with the olvanil will support mechanistic explanations for the present behavioral results.

Disclosures: W. Norze: None. **L. Rodriguez:** None. **P. Muñoz:** None. **A. Ramos:** None. **L. Mendez:** None. **C. Maldonado:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.06

Topic: F.04. Stress and the Brain

Support: NIH Grant MH108842

Title: Neural circuits and activity dynamics underlying sex-specific effects of chronic social isolation stress

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Abstract: Exposure to prolonged stress in critical developmental periods induces heightened vulnerability to psychiatric disorders, which may have sex-specific consequences. Here we investigate the neuronal circuits mediating behavioral changes in males and females after being subject to chronic adolescent social isolation stress. Escalated aggression is exhibited in stressed males, while social withdrawal is shown in stressed females. *In vivo* multichannel recordings of free-moving animals indicate that pyramidal neurons in the prefrontal cortex (PFC) from stressed males exhibit the significantly decreased spike activity during aggressive attacks, while PFC pyramidal neurons from stressed females show a blunted increase of discharge rates during sociability tests. Chemogenetic and electrophysiological evidence shows that PFC hypofunctioning and basolateral amygdala (BLA) principal neuron hyperactivity contribute to the elevated aggression in stressed males, while PFC hypofunctioning and ventral tegmental area (VTA) dopamine neuron hypoactivity contribute to the diminished sociability in stressed females. These results establish a framework for understanding the circuit and physiological mechanisms underlying the sex-specific divergent effects of early life stress.

Disclosures: T. Tan: None. **W. Wang:** None. **T. Liu:** None. **P. Zhong:** None. **M. Conrow:** None. **X. Tian:** None. **Z. Yan:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.07

Topic: F.04. Stress and the Brain

Support: NIH Grant R25HD090723-02

Title: Chronic mild stress induces differential depression-like symptoms and neural markers in high anxiety female Long Evans rats

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Abstract: Depression and anxiety disorders are comorbid in clinical populations and implicate overlapping neural systems that can be examined in validated animal models. We employed filial 8 female rats selectively outbred for extreme anxiety-like behavior and examined chronic mild stress-induced depression-like symptoms. We operationalized weight gain, sweet food consumption, piloerection, estrous cycling, and forced swim test coping behaviors as indices of depression-like symptoms (DLS). After a terminal forced swim test, brains were processed for immunohistochemistry for c-fos and serotonin 1A (5HT1A) receptor immunoreactive cells in the hippocampus and hypothalamus. We report evidence that F8 selectively outbred female rats exhibited extreme high (HAn) and low (LAn) anxiety-like behavior, similar to parental lines, on the elevated plus maze. An initial forced swim test revealed a differential response across extreme anxiety-like behavioral phenotypes, where HAn females exhibited more active coping styles than LAn females. On other DLS indices, chronic mild stress-treated HAn animals displayed enhanced active coping behaviors, attenuated weight gain, reduced sweet-food consumption, and stalled estrous cycling. Chronic mild stress treatment increased piloerection for all animals, independent of trait anxiety-line. Moreover, chronic mild stress treatment enhanced hypothalamic and hippocampal c-fos and 5HT1A receptor expression across HAn/LAn phenotypes—an effect that was greater in HAn animals. Thus, a female HAn phenotype conferred vulnerability to certain DLS indices (i.e., weight gain and attenuated sweet-food consumption) and resilience-like behavior to others (i.e., forced swim test coping behavior and increased consecutive estrous cycling). Together, our findings implicate the importance of using HAn lines to investigate neurobiological underpinnings of anxiety- and depression-like behaviors.

Disclosures: C.A. Calhoun: None. **B. Mason:** None. **C. Lattouf:** None. **V. Lewis:** None. **H. Barrientos:** None. **R. Mejia:** None. **S. Donaldson:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.08

Topic: F.04. Stress and the Brain

Title: Irisin suppresses acute stress-induced neurobehavioral impairment through hippocampal Akt/GSK3 pathway in a sex-dependent manner

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Abstract: Acute stress is an immediate physical or psychological perceived threat that induces anxiety-like behavior and short-term memory impairment by affecting the hippocampus. The hippocampus is one of the most stress-vulnerable regions of the brain, it also controls emotion

and spatial memory in rodents and humans. Acute stress suppresses glucose uptake in the hippocampus of male mice which leads to imbalanced energy homeostasis. In contrast, the exercise-induced myokine Irisin, induces thermogenesis, browning of white adipose tissue and modulates glucose homeostasis. Further, Irisin rescues memory impairment in an Alzheimer's disease (AD) model. We have previously shown that Irisin injection into the hippocampus reverses memory impairment caused by acute stress in male mice. However, the neural mechanism of action for Irisin has not been discovered. Therefore, we aimed to determine the signaling pathway that mediates the resilience effect of Irisin in the hippocampus. To this end, we used an acute restraint stress protocol for 3h in C57BL/6J mice (male and female) that resulted in memory impairment and anxiety-like behavior in a combined open field/novel object recognition (OF-NOR) test. Injection Irisin into the hippocampus prevented these effects. We then found that Irisin modulated Akt/GSK3β and PGC-1α expression only in the hippocampus of male mice. Akt/GSK3β axis is the main sensor for energy homeostasis in neurons. We found that acute stress decreased Akt activity while activation and expression of GSK3β and PGC-1α were increased. In response to energy deprivation, PGC-1α expression is increased to preserve energy production. We showed that Irisin injection into the hippocampus reversed PGC-1α expression and activated Akt signaling pathway. Combined, our findings provide a possible molecular pathway that supports behavioral findings. We therefore propose that acute stress results in abnormal energy homeostasis in the hippocampus which is reversed by Irisin injection. Together, our findings demonstrate a potential avenue to connect the positive effects of exercise on brain function, healthy aging, and neurodegeneration. Key Words: Irisin, acute stress, hippocampus, memory, anxiety-like behavior

Disclosures: **M. Jodeiri Farshbaf:** None. **A. Mondal:** None. **K. Alvina:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.09

Topic: F.04. Stress and the Brain

Support: NIH Grant R03NS109836

Title: Studying the effects of the pharmacological modulation of SERCA on behavior and monoaminergic neurochemical status in mice.

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Abstract: The sarco/endoplasmic reticulum (SR/ER) calcium (Ca^{2+})-ATPase (SERCA) pump is a key regulator of intracellular Ca^{2+} homeostasis and subsequently essential for cell survival and function. Neurons are no exception to this; intricate pathways involving SERCA-mediated Ca^{2+} signaling are implicated in brain pathophysiology. Several studies have indicated that

dysregulation of SERCA pumps may be involved in the molecular mechanisms underlying debilitating brain diseases including Alzheimer's and Parkinson's diseases, schizophrenia, bipolar disorder, ischemia and alcoholism. Thus, this family of P-type ATPases comprises an emerging molecular target for developing efficient pharmacotherapies. Interestingly, recent studies proposed that chronic pharmacological stimulation of SERCA2, mediated by the quinoline derivative CDN1163, may rescue aberrant locomotor and cognitive deficits in rodent models of Alzheimer's and Parkinson's diseases, introducing CDN1163 as a novel pharmacotherapeutic target. However, the extensive effects of CDN1163 administration on mouse behavior and neurotransmission still remain elusive. Herein, we investigated the potential consequences of both acute (i.e., 60-90min) and chronic (i.e., 17 days; once daily) CDN1163 administration on the behavior and neurotransmission in adult (i.e., 2-4 month old) C57BL/6J mice. Furthermore, we extended the study to assess dose-dependent responses in both sexes using a low dose (i.e., 10mg/kg, i.p.) and a high dose (i.e., 20mg/kg, i.p.) of CDN1163. Interestingly, we observed that chronic SERCA activation exerts behavioral effects in mice. Present data indicated an anxiogenic effect of the chronic CDN1163 regimen, as assessed in the open field test, as well as a depressive-like behavioral effect in chronically treated mice subjected to the forced swim test. Furthermore, these behavioral alterations were accompanied by multiple brain region-dependent neurochemical effects, as assessed *ex vivo* using reverse-phase High-Performance Liquid Chromatography (HPLC) in mice of both sexes.

Disclosures: A. Britzolaki: None. **C.C. Cronin:** None. **P.R. Flaherty:** None. **R.L. Rufo:** None. **P.M. Pitychoutis:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.10

Topic: F.04. Stress and the Brain

Support: CIHR

Title: Behavioural phenotype of novel sigma-1 receptor overexpression mouse line

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Abstract: The sigma-1 receptor (S1R) is an endoplasmic reticulum chaperone protein implicated in psychostimulant addiction and neurodegenerative diseases such as Alzheimer's disease and Huntington's disease. The endogenous pharmacology of S1R suggests sex-specific roles as it is modulated by sex hormones. The known interacting proteins of S1R include membrane bound proteins such as voltage-gated sodium, potassium and calcium channels and intracellular proteins such as the inositol trisphosphate receptor. In light of its diverse interactome, the mechanism by which S1R can alter behaviour is not understood. As a novel approach to investigate S1R, we have developed a S1R over expressing (OX) mouse line. Given that S1R modulates multiple

proteins, it is unlikely that S1R saturates its binding partners. This novel transgenic mouse was developed with a linearized *Thy1a1.2* driving SIGMAR1 tagged with *myc* on the C-terminus. Transgene integration was confirmed by PCR. Founder tissues show an elevated amount of S1R protein in multiple brain regions (hippocampus, midbrain, cortex, cerebellum). Male and female animals were assessed for phenotypic behaviour. The behavioural assays used were, in order, elevated plus, Y-maze, open field, forced swim, tail suspension, Morris water maze and passive avoidance. Data was analyzed by two-way ANOVA with sex and genotype being the interacting factors (male WT n = 33, male Sig-1R OX n = 17, female WT n = 16, female Sig-1R OX n = 19). Tukey's and Sidak's post-hoc analyses were used when appropriate. Genotype was a significant main effect in Y-maze alternation, distance travelled in Y-maze, distance travelled in elevated plus, distance travelled in open field, forced swim immobile time, passive avoidance probe trial latency and tail suspension immobility. Sex was a significant main effect in Y-maze alternation, Y-maze distance travelled, time spent in open arm of elevated plus, distance travelled in elevated plus, open field location, open field distance travelled, passive avoidance probe trial latency and tail suspension immobility. Sex of the animal and genotype did not result in a significant interaction in any of the behavioural tests. These results suggest a trend where S1R OX provides resistance to certain stressors such as forced swim and passive avoidance. Further, S1R OX mice tend cover more distance in the Y-maze, elevated plus maze and open field. Spatial memory learning in the Morris water maze is unaffected. Future plans are to expand the use of this mouse line into disease models and incorporate S1R pharmacological approaches.

Disclosures: K. McCann: None. **P. Chudalayandi:** None. **R. Bergeron:** None. **D. Dewar-Darch:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.11

Topic: F.04. Stress and the Brain

Title: Activity-dependent synaptic plasticity in prelimbic cortex underlies adaptive responses to adversity

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Abstract: Adverse experiences at different stages of life are relevant to promote vulnerable or resilient subjects. A vulnerable subject may develop mental diseases such as depression, anxiety or schizophrenia; a resilient subject will not. The development of these types of individuals could be due to several factors such as the nature of the experience, the stage of life at which the experience occurs, social support or genetics. However, there are no studies assessing the effect

of adversity during different stages of life and on synaptic plasticity. The present study aimed to evaluate if adverse events such as maternal separation (MS), mild social defeat in adult life (MSD) and maternal separation with mild social defeat (MS+MSD) promote vulnerable behaviors in the adult; and, whether these changes are associated with activity-dependent synaptic plasticity evaluated by c-Fos expression in the prefrontal cortex and hippocampus of rats. Behavioral results showed that conditions with MSD and MS+MSD promoted anhedonia and low motivation. Interestingly, the MS+MSD group increased the time spent in the area of social interaction. C-Fos analysis revealed greater number of positive cells in the prelimbic cortex of the MS+MSD group, which may be related to the increase of social interaction. A greater number of positive c-Fos cells were observed in the dentate gyrus when the animals underwent a single adverse event in adult life. These results suggest that medial prefrontal cortex and dentate gyrus could be systems involved in adaptive changes related to adversity.

Disclosures: C.J. Montes-Rodriguez: None. **V. Piña-Díaz:** None. **Z. Muñoz-Torres:** None. **E. Hernández-Reyes:** None. **O. Prospéro-García:** None.

Digital Abstract Session

P248. Cellular and Molecular Mechanisms in Animal Models of Stress and Anxiety

Program #/Poster #: P248.12

Topic: F.04. Stress and the Brain

Support: NIH/NIGMS Grant 1FI2GM128622-01
NIH/NIMH Grant K99MH123673-01
NIDA Intramural Research Program

Title: Nucleus Accumbens substance P underlies aversive learning

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Abstract: Responses to threat are an essential feature for animal survival and are a primary behavioral function of the Nucleus Accumbens (NAc). However, the synaptic and local circuit mechanisms in the NAc underlying these responses are not well known. In the NAc, highly aversive, stressful stimuli promote the release of the neuropeptide substance P from dopamine 1 receptor expressing medium spiny neurons (MSNs). We previously found substance P causes long-term potentiation (LTP) of excitatory inputs on dopamine 2 receptor (D2) expressing MSNs in the NAc core, which may provide a synaptic substrate for aversive learning. We hypothesized striatal substance P-driven LTP is required for aversive learning. To test this, we utilized a fear conditioning paradigm with a latent inhibition component and assessed how D2-MSN activity contributes to the learned association of a conditioned stimulus (CS, tone) with an aversive unconditioned stimulus (US, footshock). In the NAc core, c-fos-positive cell density in D2-MSNs correlated strongly with freezing behavior to the CS. In contrast, when a latent inhibition

procedure preceded conditioning and diminished cue salience, this resulted in decreased c-fos expression in D2-MSNs in response to the CS. This correlation was not observed in the NAc shell, in D1-MSNs, or in the context alone without the CS. Additionally, CRISPR-Cas9 knockout of NAc neurokinin 1 receptors (NK1R), the primary substance P receptor, diminished freezing to the CS during recall. These results suggest NAc core NK1Rs and D2-MSN activity contribute to the learned association between a cue and an aversive stimulus. In mice that underwent fear conditioning, electrophysiological measures for substance P release indicative of LTP in D2-MSNs were consistent with this finding and suggest plasticity is selective for the CS-shock relationship. We are currently examining *in vivo* D2-MSN activity to determine temporal dynamics of D2-MSN and relevant input activity during fear learning with and without substance P antagonism. Our work provides the foundation for a MSN crosstalk model for aversive learning and may suggest a cellular substrate for aberrant salience processing observed in mood disorders such as anxiety and behavioral depression.

Disclosures: T.C. Francis: None. **C. Sanders:** None. **M. Morales:** None. **G. Schoenbaum:** None.

Digital Abstract Session

P249. Stress Modulation of Hippocampal Circuits

Program #/Poster #: P249.01

Topic: F.04. Stress and the Brain

Support: Grant-in-Aid for Scientific Research from MEXT (19H05646; T.J.M)
Grant-in-Aid for Scientific Research on Innovative Areas from MEXT (19H05233; T.J.M)

Title: Stress alters rodent hippocampal neural dynamics

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Abstract: Adverse effects of chronic stress include loss of concentration, anxiety, depression, and impaired cognition including learning and memory deficits. Some of these deficits are mediated by stress-induced impaired hippocampal functionality at multiple levels of neural organization including dendritic shrinkage and neurogenesis and impaired synaptic plasticity. In agreement, studies in awake behaving rodents have reported that chronic stress alters place cell activity and disrupts global remapping which is the generation of distinct representations (place maps) across different surroundings. Surprisingly, it is not known if and how chronic stress alters hippocampal information processing during offline behaviour states, including quiet wakefulness/sleep, during which information consolidation takes place during sharp-wave ripples (SPW-Rs or ripples), high frequency (100-200 Hz) oscillatory events in the CA1 region. Here, we employed tetrode recordings in mice (N=17) and subjected them to chronic

immobilization stress (CIS), a well-established rodent model of chronic stress and assessed pyramidal cell activity and SPW-Rs in the dorsal CA1 of the hippocampus. We noticed a net decrease in pyramidal cell activity during the first exposure to a stress compared to the prior quiet wakefulness/sleep state. We also noticed lower firing rates and decreased participation by pyramidal cells during SPW-Rs, though pyramidal cell co-activity was enhanced during stress exposure. Interestingly, after repeated exposure to same stress, some of these stress-phenotypes were noticed during the rest-state on the last day of CIS, when no overt stress was delivered. These data indicate that chronic stress alters hippocampal neural dynamics and that disrupted ripple-spike interactions may underlie the impaired learning observed in chronically stressed subjects.

Disclosures: **A. Tomar:** None. **D. Polygalov:** None. **S. Chattarji:** None. **T.J. McHugh:** None.

Digital Abstract Session

P249. Stress Modulation of Hippocampal Circuits

Program #/Poster #: P249.02

Topic: F.04. Stress and the Brain

Title: Hippocampal modulation of hypothalamic circuits in the control of acute stress responses

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Abstract: An essential adaptive function of the nervous system is to organize appropriate physiological and behavioral responses in the face of stressful situations. However, this process can become dysregulated in psychiatric disease. Characterizing the neural circuits that modify the adaptive stress response is essential to understand how the brain regulates responses to stressful stimuli and how that regulation can go awry. A population of neurons in the hypothalamic paraventricular nucleus (PVN) expressing corticotropin-releasing hormone (CRH) integrate information about stressful stimuli before initiating responses such as the release of corticosteroids. The ventral hippocampus (vHPC) is theorized to modulate neuroendocrine responses to threat, but how it may modulate CRH neuron activity to control acute stress responses remains unknown. Here we use optogenetics, chemogenetic, fiber photometry and 2-photon calcium imaging in male and female adult mice to investigate how the hippocampus acutely modulates PVN CRH cells in response to an acute stressor. Our results suggest the ventral hippocampus (vHPC) exerts top-down control over the paraventricular nucleus of the hypothalamus (PVN) in order to modulate moment-to-moment neural responses to acute stressors.

Disclosures: **V.S. Turner:** None. **M.A. Kheirbek:** None. **F. Stefanini:** None.

Digital Abstract Session

P249. Stress Modulation of Hippocampal Circuits

Program #/Poster #: P249.03

Topic: F.04. Stress and the Brain

Support: MH107435

Title: Endocannabinoid Regulation of Innate Fear Responding

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Abstract: Anxiety disorders, including generalized anxiety disorder (GAD) and post-traumatic stress disorder (PTSD), are the most common mental illness in the United States. The endocannabinoid (eCB) system includes critical regulators of the processes involving fear responsivity and stress adaptivity and thus has been identified as an emerging therapeutic target to treat anxiety disorders. Recent evidence suggests that glutamatergic input from the ventral hippocampus (vHIPP) to the nucleus accumbens (NAc) underlies stress susceptibility. Here, using slice electrophysiology and optogenetics, we assess how eCBs modulate vHIPP-NAc activity ex vivo and in vivo and the role this plays in mediating stress responsivity. We have shown that optogenetic activation of vHIPP-NAc circuit increases avoidance of an innate stressor, the predator odor analog, 2MT. Avoidance of 2MT can be reduced by enhancing eCB signaling, and we have shown that eCBs negatively regulate glutamate release at vHIPP input onto specific interneuron populations in the NAc. This data ultimately increases our understanding of how eCBs mediate potentially therapeutic effects for stress-related behaviors.

Disclosures: V. Kondev: None. **A. Morgan:** None. **M.S. Najeed:** None. **S. Patel:** None.

Digital Abstract Session

P249. Stress Modulation of Hippocampal Circuits

Program #/Poster #: P249.04

Topic: H.08. Learning and Memory

Support: DAFCYT-2003IDPTNNN0020

Title: Serotonin transporter in dorsal hippocampus in prenatally stressed rats and its relation with learning and memory

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Abstract: Currently, stress is considered a state in which the body responds to environmental demands. This response is essential and crucial for survival. Stress can even occur during the gestational period. When stress occurs during gestational, period, it can disrupt the hippocampus physiology, causing cognitive deficits in the adult body, altering several neurotransmission systems, such as the serotonergic system. Thus, the objective of this study was to evaluate the release of serotonin as well as serotonin transporter (SERT) content in the dorsal hippocampus, related with learning and spatial memory in prenatally stressed (PS) adult male rats. Control and PS, 3-month-old males were used. Serotonin release was assessed and dorsal hippocampus was dissected under basal conditions. This structure was also obtained from other rats after performing learning and memory tests, using the Morris Water Maze (MWM). Results show that cognitive deficits were confirmed in PS males, compared to controls groups. Basal SERT content was higher in the dorsal hippocampus of PS animals than in control males, which is in line with the low basal extracellular concentration of serotonin observed in the PS animals. No significant changes were observed in SERT content during cognitive tests in PS animals compared to controls, suggesting that serotonin reuptake during cognitive processes is similar in both groups, as s extracellular concentration of serotonin increased in control and PS males. In conclusion, the cognitive deficiencies observed in PS males seem to be related with low serotonin extracellular concentrations, but not with changes in SERT.

Disclosures: D. Mendez-Guerrero: None. **F. Jiménez Vásquez:** None. **M. Rubio Osornio:** None. **M. Rubio Osornio:** None. **S. Orozco-Suarez:** None. **S. Retana Marquez:** None.

Digital Abstract Session

P250. Stress Regulation: Hormones, Hypothalamus and Extended Amygdala

Program #/Poster #: P250.01

Topic: F.04. Stress and the Brain

Support: NSERC
CIHR

Title: Hypothalamic sex differences in basal reelin and sexually dimorphic effects of chronic stress induced by CORT.

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Abstract: Introduction: Repeated exposure to the stress hormone corticosterone (CORT) can cause depressive-like behaviours paralleled by a downregulation of hippocampal reelin expression, a protein highly involved in neural plasticity. Reelin is also expressed in other key neural populations involved in the stress response, like the paraventricular nucleus (PVN), but

whether its expression is sex specific and involved in female's sex-specific vulnerability to stress and depression is not known.**Methods:** Male and female rats were treated with either daily vehicle or CORT injections (40mg/kg) for 21 days. Thereafter, they underwent the forced swim test and elevated plus maze to measure depressive-like behaviours before being sacrificed to permit quantification of reelin levels in hypothalamic nuclei using immunohistochemical techniques. **Results:** We found that the basal density of reelin-positive cells was significantly higher in males than female rats in the medial preoptic area ($p<0.01$) and PVN($p<0.05$). CORT treatment increased despair-like behaviour equally in both sexes compared to controls which was paralleled with a decrease in PVN reelin-positive cells in males ($p<0.001$) but not females ($p<0.581$). Interestingly, in male's PVN, 30% of basal reelin-positive cells co-stained for oxytocin while in females only 17.5%. Levels of CRF and AVP-IF cells were really low and barely co-localized with reelin and CORT did not affect co-localization of reelin with any of these markers. **Conclusion:** For the first time, this study shows that there is a sexually dimorphic subpopulation of reelin-positive neurons in the PVN that can be differentially affected by chronic stress. Further studies should be done to elucidate the potential role of these neural populations in individuals' susceptibility to stress and depression.

Disclosures: C. Liria Sanchez-Lafuente: None. **R. Romay-Tallon:** None. **J. Johnston:** None. **J. Allen:** None. **L. Kalynchuk:** None. **H.J. Caruncho:** None.

Digital Abstract Session

P250. Stress Regulation: Hormones, Hypothalamus and Extended Amygdala

Program #/Poster #: P250.02

Topic: F.04. Stress and the Brain

Support: NIAAA-R01-AA024095
Bowles Center for Alcohol Studies at the University of North Carolina at Chapel Hill

Title: Sex and regional differences in (3α,5α)3-hydroxypregnan-20-one (3α,5α-THP) regulation of hypothalamic and extrahypothalamic corticotropin-releasing factor (CRF).

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Abstract: Corticotropin-releasing factor (CRF) is the main activator of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress. CRF neurons are mainly in the hypothalamus, but CRF positive cells are also found in extrahypothalamic structures, including central nucleus of amygdala (CeA), hippocampus (HP), nucleus accumbens (NAc) and ventral tegmental area (VTA). An appropriate balance between inhibitory GABAergic and excitatory glutamatergic

inputs regulates CRF release and alterations in this system are found in several neuropsychiatric diseases, including drug addiction. 3α,5α-THP, the most potent positive modulator of $GABA_A$ receptors has previously been reported to attenuate basal and stress-induced hypothalamic CRF mRNA expression as well as ACTH and corticosterone serum levels in male rats. In this study, we investigated 3α,5α-THP regulation of CRF in hypothalamus and extrahypothalamic areas, in male and female Sprague Dawley rats, measuring CRF mRNA and peptide expression after 3α,5α-THP intraperitoneal administration (15 mg/kg). Sprague Dawley rats were injected with 3α,5α-THP (15mg/kg) in hydroxypropyl-beta-cyclodextrin (45%) or an equivalent volume of vehicle, sacrificed 45 min later, and brains were harvested for qPCR and western blot measurements of CRF mRNA and peptide. Data were analyzed statistically by 2-WAY ANOVA followed by the Tukey's post-hoc test. We found sex differences in hypothalamic CRF mRNA expression (females +74%, $p<0.01$) and CRF peptide levels (females -71%, $p<0.001$). 3α,5α-THP administration decreased hypothalamic CRF mRNA expression in males (-50%, $p<0.05$) and did not change CRF peptide expression in either sex. In HP and CeA, 3α,5α-THP administration decreased CRF peptide concentrations in male rats (HP -29%, $p<0.05$; CeA -62%, $p<0.01$). In VTA, 3α,5α-THP administration increased CRF peptide concentration in both males (+32%, $p<0.01$) and females (+26%, $p<0.01$). The results show sex and region-specific CRF signal regulation, at baseline and after 3α,5α-THP administration. This data may be key to develop sex-specific therapeutic approaches for stress-related disorders and addiction.

Disclosures: G. Boero: None. **R.E. Tyler:** None. **C.A. Todd:** None. **T.K. O'Buckley:** None. **I. Balan:** None. **J. Besheer:** None. **A. Morrow:** None.

Digital Abstract Session

P250. Stress Regulation: Hormones, Hypothalamus and Extended Amygdala

Program #/Poster #: P250.03

Topic: F.04. Stress and the Brain

Support: R15MH118692

Title: Androgen and androgen receptor regulation of corticotropin-releasing factor receptor 1 in the mouse forebrain

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Abstract: Stress related mood disorders such as anxiety and depression are two times more prevalent in women than men. Sex differences in these disorders are often associated with dysregulation of HPA axis function and have been demonstrated across multiple species including both rodents and humans. Androgen actions through androgen receptors (AR) have been shown to produce actions that decrease the HPA axis response and anxiety behaviors. Corticotropic releasing factor (CRF) and its binding to the CRFR1 receptor has been shown to

play a major role in the regulation of the HPA axis as well as anxiety and depression. We first tested whether CRFR1 neurons co-localize with AR and whether this differed by sex by using dual label immunohistochemistry. Using CRFR1-GFP mice, we found high co-localization of AR in CRFR1 neurons within the paraventricular nucleus (PVN), bed nucleus of the stria terminalis (dorsal and ventral divisions; BSTd, BSTv), medial preoptic area (MPOA), and posterodorsal nucleus of the medial amygdala (MePD). To test whether androgens are capable of altering CRFR1 levels, male CRFR1-GFP mice were sham operated, castrated (GDX), or castrated with supplementation of dihydrotestosterone (GDX-DHT), a non-aromatizable androgen that preferentially binds to ARs. The PVN showed a decreased CRFR1 cell number after GDX, but DHT treatment reversed this effect. In the BSTd, GDX-DHT treated mice showed a decrease in CRFR1 compared to both sham and GDX groups. No other brain regions showed differences in CRFR1 resulting from either GDX or DHT treatment. Our lab also assessed neural activation of CRFR1 neurons in mice that experienced 30-minute restraint stress. We found that there were fewer c-Fos CRFR1 co-localized neurons in GDX males compared to both sham and DHT treated mice in the MePD. No statistical differences were found in other brain regions. When assessing plasma corticosterone (CORT), we replicated previous findings that GDX males showed increased CORT levels when compared to GDX-DHT treated or sham operated males at both 30- and 90-minutes following restraint stress. Finally, we showed a negative correlation between the number of PVN CRFR1+ neurons and CORT levels at 90 minutes following restraint stress. Together these findings indicate androgens can directly alter levels of CRFR1 in the brain and has potential implications for sex differences in regulation of the HPA axis and stress-related mood disorder prevalence.

Disclosures: K.A. Rybka: None. **K. Sturm:** None. **R.M. De Guzman:** None. **J.S. Jacobskind:** None. **Z.J. Rosinger:** None. **D.G. Zuloaga:** None. **S. Bah:** None.

Digital Abstract Session

P250. Stress Regulation: Hormones, Hypothalamus and Extended Amygdala

Program #/Poster #: P250.04

Topic: F.04. Stress and the Brain

Support: NIH 3R01NS113104

Title: Regulation of stress-induced pair bond formation impairments in the socially monogamous prairie vole (Microtus ochrogaster) by extended amygdala corticotrophin-releasing hormone neurons

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Abstract: Although social interaction is typically a beneficial experience, social conflict can have a significantly negative effect on normal social behavior. The effect of social conflict on social withdrawal is a subject of significant investigation, but the impact of social conflict on the

formation of close social connections is not well understood. The primary goal of this study was to use the social defeat model of social conflict to investigate its effect on social bonding in the prairie vole (*Microtus ochrogaster*), a socially monogamous rodent species that forms strong social bonds between mating pairs known as a *pair bond*. One week after a three-day social defeat protocol, male prairie voles demonstrated an impairment in pair bond formation, but pair bond formation was accelerated in female prairie voles. Using chemogenetic manipulation, we have identified corticotrophin-releasing hormone (CRH)ergic neurons in the bed nucleus of the stria terminalis (BNST) as a novel neurobiological mechanism regulating pair bond formation in prairie voles. Since CRH in the BNST has been demonstrated to have a role in the behavioral response to social defeat and stress-dependent motivation, we hypothesize that this system would be a key modulator for the effect of social defeat on pair bond formation. Similar to BNST CRHergic neuronal activation leading to accelerated pair bond formation in male prairie voles, chemogenetic inhibition of these neurons in stress-naïve voles inhibits pair bond formation, demonstrating the sufficiency and necessity of this system in prairie vole pair bond formation. In contrast, inhibition of BNST CRHergic neurons in socially defeated prairie voles reverses the deleterious effect of defeat on pair bond formation. These results suggest a stress-dependent "switch" function of BNST CRHergic neurons that has an essential role in the impact of social stress on future social interaction and relationship formation.

Disclosures: M.C. Tickerhoof: None. **A.S. Smith:** None.

Digital Abstract Session

P250. Stress Regulation: Hormones, Hypothalamus and Extended Amygdala

Program #/Poster #: P250.05

Topic: F.04. Stress and the Brain

Support: NIMH RO1 MH113007

Title: Role of corticotropin-releasing factor (CRF) neurons in the oval nucleus of the bed nucleus of the stria terminalis on anxiety-like behavior

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Abstract: Corticotropin-releasing factor (CRF) is a neuropeptide responsible for regulating autonomic, endocrine, and behavioral responses to stress. A significant population of CRF-producing neurons is found in the oval nucleus of the bed nucleus of the stria terminalis (BNSTov), a brain region that mediates behavioral responses to stressors such as fear and anxiety. We used a Cre-CRF transgenic rat model expressing Cre-recombinase driven by the CRF promoter to investigate the role of CRF neurons in the BNSTov in modulating anxiety-like behavior. We infused AAV driving inhibitory or excitatory, Cre-dependent designer receptors

exclusively activated by designer drugs (DREADDs) fused to reporter protein, mCherry, into the $BNST_{OV}$ of Cre-/- and Cre+/- male adult rats. We then silenced or activated these CRF neurons with DREADDs selective ligand, clozapine-N-oxide (CNO), and measured the corresponding effects on anxiety-like behavior via elevated plus maze (EPM) and acoustic startle response (ASR). We also investigated the effect of additional stressor with light-potentiated startle (LPS) and startle sensitization (SS) experiments.
Inhibition For EPM, percentage time spent in open arms did not differ between Cre- and Cre+ rats post CNO injection. For ASR, we tested baseline ASR (pre test) and ASR after CNO (post test). Although no time or genotype effect, we observed a significant interaction between the two, suggesting that systemic injection before post test affected ASR differently in Cre- vs Cre+. With SS, we measured ASR before and after a series of sensitizing footshocks. Analysis revealed a significant time (P=0.0046) and genotype (P=0.0166) effect. In Cre- rats, we observed heightened ASR post shock compared to pre shock, an effect significantly attenuated in Cre+ rats. In addition, Cre- rats post shock ASR were significantly higher than those of Cre+ with CNO.
Activation For EPM, percentage time spent in open arms did not differ between Cre- and Cre+ rats after CNO. For ASR, there was no time or genotype effect. For LPS, we compared ASR in dark/dark and dark/light phases. Light exposure did not heighten ASR; however, it revealed a significant effect of genotype (P=0.0224) showing Cre- rats startling more than Cre+. For SS, shocks significantly heightened ASR in both groups (P=0.0329) and Cre- rats showed higher ASR overall compared to Cre+ (P=0.0343).
Overall, our results suggest that although silencing CRF neurons in the $BNST_{OV}$ does not affect baseline anxiety-like behavior in male rats, it attenuates stress-induced startle sensitization. Activating CRF neurons does not potentiate baseline anxiety-like behavior nor stress-induced startle.

Disclosures: **R. Chudoba:** None. **V. Olivera:** None. **P. Lis:** None. **C. Edmondson:** None. **S.H. Losee Olson:** None. **J.A. Dabrowska:** None.

Digital Abstract Session

P250. Stress Regulation: Hormones, Hypothalamus and Extended Amygdala

Program #/Poster #: P250.06

Topic: F.04. Stress and the Brain

Support: CIHR

Title: Chronic social defeat stress increases ghrelin infiltration in the arcuate nucleus in mice

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Abstract: Ghrelin is a stomach-derived peptide hormone that increases food intake through the central activation of the growth hormone secretagogue receptor (GHSR). Circulating ghrelin

levels rise in response to chronic social stress, inducing increased feeding and weight gain in mice. Ghrelin primarily functions in the brain to exert its orexigenic effects, however, ghrelin movement into the brain is extremely limited due to the blood brain barrier. Notably, social stressors have been found to increase permeability of the blood brain barrier. In this study, we investigated the effect of chronic social defeat stress on ghrelin movement into the brain in mice. Male mice underwent 21-days of chronic social defeat stress before being peripherally injected with fluorescently labelled ghrelin, Cy5-ghrelin. The results showed that stress exposure increased Cy5-ghrelin fluorescence in the arcuate nucleus of the hypothalamus, compared to non-stressed controls. Despite increased Cy5-ghrelin accumulation in the arcuate nucleus, stressed mice did not exhibit changes to ghrelin movement in other brain regions. Overall, the results suggest that increased food intake during stress may be facilitated by the increased ghrelin accumulation in the arcuate nucleus.

Disclosures: A. Smith: None. **L. Hyland:** None. **A. Abizaid:** None.

Digital Abstract Session

P250. Stress Regulation: Hormones, Hypothalamus and Extended Amygdala

Program #/Poster #: P250.07

Topic: F.04. Stress and the Brain

Support: Otsuka Pharmaceuticals

Title: Changes in gut microbiota are associated with anxiety and estradiol treatment in female mice

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Abstract: Declining levels of estrogens during menopause are associated with increased risk of anxiety, depression, diabetes, obesity and cancer. Similarly, removal of estrogens by ovariectomy increases weight gain and anxiety in rodents. Mounting evidence suggests gut microbiota influence anxiety and metabolism via the gut microbiome-brain axis. The present study investigated the effects of estradiol (E) and high-fat diet (HFD) on anxiety, gut microbiota and metabolism. Adult C57BL6J mice were ovariectomized and implanted sc with capsules containing E (50 μg) or vehicle (V). Within each treatment group, mice were fed a standard diet (SD) for 10 days and maintained on SD (n=16) or switched to HFD (n=16) for 26 days. Anxiety behavior was assessed with Light-Dark (LD) and Elevated-Plus Maze (EPM) tests at the end of the study. Following perfusions, whole brains were labeled for c-fos using iDISCO. Fecal DNA was sequenced for 16s rRNA genes to identify bacteria that correlated with E treatment, body

weight or were most predictive of anxiety. As reported previously, V mice on HFD became obese, while E mice remained lean, indicating that E protected against HFD-induced obesity. E reduced anxiety behavior on the LD and EPM tests. Interestingly, in HFD-fed mice, E decreased c-fos expression in the paraventricular hypothalamic nucleus and the subparafascicular thalamic nucleus, regions involved in anxiety, as well as the lateral hypothalamic and medial preoptic areas, which regulate energy homeostasis. E increased relative abundances of the families Bacteroidaceae, Alcaligenaceae and Verrucomicrobiaceae, namely the genus *Akkermansia*. E decreased relative abundances of Peptostreptococcaceae, Mollicutes and Clostridiales of the phylum Firmicutes. *Akkermansia* levels were negatively correlated with body weight, and positively associated with E treatment, suggesting this microbe protects mice from HFD-induced obesity. Increased abundances of Clostridiales and Alcaligenaceae (just prior to behavioral testing) and Peptostreptococcaceae and Erysipelotricaceae (longitudinally) were associated with low anxiety. Taken together, these findings provide insight into gut microbiota-based therapies for anxiety and metabolic disorders associated with declining estrogens in menopausal women.

Disclosures: H.N. Raman: None. **M.E. Graham:** None. **K.D. Acharya:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH. **A. Parakoyi:** None. **A. Corcoran:** None. **M. Belete:** None. **B. Ramaswamy:** None. **I. Sachar:** None. **K. Derendorf:** None. **S. Gottipati:** None. **M. Tetel:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Otsuka Pharmaceuticals.

Digital Abstract Session

P251. The Impact of Stress and Stress-Related Mechanisms On Cognition and Behavior

Program #/Poster #: P251.01

Topic: G.05. Mood Disorders

Support: A.Morgunova: This research was undertaken thanks in part to funding from the Canada First Research Excellence Fund, awarded to McGill University for the Healthy Brains, Healthy Lives initiative.
C.Flores: supported by the National Institute on Drug Abuse (R01DA037911), the Canadian Institutes for Health Research (MOP-74709; MOP-119543)

Title: Examining male and female vulnerability to chronic social defeat stress

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Abstract: Background: Major depressive disorder (MDD) is a chronic disabling disorder often induced by stress, affecting over 300 million people worldwide. Females are twice as likely to experience depression as males. The goal of this study was to characterize stress-induced

behavioral and molecular changes in adult male and female mice in the recently developed chronic non-discriminatory social defeat stress (CNSDS) model, in which a male and a female mouse are simultaneously subjected to social aggression.
Methods: The CNSDS was carried out to measure vulnerability to stress-induced behavioral abnormalities associated with depression-like traits in male and female mice. After 10 days of the social defeat paradigm, mice were assessed in the social interaction test and were segregated into susceptible and resilient phenotypes according to their interaction ratio. Mice then underwent behavioral tests, including the dark-light test. Trunk blood and brain samples were collected at the end of the experiment to quantify microRNA expression using Real-Time PCR analysis. **Results**: Female mice received very few attacks compared to males (4 versus 15). Nonetheless, a small percentage of females exposed to stress showed social avoidance, indicating stress hypersensitivity. Indeed, these susceptible females, but not susceptible males, showed increased anxiety-like behavior in the light dark-test, in comparison to their controls and resilient counterparts. **Conclusions**: Our results suggest that a subgroup of female mice are particularly sensitive to stress-induced social avoidance and anxiety-related behaviors. Results from microRNAs analysis will shed light into possible mechanisms underlying this trait.

Disclosures: **A. Morgunova:** None. **A. Mahmud:** None. **M. Giroux:** None. **S. Zhao:** None. **C. Flores:** None.

Digital Abstract Session

P251. The Impact of Stress and Stress-Related Mechanisms On Cognition and Behavior

Program #/Poster #: P251.02

Topic: F.04. Stress and the Brain

Support: DGAPA PAPIIT IN306918
PAPIME PE306918

Title: Sex differences in declarative memory, anxiety and depression behaviors after stress exposure

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Abstract: Several studies have demonstrated sex differences in neuroendocrine and behavioral responses to stress, where females have showed more impairment than males, which could explain the higher prevalence in females to develop stress related disorders. Additionally, most neural and behavioral studies not included females, however, the pattern suggest that stressed males have downregulation on neural activity while stressed females have not alter or have

upregulation of neural activity. The above, supports the idea of sex specific stress effects on behavior. The aim of this study was to compare female vs male responses to a predator scent stress (PSS). Male and female Wistar rats 12 weeks old were used (n=10 per group). PSS groups were exposed for 10 minutes in an exposure box that contained a bottle with scent tag impregnated with predator urine. Behavior was assessed with novel object recognition, open field, zero maze, and saccharin preference tests 24 hours after the final stressor. The results obtained in saccharine preference test were PSS-exposed females had lower preference for saccharine in comparison with control females and control males had a lower preference than control females. PSS-exposed females and males had a highest water consumption than control females and males. Saccharine consumption were higher in females than males no matter if they were stressed or not. In novel object recognition test the results were females had lower recognition index than males. PSS females spent more time explore the familiar object while PSS males explored more time the novel object. PSS females and males had higher latencies to explore the novel object for the first time than control females and males. In open field test PSS females and males spent less time in center area than PSS males. As well, females had more crosses in open field arena than males, but PSS females and males had fewer total crosses than control females and males. Additionally, lower locomotor activity can be explained by the increased time of grooming and immobility in PSS females and males. In zero maze test the results were PSS males had fewer entries and time in open arm than PSS females and control males even though none of the results in zero maze were statistically significative. The above suggests that PSS induced impairment on recognition memory in females and males but not in the same way. Also, PSS induced depression- and anxiety-like behaviors. That impairments could be associated with sex-specific alterations in areas important for learning and memory like the prefrontal cortex and hippocampus and for areas mediating mood and anxiety such amygdala.

Disclosures: P. Torres-Carrillo: None. **Y.B. Vidal-de la O:** None. **D.B. Paz-Trejo:** None. **L.D. Ochoa-de la Paz:** None. **H. Sanchez-Castillo:** None.

Digital Abstract Session

P251. The Impact of Stress and Stress-Related Mechanisms On Cognition and Behavior

Program #/Poster #: P251.03

Topic: H.04. Executive Functions

Support: NIH grant AA026421 (RS)

Title: Chronic Unpredictable Stress during Development Leads to Attention Deficits in Both Male and Female Rats

Authors: *F. KWARTENG[1], R. WANG[2], V. MICOV[1], K. A. HAUSKNECHT[1], M. TURK[1], K. ISHIWARI[1], S. OUBRAIM[1], J. B. RICHARDS[1], S. HAJ-DAHMANE[1], R. YU SHEN[1]; [1]Pharmacol. and Toxicology, [2]Toxicology and Pharmacol., Univ. at Buffalo, Buffalo, NY

Abstract: Chronic Unpredictable Stress during Development Leads to Attention Deficits in Both Male and Female Rats

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Background. Adversity during development (e.g. child abuse and neglect) is linked to multiple behavioral and cognitive conditions observed in later life, such as impulsive behavior and attention deficit/hyperactivity disorder (ADHD) in which the prefrontal cortex (PFC) are implicated. The PFC undergoes protracted development through adolescence and early adulthood. As such, adversity in early life could have a profound impact on this brain region, leading to disorders like ADHD. The underlying neural mechanisms of this process, however, are still unclear. The purpose of the present study was to establish a rat model to study attention deficits caused by chronic stress during development.

Materials and Methods. Four- to seven-week-old male and female Sprague Dawley rats underwent a mild chronic unpredictable stress (CUS) procedure for two weeks. Eleven different stressors (e.g., cage vibration, cage tilting, soiled bedding, restraint, and food deprivation) were applied once or twice in an unpredictable fashion. The rats then underwent a 2-choice reaction time (2-CRT) task, which could detect major attention deficits, including action impulsivity and lapses of attention. In addition, an open field test was conducted to examine if the CUS procedure also led to increased anxiety.

Results. In the 2-CRT task, both male and female rats that underwent the CUS procedure displayed increased action impulsivity, exhibited by elevated premature responses, compared with controls. Augmented lapses of attention were observed in female but not male CUS rats, shown by increased incorrect responses. Furthermore, both male and female CUS rats spent less time in the center area of the open field, indicating heightened anxiety.

Conclusion. Chronic unpredictable stress during development leads to increased attention deficits in both male and female rats. The deficits are more severe in females than in males. The rats undergoing CUS also show increased anxiety-like behavior. The elevated anxiety could exacerbate the attention deficits in these animals.

Keywords (up to 3): attention, chronic unpredictable stress, anxiety
Supported by by NIH grant AA026421 (RS)

Disclosures: F. Kwarteng: None. **R. Wang:** None. **V. Micov:** None. **K.A. Hausknecht:** None. **M. Turk:** None. **K. Ishiwari:** None. **S. Oubraim:** None. **J.B. Richards:** None. **S. Haj-Dahmane:** None. **R. Yu Shen:** None.

Digital Abstract Session

P251. The Impact of Stress and Stress-Related Mechanisms On Cognition and Behavior

Program #/Poster #: P251.04

Topic: F.04. Stress and the Brain

Support: PAPIIT IN306918
PAPIME PE306318

Title: Resilience or susceptibility: behavioral classification of rats exposed to chronic stress.

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Abstract: Stress is a systemic response for a real or perceived threat, that enhance the survival probability of an organism. Several mechanisms have been proposed as modulators of this response, however, when those mechanisms are not enough, and the stress response is sustained (chronic stress), it becomes dangerous for the organism. It is well recognized that a history of adverse experiences could be a risk factor for develop psychiatric disorders such anxiety or depression, this population is considered as susceptible to stress effects. Although, it is also well known that not all the subjects exposed to stress develop any disorder, this individuals are considered as resilient. In basic research, resilience and susceptibility to stress are highly studied in posttraumatic stress models and there are different classification methods to recognize each phenotype. But in other models (e. g. depression induced by stress) there are no ways for classify the effects of stress. Due this, the aim of this research was to investigate the behavioral effects of chronic stress in male Wistar rats to identify the set of altered behaviors to get a better classification of animals according to the severity of their alterations. First, we applied a chronic unpredictable stress battery (CUSB) in different lifetime periods (during adolescence, postnatal day 40, PND40) and during adulthood (PND 60) in four groups. Control group (without stress), stress during adolescence, stress during adulthood and stress in both periods (PND 40 and 60). Once the stress exposure finished, behavioral tasks were applied, saccharine preference (SP) and Forced Swim Test (FST) for evaluate depression-like behaviors and open field test (OFT) for anxiety-like behaviors. Finally, the classification method known as k-means was used. Results showed that in all the groups depression and anxiety like-behaviors are modulated by stress exposure. Also k-means classification method showed three clusters of altered behaviors: 1) No effects, 2) Moderate alterations and 3) Severe alterations. This data suggests that an exposition to stress during different periods could increase anxiety and depression-like behaviors but that changes are not present in all the subjects of the exposed groups. That means that as in human situations, individual differences (resilience and susceptibility) also happen in animal models of depression and it is an important factor to consider in future research.

Disclosures: K.B. Valencia: None. **Y. Vidal-Delao:** None. **O. Galicia-Castillo:** None. **D.B. Paz-Trejo:** None. **H. Sanchez-Castillo:** None.

Digital Abstract Session

P251. The Impact of Stress and Stress-Related Mechanisms On Cognition and Behavior

Program #/Poster #: P251.05

Topic: G.05. Mood Disorders

Support: Brain & Behavior Research Foundation NARSAD Young Investigator Grant 25488
University of Cincinnati Neurobiology Research Center Pilot Award
National Institutes of Health R21-MH120614

Title: Chronic stress causes transient microglia-mediated neuronal remodeling that contributes to persistent synaptic and behavioral consequences

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Abstract: Chronic psychological stress causes dendritic atrophy and synapse loss in the prefrontal cortex (PFC), leading to aberrant PFC function. This is important because these neurobiological changes promote the development of working memory deficits and behavioral despair. Our recent work indicates that that functional changes in microglia contribute to stress-induced synaptic deficits in the PFC. Indeed we showed that 14 days of chronic unpredictable stress (CUS) increased microglial phagocytosis of neuronal elements via CSF-1 signaling. However it is unclear if these changes reflect a permanent shift toward a dysfunctional microglia phenotype, or transient responses to adapt to stressors. In these studies male and female mice were exposed to varied durations of CUS to assess temporal dynamics of neuron-microglia interactions and associated behavioral effects. After 14 days of CUS male mice showed increased immobility in the forced swim test (FST) and reduced discrimination in temporal object recognition (TOR). These behavioral changes coincided with increased *Csf1* expression in whole PFC and *Csf1r* expression in sorted PFC microglia. Confocal imaging in Thy1-GFP mice showed that CUS decreased apical spine density for PFC pyramidal neurons and increased microglial phagocytosis of neuronal elements in layer I of the medial PFC. Notably, these changes were not evident in female mice. After prolonged CUS exposure (28 days) both male and female mice showed CUS-induced behavioral changes in FST and TOR. Interestingly, *Csf1* levels in the PFC and microglia-specific expression of *Csf1r* were normalized at the 28 day time point. Confocal imaging in Thy1-GFP mice revealed that apical spine density was still diminished in the PFC of males, but microglia-mediated neuronal remodeling returned to baseline levels after prolonged CUS. Furthermore, while female mice showed behavioral changes after 28 days of CUS, they did not show changes in microglia function or synaptic markers. Collectively these results suggest that stress-associated alterations in microglia function are dynamic, with increased microglia-mediated neuronal remodeling observed after 14 days, but not after 28 days. Additionally these studies indicate that while males and females have similar behavioral phenotypes after prolonged CUS exposure, they show divergent neurobiological

responses to stress within the PFC. Future studies will investigate whether changes in neuron-microglia interaction are directed by stress-induced changes in neuronal activity.

Disclosures: S.C. Woodburn: None. **J.L. Bollinger:** None. **E.S. Wohleb:** None.

Digital Abstract Session

P251. The Impact of Stress and Stress-Related Mechanisms On Cognition and Behavior

Program #/Poster #: P251.06

Topic: F.04. Stress and the Brain

Support: MEDICI, FES IZTACALA.

Title: Effect of stress on the procedural memory in adrenalectomized male rats

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Abstract: In response to stress, corticosterone (CORT) is released from the adrenal glands through the hypothalamic-pituitary-adrenal axis. In adrenalectomized animals (ADX), the role of glucocorticoids in memory has been evaluated; training rats under such conditions has been shown to interfere with memory formation for several tasks. Therefore, the effect of stress by restraint 15 min (R), or the injection of CORT 1mg / kg pre-training, was studied in ADX male rats, which performed the elevated T maze task (ETM). The ETM evaluates acquisition and retention of procedural memory. Thirty-two male Wistar rats were assigned to different groups: one subjected to ETM without adrenalectomy (ETM), and three adrenalectomized groups, one exposed to ETM, another to restraint 15 min before ETM (R-ETM) and another injected with corticosterone 1 mg / kg before ETM (CORT-ETM). In adrenalectomized subjects, glucose was taken at different times and the weights of the gonads were measured. In the ETM and R-ETM adrenalectomized groups impaired retention was detected. In adrenalectomized subjects decreased testicular weight, hypoglycemia was observed on days 1 and 2, which gradually recovered. Adrenalectomy caused memory impairment in the ETM task, when pre-training CORT was administered, the subjects performed similar to the group without adrenalectomy, so CORT is necessary for memory processing and with a dose of 1 mg / kg it was sufficient to regulate their processing. The restraint causes an increase in CORT and has been related to impairment of different types of memory. The restriction induced memory impairment in the R-LET group, it is likely that this effect is associated with the activation of other circuits. However, it has been observed that in adrenalectomized subjects there is gradual recovery of plasma CORT level, but not sufficient concentration for memory to be formed.

Disclosures: M.A. Gonzalez: None. **E.A. Rendon-ochoa:** None. **M.R.A. Gonzalez-lopez:** None. **N.L. Garcia-saldivar:** None. **S.E. Cruz-morales:** None.

Digital Abstract Session

P251. The Impact of Stress and Stress-Related Mechanisms On Cognition and Behavior

Program #/Poster #: P251.07

Topic: F.04. Stress and the Brain

Title: Blockade of dorsal striatal glucocorticoid receptor prevents memory acquisiton impairment induced by corticosterone

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Abstract: Response to stress is characterized by the release of glucocorticoids from the HPA axis. Corticosterone (CORT) the main hormone released by HPA, modulates adaptative responses and behavior, including learning and memory in rats. The behavioral outcome depends on the type of stressor, the moment and stress exposure time, and the type of memory. Several studies indicate that CORT administrated after training enhanced declarative and procedural memory, and administrated before training could impair declarative memory, although exist some discrepancies in procedural memory. Therefore, the present work aims to evaluate the effects of restraint stress or CORT administration before training on procedural memory in elevated T maze (ETM). ETM test consists of training the rats to avoid and escape from the open arms. On day 1 there are three trials of avoidance and one of escape. Avoidance and escape latency are measured on the second day. Wistar male rats were stressed for 15 min (restraint stress) or injected with CORT (i.p. 5 mg/ml) and the glucocorticoid synthesis inhibitor, metyrapone (ip, 40 mg/kg in 1 ml). On day 1, we found that CORT but not restraint, impaired acquisition in ETM. Metyrapone administration returned latencies to baseline in the group injected with CORT. On day 2 it was observed that CORT and restraint groups impaired retention. These results suggest that glucocorticoid inhibition is sufficient to block the acquisition impairment induced by CORT administration. The dorsal striatum has been implicated in the modulation of procedural memory formation; therefore, to determine its participation in CORT memory impairment, we implanted cannulas in rat's dorsal striatum to deliver the glucocorticoid antagonist, mifepristone. No significant differences were found in acquisition latencies among vehicle, CORT or restraint in the avoidance latencies. The CORT and restraint groups improved retention latencies. Together, these results suggest that activation of glucocorticoid receptors in the striatum before training is enough to impair the acquisition in ETM test.

Disclosures: A.O. Flores Sánchez: None. **E.A. Rendon-Ochoa:** None. **N.L. García-Saldívar:** None. **M.R.A. González-López:** None. **S.E. Cruz-Morales:** None.

Digital Abstract Session

P251. The Impact of Stress and Stress-Related Mechanisms On Cognition and Behavior

Program #/Poster #: P251.08

Topic: F.04. Stress and the Brain

Support: NIH Grant MH117103
NIH Grant F31DA051184
NIH Grant P51OD011132

Title: Glucocorticoid receptor binding contributes to cocaine-induced disruptions in goal-directed decision making

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Abstract: Many addictive drugs increase stress hormone levels. They also weaken the ability of organisms to select actions based on their consequences, biasing behavior towards stimulus-elicited habits. We hypothesized that chronic cocaine causes habit biases by increasing circulating stress hormone levels and activating the low-affinity glucocorticoid receptor (GR). We first confirmed that cocaine increases circulating corticosterone (CORT) in mice. We next trained mice to generate two nose pokes for food and then required them to update action-consequence associations when one response was no longer reinforced. Cocaine delivered in adolescence or adulthood impaired the capacity of mice to update action strategies, and inhibiting CORT synthesis prior to cocaine blocked cocaine-induced response biases. Next, we reduced *Nr3c1,* which encodes GR, in the orbitofrontal cortex, a region of the brain responsible for interlacing new information into established behavioral routines. *Nr3c1* silencing protected against cocaine-induced response biases. Both CORT and cocaine destabilize dendritic spines in the orbitofrontal cortex, and we lastly found that inhibiting actin polymerization selectively in the orbitofrontal cortex blocked the ability of mice to update action strategies – mimicking the behavioral effects of chronic cocaine and CORT alike. Future experiments will determine whether cocaine-induced CORT release disrupts reward-related decision making via the destabilization of dendritic spines on excitatory neurons in the orbitofrontal cortex.

Disclosures: M.K. Sequeira: None. **S.L. Gourley:** None.

Digital Abstract Session

P252. Regulating Systems

Program #/Poster #: P252.01

Topic: F.05. Neuroimmunology

Support: NIH NIGMS Training Grant GM008042

Title: Immune cell types in murine stellate ganglia identified by single-cell sequencing mirror immune cell diversity in the brain.

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Abstract: Objective To define immune cell populations in healthy, murine stellate ganglia using single-cell RNA sequencing (scRNA seq). **Rationale** Microglia are the resident macrophages of the nervous system and play a crucial role in neuronal development, immune response, and homeostasis. Like microglia, CNS-associated macrophages (CAMs) are derived from the embryonic yolk sack but are found in CNS border zones. While microglia and CAMs have been studied in the CNS, little is known about their role in the peripheral nervous system. Inflammation in stellate ganglia has been reported in patients with cardiomyopathy, severe ventricular arrhythmias, and inherited arrhythmia syndromes. The role of microglia and CAM in promoting inflammation and hyperadrenergic states in sympathetic ganglia remains poorly understood. Using scRNA seq, we explored the transcriptomic heterogeneity of immune cell populations in murine stellate ganglia. **Methods** Stellate ganglia from eight wildtype male mice were used for scRNA seq. A total of 1290 immune cells were identified by expression of known gene markers (Fcrls, Cxcr6, Cd209a, etc.) and analyzed. Cell clusters were determined through unsupervised clustering, visualized using a bi-dimensional T-distributed stochastic neighbor embedding (t-SNE) dimension reduction analysis and studied using pseudotime trajectory analysis and biological pathway analysis. **Results** Murine stellate ganglia contain diverse immune cell types consisting of microglia, CAMs, monocyte-derived macrophages, dendritic cells, and T-cells. More CAMs were present than microglia, and 4 subclusters of CAMs were identified, mirroring the heterogeneity found in the CNS using deposited data. Pseudotime analysis revealed a basal CAM state transitioning to an inflammatory state, and subsequently to a stressed CAM state. Another lineage downregulates overall gene expression but upregulates mitochondrial and oxidative phosphorylation genes possibly suggesting a terminal, apoptotic state. Finally, both CAMs and microglia expressed genes associated with sympathetic signaling, such as NPY, DBH, MAO-A, COMT, NET and VMAT2. This suggests immune cell interactions with sympathetic neurons in the stellate ganglion. **Conclusion** In murine stellate ganglia, immune cell types mirror the diversity seen in the brain. CAMs might represent the predominant immune cell type in stellate ganglia. Both CAMs and microglia express genes associated with sympathetic signaling and may be involved in modulating inflammation and local adrenergic signaling at the stellate ganglion. Targeting CAMs and microglia might be an innovative strategy for treating cardiac diseases.

Disclosures: D. Carrera: None. **V. van Weperen:** None. **R. Littman:** None. **X. Yang:** None. **O. Ajijola:** None.

Digital Abstract Session

P253. Blood Flow

Program #/Poster #: P253.01

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Turkish Academy of Sciences (TD)

Title: Organization of contractile proteins in pericytes suggest different flow regulatory functions

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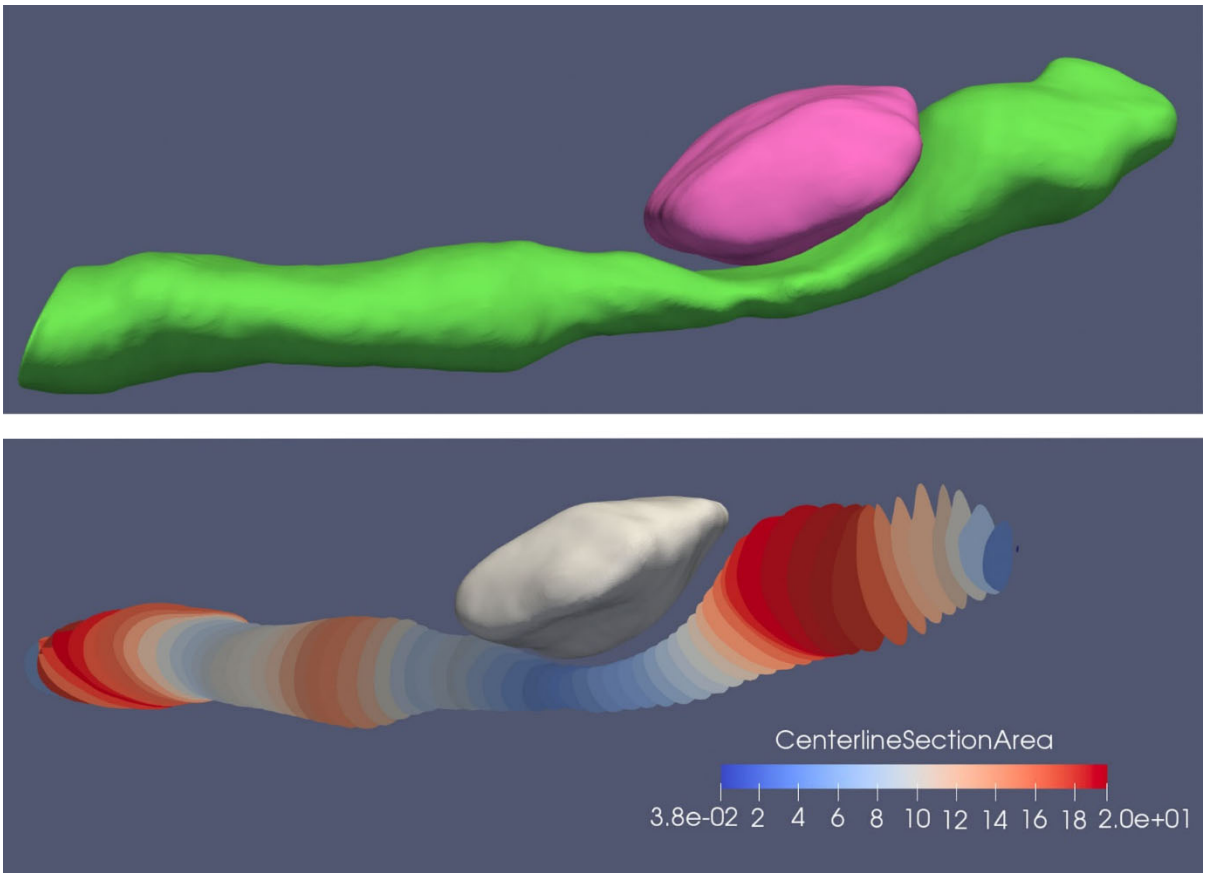
Abstract: Although it is known that capillary pericytes contribute to regulation of blood flow, the contractile proteins involved and their role in these processes are unclear. We examined the expression of alpha-smooth muscle actin (α-SMA) and myosin heavy chain-11 (Myh11) as well as their structural organization and functional coupling in pericytes located on retinal capillary network. α-SMA and Myh11 were detected by immunofluorescence on mice retinas fixed with ice-cold methanol to prevent α-SMA depolymerization. We evaluated the structural and functional coupling of the two proteins by confocal microscopy, FRET, pharmacological inhibition of the actomyosin cross bridge cycle and, evaluated the contractile force generation by 3D modeling of pericytes after α-SMA/lectin co-labeling. We found that Myh11 tightly colocalized with α-SMA in stress-fibers and is present in all pericytes along the microvasculature. However, the organization of contractile fibers differed: circular fibers in upstream pericytes were gradually replaced by oblique and horizontal orientations toward downstream branches, suggesting that upstream pericytes regulate blood flow distribution by constricting capillaries, whereas downstream ones modulate the flux rate by varying capillary resistance. Tight association of α-SMA interaction site with Myh11's head domain was confirmed by high FRET efficiency, pointing to a less than 10 nm distance between them. Suggesting a functional coupling between the two proteins, noradrenaline-induced vasoconstriction was prevented by pharmacological inhibition of actomyosin cross bridge cycle. In conclusion, capillary pericytes express Myh11 along with α-SMA and contract via actomyosin cross bridge cycle. Differential orientation of the stress fibers along the capillary segments suggests specialized blood flow regulatory functions for up- and down-stream pericytes. Legend: 3D rendering of a contracted pericyte from Z-stack images. Note the steep reduction in luminal diameter from periphery toward pericyte soma. Cooler colors indicate lower luminal area.



Disclosures: **G. Kureli:** None. **E. Erdener:** None. **T. Dalkara:** None.

Digital Abstract Session

P253. Blood Flow

Program #/Poster #: P253.02

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: USU Program Project
CHIRP (NHLBI/NIH & USU)
DoD through Center for Neuroscience & Regenerative Medicine (CNRM)

Title: Vascular and sleep deficits in a mouse model of high-altitude exposure

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Abstract: High altitude (HA) is characterized by hypobaric hypoxia, reducing effective availability of oxygen for respiration. This compromises tissue oxygenation and causes cognitive, cardiovascular and metabolic dysfunction, often with long-lasting effects even after return to normoxic conditions. In addition, HA can cause significant disruptions to normal sleep. We previously identified enhanced angiogenic activity, disruption of the blood-brain barrier, alterations in brain glucose metabolism, and increased microglia phagocytosis after HA, as well as functional deficits in hippocampal mediated behavior (Cramer et al. 2019). HA was simulated by placing ~7 week old mice in a hypobaric chamber to a simulated altitude of 5000 m (10-11% O_2, ~60% SpO_2) for chronic exposures ranging from 3-7 weeks. Assessment of vasculature was achieved through transcardial perfusion of the high contrast agent BriteVu (Scarlet Imaging, UT) followed by ex-vivo microCT imaging and analysis using Vesselucida software (MBF, VT) to quantify vascular changes including surface area, volume, diameter, length and tortuosity. We found that HA had an effect on vasculature morphology, with HA brains showing increased total blood volume of vessels, likely due to increased average vessel diameter and tortuosity. In order to assess sleep, polysomnographic recordings were carried out using EEG/EMG DSI telemetry system with HD-X02 implants (DSI, MN; n=6 per group). Throughout the 3 weeks of HA exposure animals spent increased amount of time awake during the light cycle, showed increased rapid eye movement (REM) sleep in dark cycle, and decreased slow wave sleep (SWS) in dark and light cycle (2-way ANOVA, $p < 0.001$). We also evaluated arteriolar pulsatility with use of *in vivo* 2-photon imaging and cranial windows and preliminary results suggest that penetrating arteriolar pulsatility may be reduced in the HA group. These results suggest the possibility that HA exposure may attenuate perivascular glymphatic flow. In summary, it appears that HA exposure significantly impacts vasculature and sleep architecture and can be a serious health concern and unexplored risk factor for the development of progressive neurodegenerative conditions.

Disclosures: K. Whiting: None. **V. Tsytsarev:** None. **N.P. Cramer:** None. **T. Barvir:** None. **X. Xu:** None. **S. Jaiswal:** None. **F. Lischka:** None. **A. Knutsen:** None. **C. Browne:** None. **J. Iliff:** None. **G. Yu:** None. **D.L. Dickstein:** None. **B.J. Dardzinski:** None. **D.P. Perl:** None. **Z. Galdzicki:** None.

Digital Abstract Session

P253. Blood Flow

Program #/Poster #: P253.03

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Validation of a new canine functional magnetic resonance imaging head coil

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Abstract: Validation of a new canine functional magnetic resonance imaging head coilC.-N. Alexandrina Guran[1,2], Ronald Sladky[2], Magdalena Boch[2,3], Sabrina Karl[4], Ludwig Huber[4] & Claus Lamm[2]
Disclosures: Alexandrina Guran: none. Ronald Sladky: none. Magdalena Boch: none. Sabrina Karl: none. Ludwig Huber: none. Claus Lamm: none.
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Magnetic resonance imaging (MRI) can answer questions about the neural underpinnings of behaviour and perception. To answer questions about the evolutionary origins of the brain´s organization, in terms of both structure and function, comparative neuroimaging, i.e., looking at brain organization similarities and differences between species, is an important and promising avenue. In canine neuroimaging, human knee coils are often used for image acquisition. However, they cannot be expected to yield the best possible data quality as they are not designed for canine cranial anatomy. A specific canine headcoil was developed (K9 Coil, Vienna MR Apps, Austria), optimized for the canine anatomy. Here, we compare structural (n = 9) as well as functional (resting state n = 7, and a simple visual paradigm, n = 9) imaging data collected from the K9 Coil to data collected with a human knee coil, the previous state of the art in canine MRI, while keeping the sequence settings identical. Our results show that the K9 Coil significantly outperforms the human knee coil, improving the signal-to-noise ratio across the imaging modalities by at least 35%. For structural images, increases of roughly 43% (for white and gray matter collectively) are noted, while in the functional domain, increases of roughly 74.5% in the temporal signal-to-noise ratio of resting state data, and increases of 44% of task-related functional data. These findings demonstrate that hardware improvements are instrumental in driving data quality, and thus, quality of imaging results, for modern comparative neuroimaging.

Disclosures: C.A. Guran: None. **R. Sladky:** None. **M. Boch:** None. **S. Karl:** None. **L. Huber:** None. **C. Lamm:** None.

Digital Abstract Session

P253. Blood Flow

Program #/Poster #: P253.04

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant U01HL133362

Title: Reconstruction of blood flow velocity distributions over cerebral microvascular networks by combining dynamic light scattering optical coherence tomography and two-photon microscopy

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Abstract: Substantive endeavors have been made for the study of cerebral blood flow (CBF) regulation mechanism and numerical modeling of oxygen transfer and delivery within cerebral microvascular networks. The reconstruction of absolute blood flow velocity distribution over a high-resolution cerebral microvascular network contributes to the better understanding and improved modeling of those mechanisms, but it is still a challenging task because the anatomical vascular structure and blood flow measurements are typically collected using nonconcurrent optical imaging techniques such as two-photon microscopy (TPM) and dynamic light scattering optical coherence tomography (DLS-OCT) which demand data co-registration to account for the differences in imaging setup and distortions. We propose a framework for rodent model to map a large set of CBF velocity measurements down to the capillary level from DLS-OCT on the corresponding anatomical microvascular structure obtained using TPM. The cerebral angiograms and absolute blood flow velocities were obtained from three anesthetized young-adult female C57BL/6 mice with sealed glass cranial windows installed. Dextran-conjugated Alexa Fluor 680 was administered to each subject as the vascular contrast agent for TPM. A global 3D and multi-layer 2D co-registration operations were employed on the TPM and DLS-OCT angiograms to obtain transformation matrices from the TPM space to the DLS-OCT space. Graph-based vascular structures were retrieved from TPM angiograms and transformed into the DLS-OCT space. Finally, accurate cerebral blood flow speed distributions were established on the graph-based high-resolution cerebral microvascular networks. Using the proposed framework, we were able to collect the mean red blood cell (RBC) velocity distributions from over 1000 microvascular segments of the three mice in arterioles, venules, and capillaries as a function of the branching order from precapillary arterioles to postcapillary venules. The proposed framework in this work can be applied for quantitative analyzing the dense data sets from OCT and multiphoton microscopy and pave the way for improved performance in the numerical modeling of CBF regulation mechanisms, oxygen transport, and delivery with realistic microvascular networks in both healthy and diseased rodent brains.

Disclosures: **Q. Pian:** None. **M. Alfadhel:** None. **J. Tang:** None. **G.V. Lee:** None. **B. Li:** None. **B. Fu:** None. **Y. Ayata:** None. **D. Boas:** None. **T.W. Secomb:** None. **S. Sakadzic:** None.

Digital Abstract Session

P254. Homeostasis: Anatomical Considerations

Program #/Poster #: P254.02

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH GM127251

Title: Atlas-based spatial analysis and quantitative assessment of the rat hindbrain regional response to glycemic challenge

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Abstract: Hypoglycemia unawareness may result from a failure of hindbrain glucosensing neurons to effectively respond to decreases in circulating glucose. Hindbrain epinephrine and norepinephrine neurons are necessary to prompt the glucose counter-regulatory response (CRR) that protects against hypoglycemic conditions, but there is limited knowledge of the precise atlas-based distribution of glucosensing neuronal populations. To address this, hindbrain regions were surveyed in glucoprivic (2-deoxyglucose, 250 mg/kg, i.v.) and saline-control rats. Single- and double-labeled neurons processed using antibodies against phospho-ERK1/2, dopamine β-hydroxylase, and choline acetyltransferase were photographed. These images were aligned with those of an adjacent series of Nissl-stained tissue that served as a cytoarchitectural reference, enabling the data to be mapped to a rat brain atlas (Swanson, *Brain Maps 4.0*, 2018). Additional animal cases have been surveyed, mapped, and an assessment of the hindbrain regional response to 2-DG comparing phospho-ERK1/2 labeled cell bodies 15 minutes after treatment was conducted. Cell counts were tallied to quantify the rostrocaudal distribution of activated neurons per region. In glucoprivic animals, we found increased cellular activation in hindbrain adrenergic/noradrenergic regions, suggesting these areas are associated with rapid responses to glycemic challenge. In the anterior hindbrain (e.g., Swanson Levels 51-53) phospho-ERK1/2+ neurons were distributed in the locus coeruleus and sub-coeruleus. More caudally (e.g., L55-63), activation was observed in the nucleus ambiguus and cholinergic cell groups in the vicinity of the facial nucleus. At the most caudal hindbrain atlas representations (e.g., L67-71) activation was observed in the nucleus of the solitary tract, dorsal motor nucleus of the vagus nerve, and the hypoglossal nucleus. These data indicate that rapid responses to glycemic challenge are chiefly mediated by hindbrain adrenergic/noradrenergic and cholinergic centers.

Disclosures: G. Tapia: None. **L.J. Agostinelli:** None. **S.D. Chenausky:** None. **J.V. Salcido:** None. **J.E. Esquivel:** None. **A.M. Khan:** None.

Digital Abstract Session

P254. Homeostasis: Anatomical Considerations

Program #/Poster #: P254.03

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIDDK 1R01DK106229
NIH TL1TR001440
NLM T15LM007093

Title: Identification and analysis of controlling nodes in a neural network regulating feeding behavior

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Abstract: Dysregulated eating carries a substantial economic and societal toll both in the United States and globally. Eating disorders like anorexia nervosa, bulimia nervosa, and binge-eating disorder can have severe effects on all body systems and are difficult to effectively treat. Currently, most treatments for these disorders rely on behavioral modification or surgical interventions, with few pharmacotherapeutic options. Because of this, a more detailed understanding of how the brain functions to regulate food intake is necessary. While multiple brain regions have been implicated in various aspects of food intake, to date, there is no systematic approach to quantitatively analyze the relative importance of each brain region to the overall function and control of the neural feeding network. Using the Allen Mouse Brain Connectivity Atlas combined with targeted literature searches, we identified 64 brain regions (or nodes) implicated in control over different aspects of feeding and constructed an *in-silico* model of the resulting mouse brain structural neural network. Network control principles were then applied to the feeding network, allowing us to identify key controlling nodes, as well as nodes that act as network hubs. *In-silico* lesioning of controlling nodes and the resulting effects on overall network controllability identified distinct brain regions that influence overall feeding network function. By applying network control principles to create a model of the mouse feeding network, we created an unbiased *in silico* tool that identified network nodes controlling food intake for future *in vivo* confirmation. This provides a connectome-driven strategy to identify central targets for pharmacotherapies to treat eating disorders while minimizing potential effects on overall brain function.

Disclosures: S. Miller: None. **A. Borzou:** None. **J. Hommel:** None. **N. Smith:** None. **J. Kasper:** None.

Digital Abstract Session

P254. Homeostasis: Anatomical Considerations

Program #/Poster #: P254.04

Topic: F.09. Food and Water Intake and Energy Balance

Support: Warren Alpert Distinguished Scholar Award
NIH R01 MH11408

Title: Neurons that regulate mouse torpor

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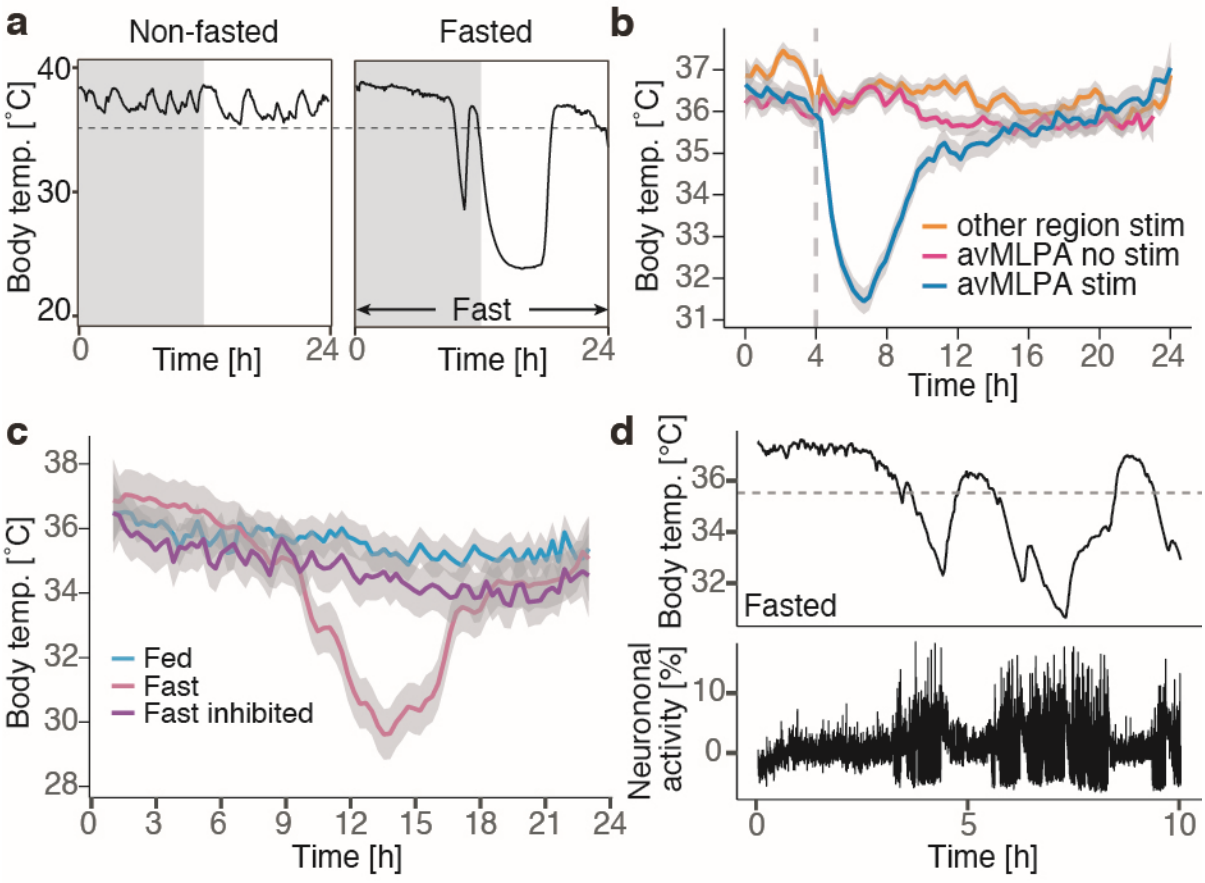
Abstract: The advent of endothermy, which is achieved through the continuous homeostatic regulation of body temperature and metabolism, is a defining feature of mammalian and avian evolution. However, when challenged by food deprivation or harsh environmental conditions, many mammalian species initiate adaptive energy-conserving survival strategies—including torpor and hibernation—during which their body temperature decreases far below its homeostatic set-point. How homeothermic mammals initiate and regulate these hypothermic states remains largely unknown. We discovered that entry into mouse torpor, a fasting-induced state with a greatly decreased metabolic rate and a body temperature as low as 20 °C, is regulated by neurons in the medial and lateral preoptic area of the hypothalamus. We show that restimulation of neurons that were activated during a previous bout of torpor is sufficient to initiate the key features of torpor, even in mice that are not calorically restricted. Among these neurons we identify a population of glutamatergic *Adcyap1*-positive cells, the activity of which accurately determines when mice naturally initiate and exit torpor, and the inhibition of which disrupts the natural process of torpor entry, maintenance and arousal. Taken together, our results reveal a specific neuronal population in the mouse hypothalamus that serves as a core regulator of torpor. This work forms a basis for the future exploration of mechanisms and circuitry that regulate extreme hypothermic and hypometabolic states, and enables genetic access to monitor, initiate, manipulate and study these ancient adaptations of homeotherm biology.



Disclosures: **S. Hrvatin:** None. **S. Sun:** None. **M.E. Greenberg:** None.

Digital Abstract Session

P254. Homeostasis: Anatomical Considerations

Program #/Poster #: P254.05

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant GM127251
NIH Grant MH114961

Title: Comparison of rostral and caudal prelimbic cortical connections with the forebrain in the adult male rat

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Abstract: Connections of the cingulate region are engaged when changing environments demand adaptive control of habitual behaviors; these functions are studied as subprocesses such as attention, executive control, and working memory. The prelimbic area (PL) is frequently

identified as a key anatomical locus for these functions. However, studies usually focus on the caudal half of the PL, despite its 3 mm span, and leave the rostral PL unexamined. A comparison of rostral and caudal PL connections will help us judge when these omissions are especially problematic. To this end, we targeted the PL with co-injected anterograde (Phaseolus vulgaris leucoagglutinin) and retrograde (cholera toxin subunit B) tracers and performed high-spatial resolution analysis to compare its forebrain connections within an atlas framework (Brain Maps 4.0; Swanson, 2018, J. Comp. Neurol.). Among cerebral nuclei, pronounced differences were seen in the nucleus accumbens (ACB). Projections from the caudal PL densely targeted both ACB shell and core divisions whereas terminals from the rostral PL were restricted to the core. Rostral PL projections appeared to target matrix compartments whereas more patch innervation was observed for caudal PL projections. Bidirectional PL connections with anterior basolateral amygdala (BLAa) showed topographic differences. Hypothalamus received light projections from the rostral PL whereas caudal PL innervated several hypothalamic structures, most of which were found in the lateral hypothalamic area. In thalamus, PL connections were bidirectional. Rostral and caudal PL connections showed remarkably little spatial overlap despite having similar target structures. Caudal PL was strongly connected to the mediodorsal nucleus medial part whereas rostral PL mainly connected with its central part. The paratenial nucleus connected with the caudal PL in its rostral part, but not its caudal extension, whereas rostral PL primarily connected with its caudal part. Overall, our work demonstrated that functional experiments involving the PL could produce variable effects depending on which rostrocaudal segment was targeted. Further anatomical and functional characterization will help fine-tune experimental design and contribute to better division schemes for the rat prefrontal cortex.

Disclosures: K. Negishi: None. **L.P. Montes:** None. **M.S. Ponce:** None. **Y.C. Wells:** None. **A.M. Khan:** None.

Digital Abstract Session

P254. Homeostasis: Anatomical Considerations

Program #/Poster #: P254.06

Topic: F.09. Food and Water Intake and Energy Balance

Support: AA028218

Title: Sex differences in binge eating behavior and expression of pituitary adenylate cyclase-activating polypeptide in the thalamic paraventricular nucleus in rodents

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Abstract: Binge eating disorder, characterized by the overconsumption of food in a discrete time period, is the most common eating disorder in the United States and affects women more than men. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide implicated in feeding and is independently processed into PACAP-27 and PACAP-38. We have recently

identified high levels of PACAP-27 (more than PACAP-38) in cells of the paraventricular nucleus of the thalamus (PVT), a limbic brain region involved in feeding. To better understand the sex difference in the rate of binge eating disorder, we used a limited access home cage model with Milk Chocolate Ensure Plus® to induce binge eating behavior in adult male and female mice. Under this 6-week paradigm, female mice (n = 7) demonstrated greater binge eating than male mice (n = 5), consuming more Ensure both as a function of bodyweight (BW) (0.22 kCal/g BW vs. 0.17 kCal/g BW, $p < 0.001$) and as a percent of total daily calories consumed (33.4% vs. 25.7%, $p < 0.05$). Next, we used quantitative real-time PCR (qRT-PCR) to examine PVT PACAP mRNA in adult male and female mice (n = 5/group) with no history of binge eating, and found that females had higher levels of PVT PACAP mRNA than males (+100%, $p < 0.05$). In adult male and female rats, both qRT-PCR and immunohistochemistry further supported this finding. Gene expression of PACAP was higher in females by 97% in the anterior PVT and 28% in the posterior PVT. Protein levels of PACAP were also higher in females (n = 5/group), for both PACAP-27 (52% vs. 44% of all cells, $p < 0.01$) and PACAP-38 (13% vs 10%, $p < 0.05$) across the entire PVT. In summary, our results indicate that females have higher levels of PACAP in the PVT and are more prone to binge eating, suggesting that PACAP in the PVT could drive binge eating behavior.

Disclosures: G.R. Curtis: None. **A.T. Gargiulo:** None. **A. Gupta:** None. **B.E. Pirino:** None. **J.R. Barson:** None.

Digital Abstract Session

P254. Homeostasis: Anatomical Considerations

Program #/Poster #: P254.07

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant GM127251

Title: High-spatial resolution atlas-based mapping of insular cortical connections to the amygdala and thalamus in the adult male rat

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Abstract: The insular cortex is known to be a hub that receives and sends information to different brain regions. It integrates autonomic and limbic responses, and is anatomically connected to amygdalar and thalamic subregions. The insular cortex, in turn, receives somatosensory, visual, gustatory, and auditory information from thalamic regions. Similar to the amygdala, the insular cortex plays a role in processing signals encoding emotional responses. Early tract-tracing studies have shown that injections in limbic areas displayed anterograde and retrograde labeling in the insular cortex. These connections have not yet been mapped in a standardized, high-spatial resolution atlas. Here, we focus on mapping the anatomical connections between portions of the insular cortex, mainly in the dorsal agranular insular area

(AId), and subregions in the amygdala and thalamus; using a standardized reference atlas (*Brain Maps 4.0* (*BM4.0*); Swanson, 2018, *J. Comp. Neurol*.). Cholera toxin B subunit (CTB) and *Phaseolus vulgaris* leucoagglutinin (PHAL) were injected in the AId. The injection site was from *BM4.0* atlas levels 9-13 (AP= +2.80 to +1.20 mm from Bregma, β) and the core was from level 10 to 11 (AP= +2.15 to +1.70 mm from β). Diaminobenzidine (DAB) was used to visualize PHAL labeling and a nickel-enhanced DAB reaction was used for CTB labeling in coronal rat brain sections. Adjacent tissue series were Nissl-stained, and boundaries were assigned based on cytoarchitecture, as described in *BM4.0*. Results were consistent with previous studies showing projections from the insular cortex to the anterior and posterior basolateral amygdalar nucleus (BLAa and BLAp), lateral amygdalar nucleus (LA), and central amygdalar nucleus (CEA). The LA had the highest expression of PHAL, followed by the CEA, BLAa, and BLAp. Cell bodies expressing CTB were only present in the LA. The AId also showed projections to subregions of the thalamus, specifically to the mediodorsal thalamic nucleus medial part, mediodorsal thalamic nucleus lateral part (MDl), paraventricular thalamic nucleus (PVT), and the intermediodorsal thalamic nucleus (IMD). Cell bodies expressing CTB were present in the MDm, IMD, MDl, with very few also present in MDc. Subregions of the amygdala and the thalamus were analyzed for *BM4.0* atlas levels 25-31. These data provide high-spatial resolution maps of the connectivity between the insular cortex and the amygdala or thalamus that can greatly aid in targeting for functional experiments using methods such as optogenetics.

Disclosures: L.P. Montes: None. **K. Negishi:** None. **A.M. Khan:** None.

Digital Abstract Session

P255. Blood Brain Barrier

Program #/Poster #: P255.01

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH grant # HL146832

Title: Fibrinogen-induced oxidative stress and inflammatory responses in astrocytes

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Abstract: Neuroinflammation is the hallmark of traumatic brain injury (TBI), which may progress years beyond the trauma causing cognitive decline, particularly reduction in short-term memory (STM). TBI is associated with elevated blood levels of fibrinogen (Fg) called hyperfibrinogenemia (HFg). We found that HFg resulted in Fg extravasation, deposition in vasculo-astrocyte interface and activation of astrocytes via interaction with astrocyte cellular prion protein (PrP^C). These effects were associated with neurodegeneration and STM reduction during TBI. However, mechanisms of HFg-induced astrocyte activation-mediated neurodegeneration is unclear. We hypothesized that HFg, via Fg-PrP^C interaction, stimulates astrocyte inflammatory responses and causes increase in nitric oxide (NO) production and

generation of reactive oxygen species (ROS). Cultured mouse brain astrocytes were treated with either serum-free medium, 4 mg/ml of Fg, or with Fg (4 mg/ml or 1 mg/ml) in the presence or absence PrP blocking peptide overnight or for 3hrs for detection of Fg and astrocyte PrP^C interaction and generation of ROS. Possible formation of fibrin was prevented by the presence of hirudin. Effects of Fg on expression of astrocyte proinflammatory genes (assessed by q-PCR) and proteins (assessed by ELISA), and Fg and PrP^C interaction assessed by proximity ligation assay (PLA) (Duolink™PLA, Millipore Sigma) were tested. Astrocyte ROS and NO were detected using carboxy-H2DCFDA (Image-IT™ LIVE Green ROS Detection Kit, Invitrogen) and Griess assay (Promega), respectively. We found that HFg caused an increase in interleukin 6 (IL-6), C-X-C motif chemokine 10, and chemokine (C-C motif) ligand 2 gene and IL-6 protein expressions. It did not affect IL-10 gene expression. PLA positive signals revealed that Fg was in close proximity (< 40nm) to astrocyte PrP^C. HFg caused an increase in ROS and NO productions. Inhibiting of PrP reduced all these Fg effects. These results confirm our hypothesis that Fg interacts with astrocyte PrP^C inducing release of inflammatory agents, production of ROS and NO, which contribute to oxidative stress and possibly trigger neurodegeneration. The latter can be a result of cognitive decline previously seen during mild-to-moderate TBI in our studies.

Disclosures: **N.H. Sulimai:** None. **J. Brown:** None. **D. Lominadze:** None.

Digital Abstract Session

P255. Blood Brain Barrier

Program #/Poster #: P255.02

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant P20GM103442
NIH Grant 1R21AG070412-01
Host-Pathogen CoBRE Pilot Grant P20GM113123

Title: Alterations in blood brain barrier permeability after chronic allergen exposure in a mouse model of mild cow's milk allergy

Authors: *D. L. GERMUNDSON, N. A. SMITH, A. BRISHTI, K. NAGAMOTO-COMBS; Univ. of North Dakota SMHS, Grand Forks, ND

Abstract: Food allergy has been implicated as a risk factor for various neurodegenerative disorders. However, the connection between allergic hypersensitivity and neurodegeneration remains elusive. We hypothesized that repeated consumption of offending allergens by mildly allergic individuals could sustain chronic inflammation, which in turn would promote degenerative pathology via neuroinflammation. To test our hypothesis, we developed a mouse model of non-anaphylactic cow's milk allergy, in which 4-week old male C57BL/6J mice were sensitized to a bovine whey allergen, β-lactoglobulin (BLG), for 5 weeks and then subsequently placed on a whey-containing diet for 2 weeks. The BLG-sensitized mice were able to consume the allergen diet without weight loss or obvious anaphylactic symptoms despite their

significantly increased levels of serum IgE. Mice were then sacrificed to investigate changes in glia, blood brain barrier permeability, and peripheral immune cell migration into the central nervous system. After BLG-sensitization, GFAP-immunoreactive astrocytes in the brain were increased in number around larger blood vessels and showed more reactive morphology. Vascular permeability in the brain was also assessed by immunostaining with an anti-IgG antibody to determine transendothelial extravasation. While sham mice showed strong positive staining within the vessels, the brains of BLG-sensitized mice were diffusely stained, likely due to a 'leaky' blood brain barrier. Additionally, in highly vascularized regions of the brain, such as the choroid plexus, BLG-sensitized mice showed increased numbers of mast cells as we have previously reported. The increase of mast cells suggested that these innate, proinflammatory immune cells may have contributed to the observed changes in blood brain barrier permeability. Taken together, these results indicated that chronic exposure of sensitized mice to BLG significantly altered brain physiology even without typical anaphylaxis symptoms. Furthermore, these findings support the notion that food allergy may serve as a risk factor for the development of neurodegenerative disorders and highlight the possibility for food allergy management as a preventative approach for reducing chronic neuroinflammation.

Disclosures: D.L. Germundson: None. **K. Nagamoto-Combs:** None. **N.A. Smith:** None. **A. Brishti:** None.

Digital Abstract Session

P255. Blood Brain Barrier

Program #/Poster #: P255.03

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Hacettepe University Scientific Research Projects Coordination Unit. Project Number: TDK-2020-18590

Title: Reduced Folate Carrier 1 is Present in Cerebral and Retinal Microvessels

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Abstract: Microvessels of the brain and retina are encircled by pericytes and vascular smooth muscle cells, called mural cells. These cells have several roles in health and disease. Recently, a meta-analysis of five transcriptomic studies of cerebral mural cells showed three genes were enriched, one of which was the reduced folate carrier 1 (RFC1, SLC19A1) gene. However, to date, RFC1 protein in mural cells of the brain has not been demonstrated. Here, first, we aimed to detect RFC1 protein expression of cerebral mural cells. Second, since retina is considered as the extension of the brain in terms of microcirculatory properties, we hypothesized that RFC1 should also be present in retinal microvessels. We used brain sections, isolated brain

microvessels, whole mount retinas and retinal digest preparations obtained from male and female naïve adult Swiss albino mice to immunohistochemically characterize RFC1 expression in microvessels and pericytes. We used 2 commercially available RFC1 antibodies targeted to 2 different regions. As positive controls we used mice liver, kidney and gut sections where RFC1 protein expression is already abundant and determined immunohistochemically. We colocalized RFC1 marker with the accepted pericyte markers such as platelet-derived growth factor receptor beta (PDGRFR-beta), Aminopeptidase N (CD13) and Neural/glial antigen 2 (NG2). Vessels were visualized by 'Fluorescein' or 'Texas Red' labeled Lectin. Hoechst 33258 was used to label cellular nuclei. We also applied methotrexate (MTX); which is used clinically as an antineoplastic and antirheumatic agent inhibiting folic acid uptake by acting as a non-covalent inhibitor of RFC1 intraperitoneally and obtained brains, as well as intravitreally and obtained retinas, to study if RFC1 protein changes. We showed immunohistochemically that RFC1 protein was present in cerebral mural cells widely, and is colocalized with accepted pericyte markers. In literature, RFC1 was determined to be present only in the retinal pigment epithelium constituting the outer blood-retinal barrier. However, for the first time, we detected RFC1 protein in the microvessels of inner blood retina barrier - the endothelium and pericytes, in line with the transcriptomic studies. Our preliminary data showed that MTX administration changed the RFC1 protein in acute or chronic time points. Therefore, we suggest that RFC1 protein, which is overlooked in microvasculature until know, is widely expressed in retinal and cerebral pericytes, and may have a critical role in health and disease.

Disclosures: **G. Gurler:** None. **G. Uruk:** None. **M. Yemisci ozkan:** None. **N. Belder:** None.

Digital Abstract Session

P255. Blood Brain Barrier

Program #/Poster #: P255.04

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01MH107333 (W.-K.K.)
NIH Grant R21MH108458 (W.-K.K.)
NIH Grant R01AI097059 (M.J.K.)
NIH Grant R33AI110163 (M.J.K)
NIH Grant R56AG052349 (E.S.D.)
NIH Fellowship 1F31NS116926 (D.G.B)

Title: A subset of smooth muscle actin-negative microvascular pericytes transition to smooth muscle actin-positive pericytes in the brain during normal aging or HIV/SIV infection

Authors: ***D. G. BOHANNON**[1], H. R. OKHRAVI[2], J. KIM[3], M. J. KURODA[4], E. S. DIDIER[4], W.-K. KIM[1];
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Abstract: Historically, the presence of both alpha-smooth muscle actin-positive (SMA+) and SMA- brain microvascular pericytes have been repeatedly referenced throughout literature, but a lack of in-depth characterization of these cells has hindered our understanding of their role(s) in neurological diseases and disorders. In this study, we found that SMA- primary human brain microvascular pericytes transition to a SMA+ phenotype in culture after two 16-d passages. Compared to untreated controls, treatment with HIV-1 BAL gp120 recombinant protein (10-20 ng/mL) for 3 d induces an increase in the number of SMA+ cells. Similar changes in SMA +/- pericyte populations were observed in the frontal cortex of male and female rhesus macaques during natural aging (juvenile, adult, aged; n=10/group) and in young adult males with SIV infection with or without encephalitis (n=5/group). The percentage of the pericytes that were SMA+ (type-2 pericytes; PC2) was found to be increased in macaques both with aging and SIV status. Furthermore, PC2-associated vessels had significantly less claudin-5 ($p<0.0001$) and significantly more fibrinogen extravasation ($p<0.0001$), resulting in a strong positive correlation between the percentage of PC2 and percentage of vessels with fibrinogen extravasation in each animal regardless of animal group or infection status ($r^2=0.76$, $p<0.0001$). Ultimately, our findings demonstrate that the in vitro observed transition from SMA- type-1 pericytes (PC1) to PC2 can occur in vivo, providing a potential explanation for the increased percentage of PC2 in the brain of naturally aging rhesus macaques and SIV-infected macaques. These results also suggest that PC2 may play an important role in BBB disruption in various neurological disease and that the PC1-to-PC2 transition may be a pathologically more relevant change in the BBB than pericyte loss.

Disclosures: D.G. Bohannon: None. **H.R. Okhravi:** None. **J. Kim:** None. **M.J. Kuroda:** None. **E.S. Didier:** None. **W. Kim:** None.

Digital Abstract Session

P255. Blood Brain Barrier

Program #/Poster #: P255.05

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Dana Foundation
NIH R21

Title: Oat1 expression in murine and human leptomeninges

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Abstract: The organic anion transporter (OAT) family of transmembrane proteins have high clinical relevance because of their essential role in regulating the blood concentration of important drugs like antivirals, nonsteroidal anti-inflammatory drugs, and antibiotics. The OAT family of proteins are membrane transporters, exchanging one molecule of hydrophilic organic anion for dicarboxylates such as glutarate or ketoglutarate. This exchange system is seen most

classically in the kidney, where OAT1 (encoded by Slc22a6) is highly expressed in the basolateral membrane of the proximal tubular cells and functions to absorb anions from the blood thereby regulating renal secretion of soluble drugs. Studies involving knockout of OAT1 and OAT3 (encoded by Slc22a8) also point to roles for the transporters in regulation and maintenance of gut microbiome homeostasis. Beyond the kidney, OATs have also been identified at various other blood-fluid barrier sites including liver, retina, choroid plexus, brain endothelium and placenta. We have recently localized OAT1 to the arachnoid layer of murine and human brain tissue. In this study we use layer- and cell-specific markers in mouse and human tissue, along with quantitative methods in mice, to better understand the location and expression level of OAT1 and OAT3 in the brain. In mice we find that the expression level of OAT1 is 1.3 times higher in the leptomeninges than in the kidney. Additionally, we find that OAT3 expression in murine meninges is drastically less than that of OAT1. The expression of OAT1 in cerebrospinal fluid (CSF) facing arachnoid mater could potentially point to a role for OAT1 in CSF filtration within the subarachnoid space which has important implications for the regulation of drug pharmacokinetics. Furthermore, meningeal expression of OAT1 is important to understand as this protein also participates in the exchange of dicarboxylic acids, the brain specific accumulation of which is a hallmark of several currently incurable genetic metabolic diseases like Canavan disease, the glutaric acidurias and others.

Disclosures: **V. Hull:** None. **Y. Wang:** None. **D. Pleasure:** None.

Digital Abstract Session

P255. Blood Brain Barrier

Program #/Poster #: P255.06

Topic: A.03. Stem Cells and Reprogramming

Support: Glut1 Deficiency Foundation

Title: Brain microvascular endothelial cells are sensitive to hypoglycemia, and partially rescued by ketone bodies

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Abstract: Introduction: Glucose represents the main source of energy of the central nervous system (CNS), as 20% of daily glucose intake is directed towards the brain. Glucose transport inside the CNS is occurring mostly via the blood-brain barrier (BBB) via the presence of several glucose transporter isoforms (GLUTs). Although glucose metabolism has been mostly investigated in the lens of the astrocyte-neurons axis, the fate of glucose and its metabolism at the BBB remains elusive. GLUT1 deficiency syndrome (GLUT1DS) is autosomal dominant haploinsufficiency characterized by mutations in *SLC2A1* resulting in impaired GLUT1 expression and/or activity. Patients suffering from GLUT1DS suffer from epileptic seizures, intellectual disabilities, and movement disorders. As of today, medical intervention involving the

adoption of a ketogenic diet (KD) remains the main course of action with satisfactory clinical outcomes. Yet, the effect of hypoglycemia and ketone bodies on the BBB function (including its glucose metabolism) remains unclear. This study investigates the effect of hypoglycemia and ketone bodies on the barrier function and glucose metabolism in vitro. Methods: CTR90F and CTR65M iPSC derived BMECs were used in this study. Changes in GLUTs expression was assessed by immunofluorescence and flow cytometry, change in glucose uptake was assessed using 14C-glucose, change in glycolytic flux using SeahorseXF24 flux analyzer, and changes in the barrier function by transendothelial electrical resistance (TEER) and permeability to fluorescein. Cells were supplemented ketone bodies (KB, 4microM beta-hydroxybutyrate, and 1mM acetoacetate) for 24 hours. Results: Our data suggest that a decrease in glucose level upregulates the expression of GLUT1 and GLUT3 isoforms in our BMECs monolayers, such decrease was accompanied by a decrease in glucose uptake, alterations in tight junction complex, as well as a decreased cell metabolic activity and glycolytic flux resulting in a partial recovery of the barrier function under mild hypoglycemia and a partial recovery of the glycolytic flux. No significant changes in glucose uptake was observed in our model following treatment with KB. Discussion: Our study suggests that BMECs may rely on glycolysis as the main source of energy, a decrease in blood glucose may have a detrimental effect on the barrier function. Supplementation with Ketone body (beta-hydroxybutyrate and acetoacetate) partially relieved such symptoms.

Disclosures: I. Pervaiz: None. **A. Al-Ahmad:** None.

Digital Abstract Session

P256. Functional Imaging

Program #/Poster #: P256.01

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Wellcome Trust 205093

Title: Exploring the relationship between functional ultrasound and neuronal activity

Authors: *A. O. NUNEZ-ELIZALDE[1], M. KRUMIN[1], C. B. REDDY[1], G. MONTALDO[2], A. URBAN[2], K. D. HARRIS[1], M. CARANDINI[1];
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Abstract: Functional ultrasound imaging (fUSi) is an appealing method for measuring blood-volume signals from the brain. However, the blood-volume signal measured by fUSi bears an unclear relationship to the underlying neuronal firing. Is this relationship linear, and stable across brain regions, subjects, and time?
To answer these questions, we performed simultaneous fUSi measurements and Neuropixels recordings in awake mice. Eight recording sessions were performed across four mice (~4 coronal slices per session). We inserted one or two Neuropixels probes per session, and related firing recorded from these probes to fUSi signals from the corresponding regions of interest.

A linear model provided a good estimate of fUSi signals based on neuronal firing. We measured the multi-unit activity (MUA) from the Neuropixel probes and convolved it with a hemodynamic filter. The filter was estimated using a linear regression model using half the data from one recording, and tested on the second half. Model predictions were accurate ($r > 0.5$). Furthermore, this model generalized across mice: it increased prediction accuracy relative to an instantaneous MUA model with no delay in all sessions in all three held-out mice. The hemodynamic filter resembled one measured optically, peaking ~1.5 s after neural activity (Pisauro, et al., *J Neurosci* 2013).
These results indicate that the relationship between neuronal firing and fUSi is stable and linear. Our current efforts aim to optimize the processing of fUSi signals so that they best capture variations in neuronal firing across both time and space.

Disclosures: **A.O. Nunez-Elizalde:** None. **M. Krumin:** None. **C.B. Reddy:** None. **G. Montaldo:** Other; AUTC. **A. Urban:** Other; AUTC. **K.D. Harris:** None. **M. Carandini:** None.

Digital Abstract Session

P256. Functional Imaging

Program #/Poster #: P256.02

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Simultaneous functional imaging of hemodynamic and mechanical properties changes during neuronal activation in the rodent brain with Ultrafast Ultrasound

Authors: ***F. LASZLO**, C. RABUT, S. PEZET, T. DEFFIEUX, M. TANTER;
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Abstract: *Introduction*
Advances in neuroscience are closely tied to the development of neuroimaging techniques. Functional ultrasound imaging (fUS), based on ultrafast Doppler, permits to image the Cerebral Blood Volume (CBV) variations and thus to monitor brain activity through the neurovascular coupling. The hemodynamic response to a functional stimulus is slow and may conceal faster transient phenomenons. A recent study (Patz, Science Advances, 2019) formulated the hypothesis that brain activity would also lead to a fast local change in tissue stiffness and therefore would give access to faster functional imaging. By merging fUS and Shear Wave Elastography (SWE), we investigate here this neuromechanical (NM) coupling hypothesis that could serve as a new functional contrast.
Methods
To study simultaneously hemodynamics and tissue mechanical properties in the brain, we programmed for the first time an ultrafast research scanner with a hybrid ultrasound sequence that combines SWE, by generating a shear wave with radiation force and monitoring its propagation with ultrafast imaging (framerate of 5000 Hz) and fUS by ultrafast imaging (framerate of 500 Hz) for power Doppler. In vivo experiments were conducted on 6 rats under perfusion of ketamine/medetomidine with craniotomy centered on Bregma - 6.2mm. Functional

visual stimulation was controlled by a chaining of 30 s of 3 Hz blinking green LED and 30 s of rest during 3 minutes to activate the Superior Colliculus (SC).

Results

High-quality Doppler movies were obtained on rats and show strong correlations between stimuli and vascular response in SC (β - 6.2 mm) with an average CBV increase of 18 % during activation. SWE proved its robustness with a reproducibility under 5 % and relevant tissue stiffness values (17,7 ± 0,4 kPa) in SC. In this zone, the difference in stiffness between stimulation and rest remained under 0.1 % indicating the absence of a fast NM effect in the activated area and thus contravening the fMRI study. However, we showed that stiffness significantly changes solely in SC with an average 6 % decrease ($p = 0.0018$) after 3 minutes of activation. In conclusion, we designed a novel reliable tool that could be used to investigate a NM coupling and showed that a functional activation leads to a slow, localized and reversible decrease in brain stiffness.

Disclosures: **F. Laszlo:** None. **C. rabut:** None. **S. pezet:** None. **T. deffieux:** None. **M. tanter:** None.

Digital Abstract Session

P256. Functional Imaging

Program #/Poster #: P256.03

Topic: I.04. Physiological Methods

Support: ERC Helmholtz

Title: Simultaneous functional ultrasound and electrophysiological recordings of awake and behaving monkeys in Supplementary Eye Field.

Authors: ***J. CLARON**[1], F. ARCIZET[2], *T. DEFFIEUX[1], M. TANTER[1], P. POUGET[3]; [1]ESPCI Paris, PSL Res. University, Physic for Medecine Paris, INSERM U1273, Paris, France; [2]Inst. de la Vision, Paris, France; [3]Paris Brain Inst., Paris, France

Abstract: Introduction To date most whole brain imaging techniques such as fMRI are based on the use of neurovascular coupling. However its combination with electrophysiological recording for a direct assessment of local brain electric activity remains complex due to electromagnetic compatibility issues. Here, by coupling functional ultrasound (fUS) and electrophysiological recording, we were able to directly compare the hemodynamic blood flow at high spatiotemporal resolutions and the local neuronal activity, we were capable to record both signals simultaneously in awake behaving monkeys doing an oculomotor task. **Methods** We used an ultrasound neuroimaging scanner (Iconeus, France). For ultrasensitive Doppler imaging, the scanning sequence consisted of 11 tilted planar ultrasonic waves with a pulse repetition frequency of 500 Hz and a linear ultrasonic probe (128 elements, 0.1 mm pitch, 15 MHz). Electrophysiology was performed using regular tungsten microelectrode with electrical signal recorded on a MAP system (Plexon). Electrophysiological signals were filtered using the basic

feature 0.5 to 135 Hz for LFPs and 40kHz for spike activity. Here we aim to investigate the relation between the Power Doppler signal, proportional to the local Cerebral Blood Volume (CBV) and neuronal firing using simultaneous fUS and electrophysiology recordings. **Results** First, the ultrasonic imaging of local tissue motion during electrode insertion enabled us to precisely locate the electrode tip with a 100 µm precision. After the positioning of the electrode in the supplementary eye field (SEF) region at 2mm depth in the prefrontal cortex, we made electrophysiological and fUS coupled recording. The SEF was chosen for its role in monitoring oculomotor movement during cognitive tasks. We compared multiple and single unit activity with corresponding fUS signal. We saw that neuronal activity always preceded hemodynamic activity. We also observed long term dissociations between CBV and neuron firing recorded within a close area. **Conclusion** The simultaneous recording of local electrophysiological signals and global functional ultrasound imaging data allows us to record distinct biological phenomena at the same time, but also to monitor the insertion and precise positioning of the electrophysiological probe in the brain, in real time. **References** Neurophysiological investigation of the basis of fMRI signal, NK Logothetis *et al.,* 2001, Nature, DOI : 10.1038/35084005 Functional ultrasound imaging of the brain reveals propagation of task-related brain activity in behaving primates, A. Dizeux *et al.*, 2019, Nature Communication

Disclosures: J. Claron: None. **F. Arcizet:** None. **T. Deffieux:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-foundator and share-holder of ICONEUS. **M. Tanter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-foundator and share-holder of ICONEUS. **P. Pouget:** None.

Digital Abstract Session

P257. Autonomic Nervous System and Regulatory Responses

Program #/Poster #: P257.02

Topic: F.07. Autonomic Regulation

Support: Galvani Bioelectronics, UK

Title: Intermittent Stimulation of the Pudendal Sensory Nerve Alters Voiding Behavior in Conscious Unrestrained Wistar Rats

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Abstract: Overactive bladder (OAB), resulting in urgency, frequency, and incontinence, is a highly prevalent condition that leads to medical complications and decreased quality of life. Pudendal (Pud) nerve stimulation (STIM) has shown promising clinical results, but effective STIM parameters to reduce OAB symptoms remain unclear. Preclinical studies demonstrated

that the effects of pudendal sensory nerve (PudS) STIM on bladder capacity were both frequency and amplitude-dependent, but these studies were in anesthetized animals. Our goal was to quantify the effect of PudS STIM on voiding behavior in conscious unrestrained female Wistar rats. Food and water consumption, body weight, void frequency (VF), and void volume (VV) were recorded. Rats were habituated in metabolism cages for ~24 h/day, twice a week, and after habituation, the left PudS was implanted with a 300 μM bipolar cuff connected to a skull-mounted headcap. After recovery, animals were placed into metabolism cages three days a week (Pre-STIM: Mon, STIM: Wed, and Post-STIM: Thu). For STIM, rats were connected to an external stimulator, impedances recorded, and STIM threshold determined at 10 Hz (minimum amplitude that produced either a visual contraction of the pelvic region or any sign of awareness). The test amplitude was set just below this threshold, i.e., without contractions or awareness. PudS STIM was delivered at 10 Hz with one of three duty cycles (1/1, 3/1, or 1/2 h on/off) for 24 h once per week for several weeks. All three PudS STIM paradigms increased VV and decreased the VF over the 24 h period during STIM compared to Pre-STIM. The 1/1 h on/off STIM also increased VV on Post-STIM. All PudS STIM paradigms increased VV and decreased VF during STIM compared to Pre-STIM with both lights on and lights off. No change in normalized food consumption was observed across all duty cycles. The 3/1 h on/off STIM decreased normalized water consumption, whereas the 1/1 and 1/2 h on/off had no effect. Our results demonstrate, as reported in acute anesthetized studies, that PudS STIM is effective in changing the voiding behavior in conscious unrestrained animals. We observed increased VV and decreased VF using three different PudS STIM paradigms. Furthermore, with the 1/1 h on/off paradigm these effects persisted the following day, i.e., a carryover effect. Our results suggest that continuous STIM may not be necessary to reduce OAB symptoms and provide positive evidence that electrical STIM of peripheral nerves responsible for the coordination of lower urinary tract function may have utility in treating OAB.

Disclosures: C.L. Langdale: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Galvani Bioelectronics. **J.A. Hokanson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Galvani Bioelectronics. **D. Degoski:** None. **P. Milliken:** A. Employment/Salary (full or part-time):; Galvani Bioelectronics. **W.M. Grill:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Galvani Bioelectronics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Galvani Bioelectronics.

Digital Abstract Session

P257. Autonomic Nervous System and Regulatory Responses

Program #/Poster #: P257.03

Topic: F.07. Autonomic Regulation

Support: NIH Grant NR013693

Title: Sex Differences in Insular Gyrus activity during a Foot Cold Pressor

Authors: *B. KRAUSE-SORIO[1], K. E. MACEY[2], R. KUMAR[3], J. OGREN[2], R. HARPER[2], P. M. MACEY[4];
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Abstract: Introduction: Cardiovascular disease (CD) is the leading cause of death with striking sex differences. Neural control of cardiovascular functioning may underlie sex differences. We found sex- and region-specific variation across the five distinct gyri of the insula, which hold distinct roles in central autonomic regulation, using fMRI during a Valsalva maneuver (predominantly sympathetic activation) and a handgrip challenge (mainly parasympathetic withdrawal). Men showed greater left-lateralized responses in anterior, middle and posterior gyri, while women displayed greater right-lateralized activation in anterior and posterior, but not middle gyri. In order to determine whether the effects are task-specific, we compared sex and regional insular activity during a cold pressor challenge, an autonomic challenge targeting sympathetic activation associated with vasoconstriction and pain.**Methods:** Twenty-two women (age; mean±std: 50±4 yrs) and 39 men (45±3 yrs) underwent fMRI scanning using a 3T Siemens Trio scanner. The BOLD response during the 1 min right-foot cold pressor challenge was compared to a preceding 2 min baseline and a subsequent 2 min recovery period. Regions of interest were left and right anterior short gyri (ASG), mid-short gyri (MSG), posterior short gyri (PSG), anterior long gyri (ALG), and the posterior long gyri (PLG, anterior-to-posterior order). Heart rate (HR), blood oxygen saturation, and BOLD signals were compared between gyri, hemispheres, anterior vs. posterior insula and sexes at 2 sec intervals.**Results:** Women had higher baseline HR than men but there was no difference in percent HR change during the challenge. The BOLD signal initially peaked in all gyri, returned towards baseline at 10-15sec and increased again. Right hemisphere gyri responded more strongly than left gyri. Anterior vs posterior responses were also right-lateralized, with greater sub-regional differentiation in men. Increased right vs left responses in the ASG were similar for women and men, but women showed higher increases than men in the middle gyri (MSG, PSG, ALG).**Conclusions:** These findings are in line with our previous results demonstrating that the anterior gyri of the insula, particularly in the right hemisphere, responded more strongly to sympathetic activation. Unlike the Valsalva and hand grip challenges, there were no sex differences in lateralization in the ASG, but men showed reduced right hemisphere responses compared to women in the mid gyri. Despite similar lateralization, a comparable stimulus elicited differences in anterior insular responses, suggesting this region may be involved in sex differences in cardiovascular symptoms.

Disclosures: B. Krause-Sorio: None. **K.E. Macey:** None. **R. Kumar:** None. **J. Ogren:** None. **R. Harper:** None. **P.M. Macey:** None.

Digital Abstract Session

P257. Autonomic Nervous System and Regulatory Responses

Program #/Poster #: P257.04

Topic: F.08. Biological Rhythms and Sleep

Support: Branco Weiss fellowship of the Society in Science–ETH Zurich
Grants from the Flanders Fund for Scientific Research (FWO projects KAN 1506716N and G079017N)
European Varela Awards (Mind & Life Europe)

Title: Cross-frequency dynamics of neural, cardiac and respiratory rhythms in the context of effortful cognition and breath focus meditation

Authors: *J. R. SORIANO, J. RODRIGUEZ-LARIOS, K. ALAERTS;
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Abstract: Background. Pairs of neural oscillators can only phase-synchronize when their peak frequencies form harmonic relationships (e.g., $f_2 = f_1/2$; alpha = 10 Hz; theta= 5 Hz; ratio alpha:theta = 2:1). It is hypothesized that transient shifts in peak frequencies of different neural oscillators form the principal mechanism by which cross-frequency coupling and decoupling (i.e., facilitating information integration and communication) is implemented in the brain. Brain oscillatory activity in the alpha and theta bands has been shown to undergo peak frequency shifts upon conditions of cognitive effort, breath focus meditation practice and rest. Thus, considering the growing evidence that an interplay between neural and other main physiological oscillations (e.g. cardiac, respiratory and gastric rhythms) also takes place in a state-dependent manner, a similar mechanism of cross-frequency relationships has been proposed to characterize brain-body interactions. **Methods.** In line with a systemic approach to study human oscillatory physiology, we here explored cross-frequency relationships between neural-cardiac (i.e., alpha and heart rate; HR), as well as neural-respiratory oscillators (i.e., alpha and respiration rate; RR). Electroencephalography, electrocardiography and respiration were recorded from 18 young adults (mean age 23.56 ; 11 women) during three 5-min conditions of (i) rest, (ii) breath focus meditation and (iii) a cognitively demanding arithmetic task. Then, transient frequency changes per oscillator and the ratio between frequencies of oscillator pairs were computed. **Results.** The transient incidence of alpha-HR and alpha-RR cross-frequency relationships at or around the harmonic (8:1 for alpha-HR, 32:1 for alpha-RR) was significantly higher during the arithmetic task compared to rest and breath focus meditation. On the contrary, the incidence of approximately non-harmonic relationships (multiples of the respective harmonic ratio multiplied by the irrational number golden mean, 1.618; i.e., ratios of 12.94:1 for alpha-HR, 51.78:1 for alpha-RR) was shown to be higher during breath focus meditation compared to both rest and arithmetic task. **Conclusion.** These findings extend insights into the physiological underpinnings of cognition considering the integration of dynamically interacting body subsystems. More specifically, the cross-frequency relationships predominant in each condition suggest that during effortful cognition there is a greater degree of coupling between neural and cardiac/respiratory

oscillators. Conversely, breath focus meditation seems to be characterized by decoupling between these oscillators.

Disclosures: J.R. Soriano: None. **J. Rodriguez-Larios:** None. **K. Alaerts:** None.

Digital Abstract Session

P257. Autonomic Nervous System and Regulatory Responses

Program #/Poster #: P257.05

Topic: F.07. Autonomic Regulation

Title: Relationship between autonomic responses and balance control during stationary standing balance tasks

Authors: *K. J. HALL[1], M. LAYLOR[1], K. VAN OOTEGHEM[1], P. POLASTRI[2], J. VARGHESE[1], W. MCILROY[1];
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Abstract: A critical role for the autonomic nervous system (ANS) in the control of balance reactions has been proposed previously with evidence for potential involvement being inferred from the observation of phasic galvanic skin responses (GSR) evoked by external balance perturbations. The current study explored whether the coupling between ANS reactivity and balance reactions would be observed during spontaneously occurring instability while standing. It was hypothesized that time varying changes in GSR (ANS reactivity) would be associated with time varying changes in mediolateral center of pressure (ML-COP) (somatomotor reactivity). Nine individuals (5 females, 4 males; aged 19-37 years) were recruited. To induce varying balance demands during standing the study compared ML-COP and GSR data across different task conditions varying the availability of vision and width of the base of support. Subjects completed 3, 30 second trials for each of the following stance conditions: standard, narrow and tandem eyes closed, tandem eyes open, tandem eyes open with dome to shield visual input and restricted peripheral visual field. ANS activity was evaluated by measures of GSR recorded from Ag-AgCl electrodes on the middle phalanges of digits 2 and 4 on the left hand; balance measures include ML-COP excursion frequency and amplitude recorded from two force plates embedded in the floor underneath each foot. Subjects were instructed to stand as still as possible with arms crossed in front of their chest. When comparing mean task differences across subjects there was an expected increase in postural sway from tasks with wide stance and no sensory restrictions (least challenging) to those with narrow stance and no vision (most challenging). The correlation analysis revealed a significant positive relationship between ML-COP variability and GSR variability when comparing across tasks ($r=0.94$, $df=5$, $p<0.05$). In addition, correlations coincided within each subject and revealed a significant positive correlation in 7 participants ($r=0.47, 0.57, 0.62, 0.62, 0.81, 0.64, 0.69$ respectively, $df=19$, $p<0.05$) and no significant relationship in 2 participants ($r=0.36, 0.29$ respectively, $df=19$, $p>0.05$). The current study revealed a significant relationship between ML-COP and GSR during balance tasks, revealing the ANS reactivity associated with naturally occurring instability when standing still, which is

proportional to the degree of instability. Next steps will explore the temporal association between the time varying changes in COP and GSR to establish if ANS reactivity phase leads or lags the evoked motor reactions.

Disclosures: K.J. Hall: None. **M. Laylor:** None. **K. Van Ooteghem:** None. **P. Polastri:** None. **J. Varghese:** None. **W. McIlroy:** None.

Digital Abstract Session

P257. Autonomic Nervous System and Regulatory Responses

Program #/Poster #: P257.06

Topic: F.07. Autonomic Regulation

Support: National Institute of Nursing Research NR-017435
National Institute of Heart, Lung and Blood Institute HL135562

Title: Intact Parasympathetic Withdrawal During Handgrip Challenge Reflected by Insular Functional Organization And Heart Rate Variability In Obstructive Sleep Apnea

Authors: *A. PAL[1], J. A. OGREN[2], F. MARTINEZ[1], R. S. AYSOLA[3], R. KUMAR[4], L. A. HENDERSON[5], R. M. HARPER[2], P. M. MACEY[1];
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Abstract: Study Objectives: Insular gyri regulating autonomic function is disrupted in obstructive sleep apnea (OSA), which may contribute to cardiovascular comorbidities. Distinct activity patterns in insular gyri emerge to autonomic challenges, including anterior and right-side dominance during sympathetic activation; yet, OSA patients show only modest changes to such organization to Valsalva. We assessed neural responses of insular gyri in OSA and healthy Control participants to a static handgrip (HG) pressor task that principally is accompanied by parasympathetic withdrawal. We also assessed the parasympathetic nervous system activity compared to normal resting values (PNS index) aspect of heart rate variability in a separate cohort to estimate parasympathetic withdrawal during HG in OSA vs Control. **Methods:** We measured insular gyral response patterns with blood oxygen level-dependent functional MRI (fMRI). We studied 48 newly-diagnosed OSA (age mean±std:46.5±9years; AHI±std:32.6±21.1events/hour; 36 male) and 63 healthy (47.2±8.8years; 40 male) participants. Subjects performed four 16s HGs (1min intervals, 80% subjective maximum strength) during scanning. fMRI time-trends from five insular gyri—anterior short (ASG); mid short (MSG); posterior short (PSG); anterior long (ALG); and posterior long (PLG)—were assessed with repeated-measures ANOVA (p<0.05). Outside the scanner, we collected ECG data from 34 newly-diagnosed OSA (51.5±13.9years; 21.0±15.3events/hour; 20male) and 37 healthy (44.1±13.8years; 12male) participants during three 30s HGs (90 s intervals, 80% maximum strength) to estimate the PNS index calculated by Kubios. HG compared to baseline PNS indices

were averaged for 3 HGs in each subject, followed by group comparisons between OSA and Controls using ANOVA. **Results:** Females showed greater right anterior dominance in the ASG, but no fMRI differences emerged between OSA and controls relative to the functional insular organization in response to HG. Males showed greater left anterior dominance of the ASG, but there were no differences between OSA and controls. HG compared to baseline PNS indices, reduced by 0.8 a.u. (Control) and 0.7 a.u. (OSA), but with no significant OSA vs. Control differences. **Conclusions:** PNS index changes consistent with parasympathetic withdrawal during HG did not differ in OSA vs. Controls. Insular gyral functional organization responses to HG in OSA and Controls did not diverge significantly, further supporting an interpretation that parasympathetic withdrawal markers in the insula are largely intact in OSA, despite earlier studies showing morphologic injury to the overall insular structure in OSA.

Disclosures: A. Pal: None. **J.A. Ogren:** None. **F. Martinez:** None. **R.S. Aysola:** None. **R. Kumar:** None. **L.A. Henderson:** None. **R.M. Harper:** None. **P.M. Macey:** None.

Digital Abstract Session

P257. Autonomic Nervous System and Regulatory Responses

Program #/Poster #: P257.07

Topic: F.07. Autonomic Regulation

Support: Canadian Institutes of Health Research

Title: Sex Differences in repeated restraint stress-induced hyperthermia in rats

Authors: *T. J. PHILIPPE, M. DORDEVIC, V. VIAU;
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Abstract: Experience with a controllable challenge can protect an organism from the negative consequences of stress. Thus, determining the mechanisms of adaptive responses has important implications for understanding stress resistance and vulnerability. In our model of stress habituation, male and female rats emerge from repeated restraint exposure to show comparable declines in glucocorticoid stress responsivity, but also marked differences in thermoregulatory responses to serotonin (5-HT) 1A receptor activation. Since this receptor also modulates stress-induced hyperthermia, at least under acute conditions, here we explored the extent to which core body temperature responses are deferentially modulated by repeated restraint stress in male and female rats. Core body temperature was continuously monitored (5 min intervals) in 90 day old rats bearing intraperitoneal telemetric temperature sensors (Anipill, BodyCAP), implanted 7 days before testing (n = 8/group) in stress naïve animals, and those exposed to 2h periods of restraint, repeated daily (9:00 to 11:00 AM) for 5 consecutive days. As a function of repeated stress exposure, males, but not females showed reliable reductions in restraint-induced hyperthermia. Furthermore, females continued to show anticipatory thermal responses for up to 72 h following the last restraint exposure. Taken together, these findings suggest sex differences in stress-metabolic adaptations, and for this to occur as a consequence of changes in 5-HT regulation of

sympathetic nervous system outflow in male and females. While these potential differences in 5-HT signaling may well serve homeostatic responses to controllable stress, they may also be relevant to sex disparities in mood disorders associated with chronic stress exposure.

Disclosures: **T.J. Philippe:** None. **M. Dordevic:** None. **V. Viau:** None.

Digital Abstract Session

P257. Autonomic Nervous System and Regulatory Responses

Program #/Poster #: P257.08

Topic: F.07. Autonomic Regulation

Support: KAKENHI Grant 15K08664

Title: Downstream projection of Barrington's nucleus to the spinal cord in mice

Authors: ***M. KAWATANI**[1], W. C. DE GROAT[2], K. ITOI[3], K. UCHIDA[4], K. SAKIMURA[5], A. YAMANAKA[6], T. YAMASHITA[7], *M. H. KAWATANI[8];
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Abstract: Barrington's nucleus controls micturition behavior through the downstream projection to the spinal cord. In the Barrington's nucleus, two types of projection neurons, which were classified by the expression of *Crh* (Bar^{CRH}) and *Esr1* (Bar^{ESR1}), have suggested playing distinct roles on micturition behavior by manipulations of neural activity. The difference of their functional roles was explained by their projection targets, i,e, Bar^{CRH} preferentially projected to the L6-S1 intermediolateral (IML) region in the spinal cord, and Bar^{ESR1} projected to L6-S1 IML and dorsal commissure nucleus (DCN) region in the spinal cord. However, the synaptic connections from Bar to the spinal cord have not been fully elucidated. To understand the detailed mechanisms of synaptic integration in the spinal cord, we conducted *in vitro* slice patch-clamp recordings from spinal neurons, which are involved in the regulation of pelvic organ function. We examined the synaptic connectivity from Bar populations by using ChR2 assisted circuit mapping (CRACM) techniques. As a result, we revealed a direct glutamatergic synaptic connection from both types of Bar populations to DiI-labeled sacral pre-ganglionic neurons (S-PGN). Comparing the properties of opto-evoked excitatory post synaptic current (oeEPSC) to the S-PGN, Bar^{CRH} and Bar^{ESR1} send similar strong output to some populations of S-PGN. The stimulation of Bar^{ESR1} sometimes evoked modest intensity of oeEPSC, which might excite interneuron in the slice preparation. In addition, Bar^{ESR1} stimulation evoked opto-evoked inhibitory post synaptic current (oeIPSC) response to small portions of S-PGN. Optical stimulation of Bar^{CRH} and Bar^{ESR1} terminals elicited monosynaptic oeEPSC and polysynaptic

oeIPSC to the sacral DCN neurons, which might include interneurons projected toward either IML regions or ventral horn. Application of capsaicin or some neuropeptides (Substance P, somatostatin, gastrin-related-peptide, corticotrophin-releasing factor) modulate spontaneous post synaptic currents, but did not significantly alter oeEPSCs by Bar terminal stimulation.In current-clamp recordings, bath application of capsaicin increased opto-evoked responses to trains stimulations of Bar terminals. In conclusion, we provide the principles of synaptic connectivity from Bar to the spinal cord and suggest one part of the mechanisms of synaptic integration in the spinal cord.

Disclosures: **M. Kawatani:** None. **W.C. de Groat:** None. **K. Itoi:** None. **K. Uchida:** None. **K. Sakimura:** None. **A. Yamanaka:** None. **T. Yamashita:** None. **M.H. Kawatani:** None.

Digital Abstract Session

P257. Autonomic Nervous System and Regulatory Responses

Program #/Poster #: P257.09

Topic: F.08. Biological Rhythms and Sleep

Support: R01CA194924
P20GM121322
U54GM104942
5R01NS092388

Title: Circadian Influences on Chemotherapy Efficacy in a Mouse Model of Brain Metastases of Breast Cancer

Authors: ***W. H. WALKER**[1], J. R. BUMGARNER[1], S. A. SPROWLS[2], J. A. LIU[1], O. H. MELÉNDEZ-FERNÁNDEZ[1], J. C. WALTON[1], P. R. LOCKMAN[2], A. C. DEVRIES[3], R. J. NELSON[1];
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Abstract: In general, chemotherapy is more effective in the treatment of peripheral tumors than brain metastases, likely reflecting the reduced ability of chemotherapy to cross the blood brain barrier (BBB) and/or blood tumor barrier (BTB) at an efficacious concentration. Recent studies in *Drosophila* have demonstrated circadian regulation of BBB permeability. Thus, we sought to determine whether BBB/BTB permeability to chemotherapeutic agents displays circadian regulation. We predicted that optimally timed chemotherapy would increase anti-tumor efficacy in a murine model of brain metastases of breast cancer (BMBC). First, female NU/NU mice received a 1oo µl intracardiac injection of the human brain seeking Her2+ breast carcinoma cell line, JIMT-1BR3-Luc ($1.75x10^5$ cells). Thirty days following tumor inoculation, we characterized daily alterations in ^{14}C-paclitaxel within BMBC following injections given at four time points across the day: (zeitgeber time (ZT) 0 (lights on), ZT5, ZT14 (lights off), ZT17). Peak and trough concentrations of ^{14}C-paclitaxel within BMBC occurred mid-dark phase and at

the beginning of the light phase, respectively. Next, we assessed the functional significance of increased ^{14}C-paclitaxel concentrations within BMBC by monitoring the onset of neurological symptoms and cell death within BMBC. Mice received a 1oo µl intracardiac injection of the cell line, JIMT-1BR3-Luc ($1.75x10^5$ cells). Following 14 days a tumor growth, mice were randomly assigned to treatment groups, ZT0 or ZT17, and received once a week injections (2 weeks total) of paclitaxel (13 mg/kg) corresponding to either the peak or trough of BBB/BTB permeability (determined in Exp.1). Mice that received chemotherapy injections during the dark phase demonstrated increased cell death within BMBC and delayed onset of neurological symptoms relative to mice that received injections during the light phase. Together, these data provide strong evidence that chrono-chemotherapy is a novel and potentially effective treatment strategy for BMBC, and emphasizes the importance of time of day when administering chemotherapy to patients.

Disclosures: W.H. Walker: None. **J.R. Bumgarner:** None. **S.A. Sprowls:** None. **J.A. Liu:** None. **O.H. Meléndez-Fernández:** None. **J.C. Walton:** None. **P.R. Lockman:** None. **A.C. DeVries:** None. **R.J. Nelson:** None.

Digital Abstract Session

P258. Circadian Rhythms: Signals, Circuits and Behavior

Program #/Poster #: P258.01

Topic: F.08. Biological Rhythms and Sleep

Title: Study of the anticipatory activity to nebulized nicotine in female mice Strain C57BL/6

Authors: *Z. M. ROSAS[1], Y. BONASTRE[2], R. C. ZEPEDA[1], M. FLORES[3], O. LÓPEZ[3], T. MEZA[4], A. CORTÉS[5], T. MOLINA[6], C. JUÁREZ PORTILLA[2];
[1]Univ. Veracruzana, Xalapa, Mexico; [2]Ctr. de Investigaciones Biomédicas, Xalapa, Mexico; [3]Inst. de Ciencias de la Salud, Xalapa, Mexico; [4]Facultad de Medicina, Xalapa, Mexico; [5]Facultad de Biología, Xalapa, Mexico; [6]Inst. Interdisciplinario de Investigaciones, Xalapa, Mexico

Abstract: Study of the anticipatory activity to nebulized nicotine in female mice Strain C57BL / 6 Zenaida Rosas Ovando, Yumey Bonastre, Rossana C Zepeda, Mónica Flores, Óscar López, Thuluz Meza, Albertina Cortés, Tania Molina, Claudia Juárez PortillaAnticipatory activity allows to organisms to be prepared to future resources; it is characterized by increasing their locomotor activity from 1-2 h prior to a wide variety of high motivational value stimuli. It has been suggested that drugs of abuse can be synchronizers of circadian rhythms, as subjects show spontaneous locomotor activity 2 h prior drug arrival. In addition, intensity of effects as well as the potential to develop addiction depends on the sex of users. For instances, several reports have shown that women consume higher doses of certain drugs such as methamphetamine, cocaine and nicotine than men. Experimental female rats intake drugs faster, from the operant chamber, than male rats. Under these considerations, the goals of the present work were 1) to assess whether female mice show anticipatory activity to daily-nebulized

nicotine, and 2) to compare the intensity of the anticipatory activity to nicotine between female and male mice (previous data). Female C57/BL6 mice were divided in two groups, control group was nebulized with water and experimental group received nebulized nicotine at ZT4 during 20 minutes over 14 days. Animals were recorded 4 h prior to nebulization time and one h later for all experimental days. Videos were analyzed manually and behavior such as movement, grooming were identified considering the time in which they were performed. Results show that females anticipate 1 h prior nicotine availability. The anticipatory activity was significantly higher than anticipation of male mice. This latter result suggests that the ovary hormones could influence the increment in the intensity of anticipation from females.

Disclosures: Z.M. Rosas: None. **Y. Bonastre:** None. **R.C. Zepeda:** None. **M. Flores:** None. **O. López:** None. **T. Meza:** None. **A. Cortés:** None. **T. Molina:** None. **C. Juárez Portilla:** None.

Digital Abstract Session

P258. Circadian Rhythms: Signals, Circuits and Behavior

Program #/Poster #: P258.02

Topic: F.08. Biological Rhythms and Sleep

Support: DFG Grant STE531/18-3
DFG Grant STE531/26-1: SPP 2041
Förderlinie Graduiertenkollegs: Biological clocks, Univ. Kassel

Title: Examination of correlations between delta-, theta-, alpha-, beta-, and gamma-wave activity of the circadian clock and locomotor activity rhythms in the Madeira cockroach Rhyparobia maderae

Authors: *J. A. PLATH[1], J. Y. GESTRICH[3,1], P. ROJAS[2], M. BARTHOLMAI[1], A. MASSAH[1], C. LESEIGNEUR[1], M. J. A. PLATH[1], M. GARCIA[2], M. STENGL[1];
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Abstract: During the daily sleep-wake rhythm typical oscillatory brain waves, field potentials of mostly unknown origin, occur in mammalian brains. Recent insect studies suggested that brain waves are an evolutionary conserved property not limited to mammalian brains. In the Madeira cockroach we investigated which brain waves are generated by the circadian clock network and postsynaptic brain neuropils suited to regulate sleep-wake cycles. The cockroach clock was allocated previously to the accessory medulla (AME), a small neuropil in the brain's optic lobe. It is innervated by ~240 neuropeptidergic clock neurons, receives sensory inputs and sends outputs to different central brain neuropils to regulate daily activity rhythms. In search for clock-contacted premotor areas suited to regulate sleep-wake cycles we performed backfills from central pattern generators in the thorax combined with immunocytochemical staining of neuropeptidergic clock outputs. To investigate circadian modulation of brain wave frequencies,

we performed long-term loose-patch recordings from the AME (n = 28). *In vivo* brain wave recordings from the AME were combined with simultaneous recordings from premotor areas and/or with electromyograms from the leg muscle in intact animals (n = 8). Also, action potentials and local field potentials from the AME-clock and leg electromyograms as motor outputs were monitored simultaneously (n = 3). Previous results indicated that leg muscle activity approximates locomotor activity, thus, can serve as motor output assay (n = 10). We aimed to characterize whether / how brain waves and action potential frequencies in the clock change over the day in correlation with activity rhythms in premotor areas and motor outputs. Multiscale activity (ms to s) showed distinct activity peaks at dawn (Zeitgeber time/ZT 23.5), midday (ZT 6.5), dusk (ZT 10.5), and midnight (ZT 18.5). Interestingly, midday, evening, and midnight peaks were dominated by mid-scale events (2 - 290 ms), while action-potential-scale (1 - 2 ms) and longer events (290 ms - 9 s) were present mostly during the morning peak. Preliminary results showed that delta and theta waves were mostly found at dusk and late day to evening during photic phase shifting. Alpha and beta frequencies dominated during midday and also gamma was stronger during the day than night. Preliminary correlations between changes in locomotor activity and changes in the activity of the AME were observed (n=3). Taken together, we found ZT-dependent multiscale circadian activity of the AME also manifesting in mammalian-type brain waves that appear to correlate with either sensory input or motor output of this insect circadian clock.

Disclosures: J.A. Plath: None. **J.Y. Gestrich:** None. **P. Rojas:** None. **M. Bartholmai:** None. **A. Massah:** None. **M.J.A. Plath:** None. **M. Garcia:** None. **M. Stengl:** None. **C. Leseigneur:** None.

Digital Abstract Session

P258. Circadian Rhythms: Signals, Circuits and Behavior

Program #/Poster #: P258.03

Topic: F.08. Biological Rhythms and Sleep

Support: Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA

Title: The dual orexin receptor antagonist DORA-22 improves cage change-induced sleep alterations during the inactive phase of nocturnal rats

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Abstract: Dual orexinergic antagonists (DORAs) have been recently developed as a pharmacotherapy alternative to established hypnotics. It is unclear if DORAs improve insomnia-related sleep initiation and maintenance alterations during the rodent light/inactive period, which

is most analogous to when sleep disruption such as insomnia occurs in humans. Rats were first administered DORA-22 (Merck & Co., Inc.; 30mg/kg) or the GABA receptor-targeting hypnotic eszopiclone (10mg/kg), and then exposed to hourly clean cage changes, allowing behavioral evaluation of the propensity to sleep/sleepiness (hourly NREM sleep onset latency measures). Sleepiness was also measured following this insomnia model exposure using a rodent multiple sleep latencies protocol. A common complaint concerning hypnotic use is lingering hypersomnolence, which may impact performance, and this is a concern in pharmacotherapy of the elderly. Therefore, a second study was designed to determine a dose of DORA-22 which would initially promote sleep but exhibit minimal extended hypnotic efficacy. To determine this, animals were administered various doses of DORA-22 doses (1, 3, and 10mg/kg), then exposed to six hours of an insomnia model variant comprised of a single cage previously dirtied by a conspecific, followed by return to home cage for three hours of undisturbed recovery. In our first study, both DORA-22 and eszopiclone initially promoted sleep (hours 1 and 2), with DORA-22 exhibiting a more rapid hypnotic onset; and exhibited extended efficacy, evident six hours after administration. In our dose response second study, all DORA-22 doses initially promoted sleep; only the lowest dose (1mg/kg) did not exhibit extended hypnotic efficacy at the six-hour timepoint at the end of insomnia model exposure. In summary, we report that DORA-22 improved sleep measures when animals were exposed to mild stress-inducing stimuli (cage exposures), establishing a translatable model of hypnotic treatment in the light/inactive period, similar to when sleep disturbances occur in humans. Furthermore, a minimal dose of DORA-22 may initially promote sleep, yet exhibit few lingering (extended) effects that may impair next day mood and performance.

Disclosures: M.C. Gamble: None. **F. Katsuki:** None. **J.G. McCoy:** None. **R.E. Strecker:** None. **J.T. McKenna:** None.

Digital Abstract Session

P258. Circadian Rhythms: Signals, Circuits and Behavior

Program #/Poster #: P258.04

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant R01AG065830
NIH Grant NR01GM133032
NIH Grant R01MH103361

Title: Functional characterization of p90 ribosomal S6 kinase (RSK) signaling in the murine suprachiasmatic nucleus

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Abstract: The phasing of the master mammalian circadian clock, located in the suprachiasmatic nucleus (SCN) of the hypothalamus, is tightly regulated by the external light cycle. Within the

SCN, a key event in the light entrainment process appears to be the activation of the p44/42 mitogen-activated protein kinase (MAPK) cascade. Our previous work has shown that photic stimulation leads to activation of the MAPK pathway effector p90 ribosomal S6 kinase (RSK) in the SCN, thus raising the prospect that RSK signaling contributes to clock entrainment. Here, we tested this idea by employing an in vivo infusion approach to deliver the RSK inhibitor SL0101 [kaempferol 3-O-(3", 4''-di-O-acetyl-alpha-l-rhamnopyranoside)] to the SCN. Importantly, intraventricular infusion of SL0101 (25 mM) led to a potent suppression of light-evoked RSK activity in the SCN, and a marked attenuation of light evoked clock entrainment, as assessed using wheel running activity. In specific, in vehicle-infused C57/Bl6 mice, light exposure during the early subjective night led to a phase delay (~ -160 min) in activity onset. Contrary to this, disruption of RSK activity led to a significant relative decrease (~ - 110 min) in the phase-delaying effect of light, revealing that RSK signaling influences the magnitude of the light-evoked phase shift. Current phase-shifting experiments are focused on an examination of the contribution of RSK signaling to the late-night phase-advancing effects of light. Further, potential transcriptional and post-transcriptional mechanisms by which RSK signaling affects clock entrainment are currently being investigated. Finally, to examine whether RSK signaling affects the inherent timing properties of the master clock, cultures of SCN tissue from per1-Venus reporter mice were chronically treated (~ 4 days) with SL0101. We did not detect an effect of RSK inhibition on either the period or amplitude of the SCN, thus indicating that RSK signaling contributes to clock entrainment but does not influence the inherent pacemaker properties of the SCN.

Disclosures: H. Yoon: None. **A. Kalidindi:** None. **K.R. Hoyt:** None. **K. Obrietan:** None.

Digital Abstract Session

P258. Circadian Rhythms: Signals, Circuits and Behavior

Program #/Poster #: P258.05

Topic: F.08. Biological Rhythms and Sleep

Title: Cannabinoid receptor signaling supports sleep throughout development in males and females

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Abstract: Sleep is an essential process that supports cognitive functions, such as learning and memory. The endocannabinoid (eCB) system has emerged as a key regulator of sleep stability and quality. Increasing evidence indicates that the eCB system is differentially regulated between males and females and during development. We present our characterization of the eCB system in sleep in developing and adult male and female mice. Synaptic cannabinoid receptor 1 (CB1) is activated by endogenous bioactive lipids anandamide (AEA) and 2-arachidonoylglycerol (2-

AG). Targeted mass spectrometry indicate that synaptic 2-AG is upregulated by sleep deprivation in juvenile mice, but not adults, whereas synaptic AEA increases during sleep in adults but not juveniles. These results suggest that eCBs may differentially impact sleep during development. To test this idea, we used a piezoelectric device to non-invasively measure wake and sleep behavior in mice following pharmacological manipulations of the eCB system. Juvenile (p21-p42), adolescent (p42-p56) and adult (p100) mice were injected with PF-3845, a selective FAAH inhibitor to increase AEA, MJN-110, a selective MAGL inhibitor to increase 2-AG levels, or AM-251, a CB1 inhibitor. MJN-110 or PF-3845 treatment increased sleep amount and stability in males at each age but had minimal effects in females. CB1 inhibitor AM-251 decreased sleep amount and stability in both males and females at each age. These results suggest that an eCB tone acting on CB1 supports sleep in developing and adult males and females, and that pharmacological enhancement of the eCB tone further promotes sleep in males only. Further studies are ongoing to determine the molecular basis of the sex-based differences. These results show a clear link between sleep behavior and endocannabinoid signaling which will be useful in designing therapeutic interventions targeting the eCB system.

Disclosures: S.M. Gay: None. **S.C. Martin:** None. **N. Pazhayam:** None. **M. Ye:** None. **M. Armstrong:** None. **N. Reisdorph:** None. **G.H. Diering:** None.

Digital Abstract Session

P258. Circadian Rhythms: Signals, Circuits and Behavior

Program #/Poster #: P258.06

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01 Grant NS102209

Title: Kynurenic Acid Synthesis Inhibitor PF-04859989 Prevents Acute Kynurenine-induced Sleep Disturbances in Rats

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Abstract: Individuals who suffer from neurocognitive disorders, such as age-related dementias or schizophrenia, often suffer from sleep disturbances and comorbid parasomnias. Kynurenic acid (KYNA) is a tryptophan metabolite implicated in the pathophysiology of these disorders. Modest increases in KYNA, which acts as an antagonist at *N*-methyl-*D*-aspartate (NMDA) and α7 nicotinic acetylcholine (α7nACh) receptors, and an agonist at the aryl hydrocarbon (AhR) receptors, result in cognitive impairments and alterations in sleep-wake behavior (Pocivavsek et al. *Sleep* 2017). We presently sought to determine if pharmacological inhibition of the KYNA synthesizing enzyme, kynurenine aminotransferase II (KAT II), may serve as a potential avenue

to overcome sleep deficits. We explored the hypothesis that elevated KYNA adversely impacts sleep quality. Adult male and female Wistar rats (N = 9-11 per sex using a within animal treatment design) were implanted with telemetric devices to acquire electroencephalogram (EEG) and electromyogram (EMG) recordings and challenged with kynurenine, the direct precursor to KYNA. At the beginning of the light phase, rats received injections of either 1) vehicle, 2) kynurenine (100mg/kg; i.p.), 3) the systemically active KAT II inhibitor, PF-04859989 (30 mg/kg; s.c), or 4) PF-04859989 and kynurenine in combination. Analysis of vigilance state-related parameters categorized as wake, rapid eye movement (REM) and non-REM (NREM) were assessed for 24 h after treatment. The kynurenine challenge significantly reduced REM duration by 15% compared to vehicle treatment ($P<0.05$) during the light phase. PF-04859989 given prior to the acute kynurenine challenge elicited unchanged REM sleep duration compared to vehicle treatment or PF-04859989 treatment alone during the light phase in males. Interestingly, PF-04859989 increased NREM duration ($P < 0.05$) and decreased wake duration ($P < 0.05$) across 24 hours in both vehicle and kynurenine-treated trials, suggesting that the KAT II inhibitor may have slight sedative properties. Taken together, REM was restored when PF-04859989 was administered prior to the kynurenine challenge, suggesting that KAT II inhibition was sufficient to prevent the kynurenine-induced reduction in REM. Changes in vigilance state parameters elicited by the KAT II inhibitor may indicate mild somnolence. The present and future complementary experiments provide mechanistic value to understanding the role of KYNA in modulating sleep behavior and demonstrate KAT II inhibition as a potential therapeutic avenue for improving sleep disturbances associated with neurocognitive disorders.

Disclosures: K.M. Rentschler: None. **A.M. Baratta:** None. **H. Nicholson:** None. **J.A. Mong:** None. **A. Pocivavsek:** None.

Digital Abstract Session

P259. Sleep Systems

Program #/Poster #: P259.01

Topic: F.08. Biological Rhythms and Sleep

Support: ISF 1326/15
Adelis Foundation (YN)

Title: Sleep deprivation differentially modulates rat auditory cortex processing in a manner similar to NREM sleep

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Abstract: <u>Background:</u>
Despite the prevalence of sleep deprivation (SD) in modern lifestyle, how SD affects brain activity and behavioral deficits remain poorly understood. It has been suggested that SD-associated deficits may stem from sleep-related neural processes that invade the waking brain.

However, which aspects of neural processing are involved, and whether they also affect early sensory regions, remain unclear.

Results:

We presented sounds (tones and click-trains at different rates) to male Wistar rats (n=7) undergoing 5 hours SD, followed by 5 hours sleep opportunity, and recorded EEG, EMG, as well as spiking activity in the auditory cortex. We found that SD affected particular aspects of auditory processing -- mainly modulating late components of the auditory response and population synchrony. Accordingly, spontaneous firing rate (FR) and onset response magnitude were relatively unaffected by sleep pressure (6±1.3% and 1.1±1.1% increase, respectively, when comparing last vs. first 1.5h of SD, n=193 units, mean±sem). In contrast, post-onset FR (40-100ms), entrainment to fast click trains (20-40 Hz) and population coupling (a measure of population synchrony) were strongly modulated by sleep pressure (-35±2%, -20±2% and 17±2%). Qualitatively similar effects were observed during NREM sleep, showing even stronger modulation of post-onset FR, clicks entrainment and population coupling, when compared to wakefulness (-62±2%, -57±2%, 33±2%). Interestingly, auditory processing during REM sleep was similar to that during wakefulness (≤10% change).

Conclusions:

Specific aspects of auditory cortical processing, including population synchrony, post-onset FR and adaptation to fast modulations in stimulus intensity, are strongly affected by sleep pressure. Such changes are qualitatively similar to those observed during NREM, but not REM sleep. This implies that a NREM-like synchronized cortical state may invade the waking brain and contribute to impaired neural processing during SD.

Disclosures: A. Marmelshtein: None. **Y. Nir:** None.

Digital Abstract Session

P259. Sleep Systems

Program #/Poster #: P259.02

Topic: F.08. Biological Rhythms and Sleep

Support: Israel Science Foundation grant 1326/15
Adelis Foundation
European Research Council (ERC-2019-CoG 864353)

Title: Robust widespread early feedforward auditory cortical responses co-occurring with impaired late top-down signaling in human sleep: an intracranial iEEG/LFP/single-unit study

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Abstract: Reduced responsiveness to the environment is a hallmark of sleep, yet it remains unclear how responses along cortical sensory pathways during different sleep stages compare to those during wakefulness. Previous non-invasive studies in humans and intracranial studies in animal models have yet to resolve where along the sensory hierarchy, in which signals, and what factors affect sensory responses during sleep. Here, we performed recording sessions in 13 drug-resistant epilepsy patients implanted with clinical depth electrodes. Upon informed consent, we recorded intracerebral EEG, LFPs, and neuronal spiking activity along with polysomnography while we intermittently presented a battery of simple (click-trains) and complex sounds (e.g. words, sentences, music) during wakefulness and sleep (8 full-nights and 6 naps). We found that auditory spiking responses (n=305 in 55 units) as well as LFP high gamma (80-200Hz) responses (n=556 in 74 microwires) were largely preserved during sleep. Sleep responses exhibited moderate amplitude attenuation (median attenuation = -30% and -0.22dB for spiking activity and gamma, respectively), yet remained tightly locked to soundwave envelopes (median correlation coefficient r=0.39 in wake and 0.37 in sleep). By contrast, LFP alpha-beta (10-30Hz) power decrease during auditory stimulation (n=252 in 57 microwires) were prevalent in wakefulness but significantly disrupted in sleep (median attenuation = -1.4 dB). The degree of NREM sleep attenuation was robustly correlated with response latency in each microwire ($r=-0.8$, $p<0.001$). In addition, stronger attenuation was observed in downstream regions than in A1 itself for units' responses, in late sustained responses than in early onset responses ($p<0.001$), and during intervals with high slow wave activity (median attenuation=-5%, $p<0.001$). During REM sleep, we observed entrainment of iEEG/LFP to 40Hz click-trains at wake-like levels, unlike its partial attenuation during NREM sleep (median attenuation = 15% and 30% for LFP and iEEG respectively). Altogether, robust auditory responses persist during sleep across the temporal lobe well beyond primary auditory cortex while LFP alpha-beta power decrease, possibly reflecting top-down processes, is deficient.

Disclosures: H. Hayat: None. **A. Marmelshtein:** None. **A. Krom:** None. **Y. Sela:** None. **A. Tankus:** None. **I. Strauss:** None. **F. Fahoum:** None. **I. Fried:** None. **Y. Nir:** None.

Digital Abstract Session

P259. Sleep Systems

Program #/Poster #: P259.03

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant NS094062

Title: The effects of hypoxia on the activity of hypoglossal motoneurons during non-REM and REM sleep

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Abstract: We have developed an animal model of hypoxia in the chronically-instrumented, unanesthetized cat preparation during the course of experiments which were designed to explore neuromechanisms that were responsible for the suppression of hypoglossal motoneuron activity during REM sleep under normoxic and hypoxic conditions. In the present study, using this animal model with extracellular recording techniques, we examined the effects of hypoxia on the activity of hypoglossal motoneurons during spontaneously-occurring states of non-REM and REM sleep. Adult cats were prepared for monitoring behavioral states of sleep and wakefulness as well as extracellular recordings of hypoglossal nerve. During the experiment, the oxyhemoglobin saturation (SpO_2) level and heart rate were continuously monitored. Single episodes of hypoxia were induced, via a breathing mask positioned in front of the animal's nose and mouth, by a ventilatory system that controls "on" and "off" flows of nitrogen gas and air. The hypoglossal nerve activity was examined during naturally-occurring non-REM and REM sleep under normoxic and hypoxic conditions. Under normoxic conditions, there was a mean decrease of 12.5±2.2% (n=31) in the hypoglossal nerve activity during REM sleep compared to non-REM sleep. However, under hypoxic conditions (75% SpO_2), the nerve activity decreased by 21.0±2.5% (n=28) during REM versus non-REM sleep. The reduction in the hypoglossal nerve activity during REM sleep under hypoxic conditions was significantly greater than that under normoxic conditions (P= 0.012, unpaired t-test). In addition, there was a mean increase of 9.9±1.8% in the nerve activity during non-REM sleep under hypoxic conditions compared to normoxic conditions. The present results demonstrate that there is an increase in the activity of hypoglossal motoneurons during non-REM sleep under hypoxic conditions, and a significantly greater suppression in the activity of these motoneurons during REM sleep under hypoxic conditions compared to normoxic conditions. These data support our hypothesis that during REM sleep under hypoxic conditions (e.g., Obstructive Sleep Apnea), disfacilitation (withdrawal of excitatory drives present during non-REM sleep) and postsynaptic inhibition are responsible for the suppression of hypoglossal motoneuron activity and ensuing atonia of the genioglossal muscle.

Disclosures: C.D. Tobin: None. **S.J. Fung:** None. **M. Xi:** None. **M.H. Chase:** None.

Digital Abstract Session

P259. Sleep Systems

Program #/Poster #: P259.04

Topic: F.08. Biological Rhythms and Sleep

Support: SNF Grant P0ZHP1-178697
SNF Grant PCEFP1- 51181279
Foundation for Research in Science and Humanities STWF-17-008
Olga Mayenfisch Stiftung

University of Zurich FK-18-047
University of Zurich CRPP "Sleep and Health"
University of Zurich, Faculty of Medicine

Title: Daytime Sleep in Infants - A Marker of Brain Maturation?

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Abstract: *Objective.* A close link has been proposed between infant sleep and developmental processes of brain and body physiology. Even though daytime sleep accounts for one-third of infants' overall sleep, it is often understudied due to difficulties with its accurate quantification. We aimed to characterize daytime sleep (duration, regularity) across infancy and tested whether it relates to nighttime sleep electroencephalography (EEG) power, behavioral development, and gut bacteria.
Methods. We measured sleep behavior (11 days with actigraphy and 24-h diary), behavioral development, and gut bacteria composition in a longitudinal population of 162 infants at ages 3, 6, and 12 months. In a subset of 32 infants age 6 months, nighttime sleep EEG was recorded (128 electrodes, EGI, recording duration for 2 hours, 500 Hz sampling). A principal component approach was used to generate sleep composites, including one, which encompasses several aspects of daytime sleep. We applied standard EEG processing (bandpass filter 0.5-50 Hz, down-sampling to 128 Hz, average referencing, sleep stage scoring, semi-automated artifact rejection, spectral analysis with Fast Fourier transform routine), and computed EEG power for slow wave activity (1-4.5 Hz). Behavioral development was quantified using parent-reports (Ages and Stages Questionnaire). We determined gut bacteria composition from stool samples using 16S rRNA gene sequencing and quantified 3 measures: alpha diversity, enterotype (classification into simplified profiles), and relative maturational status (random forest). Multilevel models were applied across all 3 time points and general linear models for each time point separately.
Results. Daytime sleep duration decreased by 40% across the first year of life. Daytime sleep was associated with behavior (sum score, $p = 0.03$), such that infants with less daytime sleep scored higher on behavioral development. This effect was most pronounced at 3 months of age. Infants with less daytime sleep had significantly higher gut bacterial diversity ($p = 0.03$), again, specifically prominent at 3 months. Less daytime sleep was associated with lower slow wave activity in temporal and parietal areas of the scalp during nighttime sleep ($p = 0.007$, cluster corrected).
Conclusions. Our results indicate that daytime sleep duration and regularity in infants is associated with markers of behavioral development and gut bacterial diversity. As slow wave activity signifies sleep pressure, we propose that infants with less daytime sleep have a more

mature neurophysiology and advanced ability to sustain prolonged wakefulness, resulting in lower sleep pressure at nighttime sleep.

Disclosures: **S.F. Schoch:** None. **J.L. Castro-Meija:** None. **D.S. Nielsen:** None. **L. Krych:** None. **W. Kot:** None. **B. Leng:** None. **M. Kohler:** None. **R. Huber:** None. **G. Rogler:** None. **L. Biedermann:** None. **J. Walser:** None. **S. Kurth:** None.

Digital Abstract Session

P260. Sleep and Behavior

Program #/Poster #: P260.01

Topic: F.08. Biological Rhythms and Sleep

Support: Human Frontier Science Program LT000440
Sagol School of Neuroscience post-doctorate program
Yad Hanadiv / Rothschild Foundation
NSF-BSF collaborative grant 1756473
ERC-2019-CoG 864353
NINDS grant U01NS108930
NINDS grant R01NS084017

Title: Closed-loop intracranial stimulation during sleep enhances cortico-hippocampal synchronization and memory consolidation in humans

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Abstract: A long line of research has established a role for sleep in memory consolidation, yet the specific mediating mechanisms remain unclear. At present, there is a formidable gap between animal studies — which use direct recordings and manipulations of neural circuits to link hippocampal-cortical synchronization to memory consolidation — and non-invasive human studies. Building upon rodent studies in which precisely timed electrical stimulation during sleep reinforced the endogenous coordination between hippocampal and neocortical activities, we developed a closed-loop system that monitored activity in the human medial temporal lobe (MTL) to trigger brief pulses of electrical stimulation in neocortical white matter. Upon informed consent, 14 pharmacoresistant epilepsy patients implanted with intracranial electrodes for clinical monitoring participated in intracranial stimulation and recording sessions during sleep. Closed-loop *sync-stimulation*, robustly locked to MTL slow-wave (SW) active phase, enhanced subsequent slow wave (N = 248 iEEG contacts, $P<10^{-30}$) and spindle activity ($P<10^{-29}$) across the brain, as well as their coupling ($P<10^{-9}$). These effects were not observed in a control group receiving mixed-phase stimulations (across all MTL slow-wave phases, N = 215 iEEG contacts,

p>0.5). Following *sync-stimulation*, neuronal units (N = 280) across multiple neocortical sites significantly synchronized their firing to MTL SW activity, evident by increased ($P<10^{-8}$) phase locking to MTL field potentials. We further examined the cognitive effects of this intervention in a subset of patients (N = 9) who learned paired associations between images in the evening and performed recognition tests immediately after learning and in the morning following sleep. Each patient participated in two night sessions (order counterbalanced) either with closed-loop stimulation during sleep or without. Six of seven participants in the *sync-stimulation* group exhibited improved memory accuracy following sleep-intervention relative to the no-stimulation nights. No improvement was observed in the participant group receiving mixed-phase stimulation. Importantly, memory accuracy improvement across all participants was tightly correlated (Tau=0.45, $P<10^{-19}$) with the extent of unit-firing synchronization to the MTL and with spindle-rate enhancement (R = 0.77, P = 0.013), but not to a change in interictal spikes activity (P = 0.15). Our results provide causal evidence in humans for a model in which cortico-hippocampal synchronization during sleep mediates memory consolidation.

Disclosures: **M. Geva-Sagiv:** None. **E.A. Mankin:** None. **D. Eliashiv:** None. **S. Epstein:** None. **N. Cherry:** None. **G. Kalender:** None. **N. Tchemodanov:** None. **Y. Nir:** None. **I. Fried:** None.

Digital Abstract Session

P260. Sleep and Behavior

Program #/Poster #: P260.02

Topic: F.08. Biological Rhythms and Sleep

Support: R01 NS102209
P50 MH103222

Title: Prenatal kynurenine elevation elicits sex-dependent changes in sleep and arousal during adulthood: Implications for schizophrenia

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Abstract: Dysregulation of the kynurenine pathway (KP) of tryptophan catabolism has been implicated in psychotic disorders including schizophrenia (SZ) and bipolar disorder. Kynurenic acid (KYNA) is a KP metabolite synthesized by kynurenine aminotransferases (KATs) from its biological precursor kynurenine, and acts as an endogenous antagonist of N-methyl-D-aspartate and α7-nicotinic acetylcholine receptors. KYNA has been linked to cognitive impairments in SZ and may also contribute to sleep disturbances in patients. To further understand the role of KYNA in SZ etiology, we employ the embryonic kynurenine (EKyn) model in rats and fed kynurenine (kyn; 100 mg/day) to pregnant Wistar dams from embryonic day (ED) 15 to ED 22

(control: ECon; kyn-treated: EKyn) to elevate KYNA in the fetal brain. The present study was designed to 1) define sleep-wake behavioral dysfunction during light and dark phases and 2) test the effectiveness of PF-04859989, a KAT II inhibitor, which reduces brain KYNA, in adult (postnatal day 56 - 85) offspring from ECon and EKyn litters. We assessed the impact of this developmental exposure by examining vigilance state parameters in adult male and female rats using electroencephalogram (EEG) and electromyogram (EMG) telemetry and determined sex differences in sleep and arousal in EKyn offspring. EKyn males exhibited reduced rapid eye movement (REM) sleep (-20%, $P<0.05$), while EKyn females displayed hyperarousal compared to controls, evidenced as 1.4-fold longer average duration of wake bouts ($P<0.01$) demonstrating consolidated wakefulness. To complement sleep-wake behavioral analysis, relative cage activity was significantly reduced in EKyn females (-42% during light phase, -18% during dark phase, $P<0.05$), but no differences were determined between EKyn males and controls ($P=0.36$). When adult ECon and EKyn rats were also assessed in the open field paradigm to determine general locomotor activity, anxiety, and willingness to explore a new arena, no major deficits were determined. Preliminary analysis of data obtained after acute PF-04859989 (30 mg/kg) treatment in EKyn offspring indicate a significant increase in REM duration, suggesting that an acute decrease in brain KYNA may restore deficiencies in REM sleep and mitigate sleep deficits. Taken together, our findings demonstrate striking sex-dependent sleep alterations in EKyn offspring, and we are continuing to investigate the interplay between sleep and KYNA in a translationally relevant model of neuropsychiatric illness.

Disclosures: S. Milosavljevic: None. **K.M. Rentschler:** None. **A.L. Ditty:** None. **N.T.J. Wagner:** None. **C.J. Wright:** None. **J.A. Mong:** None. **A. Pocivavsek:** None.

Digital Abstract Session

P260. Sleep and Behavior

Program #/Poster #: P260.03

Topic: F.08. Biological Rhythms and Sleep

Support: NIH RISE: 5R25GM061151-19.
NIH COBRE: P20GM103642

Title: Dose-dependent effect of ethanol on sleep patterns in Drosophila melanogaster

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Abstract: Alcohol consumption is known to affect many physiological processes necessary for the proper functioning of the organism. In the brain, alcohol binds to several molecular targets to produce an overall suppression of neuronal activity. This leads to a series of neuroadaptive mechanisms implemented to counterbalance ethanol-induced neuronal suppression and help the system restore homeostasis. These neuroadaptive changes have been associated with the development of alcohol tolerance, dependence and ultimately addiction. In addition, these

adaptations are also believed to be the root of a series of sleep disturbances, which often manifest during the development of alcoholism. As both, alcohol addiction and sleep regulation are under homeostatic control, we hypothesize that these processes share a common mechanism. Here, we use Drosophila melanogaster as a biological model to study alcohol-sleep responses. We compare two different methods of alcohol administration and examine the ethanol concentration that each fly had after the experiment in order to study the effect alcohol has on sleep. Our results suggest that ethanol influences sleep, but this effect varies according to the manner in which it is administered. For future studies, we believe that the integration of genetic analyses with physiological modulation of neural activity within specific sleep circuits has tremendous potential to uncover the functionally relevant molecular targets whose action contributes to the deleterious effect of alcohol on sleep.

Disclosures: M.E. Ramirez Roman: None. **G. Ayala-Santiago:** None. **J. Agosto:** None. **A. Ghezzi:** None.

Digital Abstract Session

P260. Sleep and Behavior

Program #/Poster #: P260.04

Topic: F.08. Biological Rhythms and Sleep

Support: I01 BX004500
I01 BX002774
I01 BX001356
I01 BX001404
IK2 BX002130
IK2 BX004905
R21 NS079866

Title: Basal forebrain parvalbumin neurons modulate cortical E/I balance, cortical responses to sensory stimuli, and vigilant attention

Authors: *F. L. SCHIFFINO, D. D. AGUILAR, F. KATSUKI, L. K. RADZIK, J. T. MCKENNA, R. E. BROWN, *R. E. STRECKER, J. M. MCNALLY;
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Abstract: Recent work has shown that manipulation of a subpopulation of BF GABAergic neurons, those containing the calcium-binding protein parvalbumin (PV), allows exquisite control of cortical fast oscillations. Optogenetic excitation of BF-PV neurons in mice increases cortical fast oscillations, locomotion, wakefulness, and triggers arousals from sleep; BF-PV inhibition produces opposite effects. Here we investigated whether BF-PV neurons regulate more complex behaviors such as attention, learning, and the cortical processing of sensory stimuli. Brief (2s) continuous low power laser photoexcitation of BF-PV neurons (473nm@5mW) enhances cortical entrainment to repetitive presentations of 40Hz sensory stimuli; in contrast,

longer duration excitation (e.g., 2min) elevates broadband cortical gamma band activity (30-80Hz) and impairs the cortical entrainment to 40Hz sensory stimuli. Thus, brief and long durations of BF-PV excitation produce opposite effects. This suggests that appropriately timed activity of BF-PV neurons can enhance cortical responses to stimuli, while either inhibition or prolonged excitation of BF-PV neurons can impair cortical sensory responses. This strongly supports an inverted U-shaped dose response effect of BF-PV activity on cortical responsiveness and is consistent with models of the relationship between arousal and performance (i.e., Yerkes-Dodson law). These results also show how proper E/I balance is necessary for optimal cortical processing. Next, we used brief (1s) continuous laser excitation of BF-PV in a mouse vigilant attention task. Excitation of BF-PV neurons (1s,473nm@5mW) that preceded the signal by 0.5s improved vigilant attention as indicated by quicker reaction times. In contrast, both sleep deprivation (8h) and ArchT mediated inhibition of BF-PV neurons (1s,530nm@10mW) slowed reaction times. Importantly, brief BF-PV photoexcitation prior to the signal rescued deficits observed in sleep deprived mice. Finally, preliminary fiber photometric data show that continuous excitation of BF-PV increases population activity of pyramidal neurons in prelimbic cortex (CAMKII-GCaMP6f), suggesting altered E/I balance. Control experiments indicate that excitation of BF-PV neurons does not alter motivation (e.g., hunger) and is not rewarding, indicating that excitation of BF-PV neurons may enhance cognition with limited side effects and low addictive potential. The combined findings support the idea that BF-PV neurons briefly activate cortex in anticipation of, or in response to, meaningful stimuli; in turn this enhances cortical processing and facilitates attention-dependent performance.

Disclosures: **F.L. Schiffino:** None. **D.D. Aguilar:** None. **F. Katsuki:** None. **L.K. Radzik:** None. **J.T. McKenna:** None. **R.E. Brown:** None. **R.E. Strecker:** None. **J.M. McNally:** None.

Digital Abstract Session

P260. Sleep and Behavior

Program #/Poster #: P260.05

Topic: F.08. Biological Rhythms and Sleep

Support: SFB1340 (HS)
R01MH107390 (DEK)
R01MH114017 (DEK)

Title: Sleep and Survival of Drosophila melanogaster SerT mutants

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Abstract: The annual prevalence of mental health illness in the United States is on the rise, with anxiety and depression holding the top two spots, at 19.1% and 7.2%, respectively. The serotonergic system, and more specifically the serotonin reuptake transporter (SerT), are the most common pharmacological targets of anxiety and depression treatments. To better understand the relevance of altered serotonin signaling due to changes in serotonin transporter function, we generated SerT mutants in *Drosophila melanogaster* SerT using imprecise excision mutagenesis. SerT mutant sleep behavior and survival were measured as anxiety and depression are often accompanied by sleep irregularities and abnormal eating patterns. SerT mutants showed an increase in night sleep bout duration and a decrease in sleep latency. We provide evidence that the level of SerT influences sleeping patterns, since changes in SerT level influence sleep differentially. These behaviors were partially rescued in heterozygous flies. Rescue experiments using UAS-SerT were performed to ensure that the phenotypic differences observed in behavior were truly due to the lack of SerT, which in sleep is regulated by a subset of serotonergic neurons. In the desiccation experiments, SerT mutants survived longer than the wildtype flies, which is consistent with a previously published SerT hypomorph. Understanding the function of SerT and the effects of increased extracellular serotonin contributes to our knowledge about current treatments for mental illness.

Disclosures: **R.C. Arnold:** None. **N. Dalili:** None. **M.M. Sampson:** None. **S. Jois:** None. **A. Kaiser:** None. **H. Scholz:** None. **D.E. Krantz:** None.

Digital Abstract Session

P260. Sleep and Behavior

Program #/Poster #: P260.06

Topic: F.08. Biological Rhythms and Sleep

Support: NIH grant NS092388
NIGMS 5U54GM104942-03

Title: Exposure to Dim Light at Night During Recovery from Cardiac Arrest Affects Behavior and Brain Vasculature

Authors: O. H. MELÉNDEZ-FERNÁNDEZ, S. M. BROADWAY, W. H. WALKER, II, J. A. LIU, J. R. BUMGARNER, N. ZHANG, *J. C. WALTON, A. C. DEVRIES, R. J. NELSON; Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Electrical lighting and devices are now ubiquitous. Whereas these devices and ability to illuminate the night contributes to societal productivity, the negative impact of exposure to light at night (LAN) is becoming uncovered. Exposure to LAN disrupts of the endogenous circadian biological clock, and consequently, has many downstream effects. Mice exposed to dim LAN (dLAN) have decreased expression of vascular endothelial growth factor-A (VEGF), increased microglial expression, and reduced brain derived neurotrophic factor. Moreover, the development of major depressive disorder has been associated with varying VEGF

concentrations in serum and plasma, suggesting that vasculature is disrupted by LAN. These results suggest that LAN alters brain physiology and behaviors. In the present study, the effects of dLAN on mouse behavior, and neurovasculature after cardiac arrest (CA) were examined. We hypothesized that exposure to dLAN after CA would impair behavior and hippocampal vascularization in mice. Male and female Swiss Webster mice (8 wks of age; n=160) underwent a cardiac arrest/cardiopulmonary resuscitation (CA/CPR) or sham procedure, and were placed into either dark night housing [14 h light 125 lux):10 h dark (0 lux) n=40m/40f)] or dim white light at night conditions [14 h light (125 lux):10h dim light (5 lux) (n=40m/40f)] for one week. Following that week, all mice were returned to dark night conditions for 1 week. Behavioral testing and tissue collection were conducted at the end of this week. Anxiety-like behaviors were assessed using the elevated plus maze (EPM). Prior to euthanasia, mice were administered tomato lectin via a tail vein injection to visualize and quantify vasculature. Females that underwent CA and exposed to dLAN reduced anxiety-like behaviors in the EPM compared to their counterparts in dark nights. Further, females exposed to dLAN reduced vascularization in the CA2 and dentate gyrus regions of the hippocampus, which was exacerbated by CA. Males exposed to dLAN reduced their latency to enter open arms in the EPM compared to their counterparts, however they displayed no differences in total open arm entries. Males displayed vascular differences in the dentate gyrus in response to dLAN exposure, but not in response to CA. These data suggest that the effects of dLAN exposure after CA on behavior can be reversed by return to dark nights, however, vascular effects may not be reversed in that time frame. Supported by NIH grant NS092388.

Disclosures: O.H. Meléndez-Fernández: None. **S.M. Broadway:** None. **W.H. Walker:** None. **J.A. Liu:** None. **J.R. Bumgarner:** None. **N. Zhang:** None. **J.C. Walton:** None. **A.C. DeVries:** None. **R.J. Nelson:** None.

Digital Abstract Session

P260. Sleep and Behavior

Program #/Poster #: P260.07

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R21 HD099686-02
Dup15q Alliance

Title: Electrophysiological Biomarkers of Sleep in Children with Duplications of 15q11.2-13.1 (Dup15q syndrome)

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Abstract: Duplications of 15q11.2-13.1 (Dup15q syndrome) are highly penetrant for autism, intellectual disability, delayed development and epilepsy. Several genes in the 15q region,

particularly UBE3A and a cluster of $GABA_A$ receptor genes, are critical for early brain development, including synaptic function and inhibitory neurotransmission. During wakefulness, children with Dup15q syndrome have increased beta oscillations (12-30 Hz), that likely reflect aberrant GABAergic neurotransmission. Healthy sleep physiology is necessary for robust cognitive development, and prior studies have shown that non-REM sleep rhythms are highly dependent on GABAergic neurotransmission. Yet, no studies to date have quantified NREM sleep physiology parameters in children with Dup15q syndrome. Therefore, in order to examine sleep physiology, we analyzed 7 hours of overnight sleep EEG recording from a cohort of children with Dup15q syndrome (n=15, mean age: 5.7 years) and age-matched neurotypical controls (n=12, mean age: 5.8 years). We computed beta power in sleep, spindle density and percentage of slow wave sleep (SWS), and compared these features between the two groups. Compared to typically developing, age-matched controls, children with Dup15q syndrome showed significantly elevated beta power in sleep (frontal: $p=0.001$, central: $p=0.01$ and occipital: $p=0.0009$ channels), reduced spindle density ($p<0.0001$) and reduced SWS (frontal: $p<0.0001$, central: $p=0.0003$ and occipital: $p=0.0005$ channels). These abnormal sleep features may thus serve as quantitative electrophysiological biomarkers of sleep in children with Dup15q syndrome. Insights from this study not only promotes a greater mechanistic understanding of the pathophysiology defining the syndrome but also lays the foundation for studies that investigate relationship between sleep and cognition. Abnormal sleep physiology may undermine cognitive development in Dup15q syndrome and may serve as a quantifiable and modifiable target for behavioral and pharmacological interventions.

Disclosures: V. Saravanapandian: None. **D. Nadkarni:** None. **S. Jeste:** None.

Digital Abstract Session

P261. Diet, Obesity and Body Weight

Program #/Poster #: P261.01

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant DK120298
NIH Grant R01DK102918
NIH Grant AG059778

Title: Deploying genetic diversity to explore the impact of diet induced obesity on AgRP neuronal activity

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Abstract: Neurons in the arcuate nucleus of the hypothalamus (ARH) that co-express the neuropeptides agouti-related peptide (AgRP) and neuropeptide Y (NPY) are essential for driving food intake. Consistent with their role in energy balance, their activity is tightly correlated with nutritional status: increased activity is associated with hunger while decreased activity is

associated with satiety. Further, a rapid response to food cues suggests that this response does not require ingestion and supports evidence for “top-down” synaptic modulation of AgRP neurons. Our lab has previously demonstrated that AgRP neuronal activity is sensitive to diet thus diet-dependent plasticity may be a causal factor in obesity. However, up to 70% of the variability in BMI is attributable to genetic factors, most of which remains unexplained. Notably, genes within BMI-associated loci in human GWAS are highly enriched for the brain and CNS, suggesting the susceptibility to obesity may be strongly influenced by GxE effects on neuronal function. However, this research has primarily been conducted using inbred mouse models that lack genetic diversity, and do not capture the considerable influence that genetic factors play in controlling obesity and bodyweight. Here, we utilize mouse strains resilient and susceptible to diet-induced obesity to explore the impact of high-fat diet (HFD) feeding on AgRP neuronal function. Genetically resilient mice (CAST/EiJ, PWK/PhJ, and WSB/EiJ) and susceptible (NZO/H1ltJ) mice of both sexes were assessed for feeding and anxiety-like behavior; we additionally assessed AgRP neuronal excitability and synaptic plasticity in identified NPY^{+} neurons using an F1 panel. Predictably, compared to B6 mice, genetically resilient mice consume less food while susceptible mice consume significantly more. This effect is sexually dimorphic as females in both the resilient and susceptible strains are less likely to gain weight on an obesogenic diet. AgRP neuronal activity mirrors diet-induced weight gain in most susceptible strains, with the exception of the particularly resilient WSB/EiJ. Synaptic plasticity onto AgRP neurons suggests that mechanisms that drive AgRP excitability in fasted B6 mice are similar to resilient and susceptible mice, while diet-induced excitability originates from a divergent etiology. Ongoing work will explore hypothalamic gene expression and the prevalence of inflammatory markers (GFAP and IBA1) in these mice. Overall, we hope these studies will identify genetic traits linked to weight gain and obesity, which might provide insight into the diversity of the human response to DIO.

Disclosures: **A.C. Korgan:** None. **S.L.A. Martin:** None. **K.M.S. O'Connell:** None.

Digital Abstract Session

P261. Diet, Obesity and Body Weight

Program #/Poster #: P261.02

Topic: F.09. Food and Water Intake and Energy Balance

Support: Scholarship (2020/03604-8) by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP
Research Grant (2019/01306-2) by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP.

Title: Effects of maternal hyperglycemia and snack intake on male and female offspring high-protein diet preference on infancy

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Abstract: Changes on maternal metabolism and nutrition can increase offspring susceptibility to develop obesity and associated metabolic disorders. This risk is also related to offspring nutrition in postnatal life. Poor food choice has a significant impact on many non-communicable chronic diseases. There is growing evidence suggesting food choice has its origins early in life. In rats, brain circuits involved in feeding behavior control starts developing in the second half of pregnancy and continues until the first 4 weeks of postnatal life, thus making the offspring vulnerable to changes on maternal metabolism during pregnancy and lactation. However, the effects of the association between maternal hyperglycemia and inappropriate nutrition during pregnancy and lactation on offspring food preference have not been explored yet. Therefore, the present study aimed to evaluate the effects of maternal hyperglycemia associated with snack intake on male and female offspring high-protein diet (HPD) preference on infancy. To achieve this goal, pregnant Sprague-Dawley rats were divided into two groups: Control (n = 19) fed with standard chow and Snack (n=21) fed standard chow plus potato chips and 1,5% sucrose solution until lactational day 14. On pregnancy day 7, both experimental groups were then subdivided into normoglycemic dams and dams that were rendered hyperglycemic by streptozotocin administration (STZ, 35mg/kg, i.p.). Thus, four experimental groups were formed: Control (n =11), Control-Snack (n = 9), STZ, (n = 10), STZ-Snack, (n = 11). On postnatal day (PND) 30, one male and one female from each litter were housed on individual cages and fasted for 12 hours. Food preference was then evaluated by offering two different kinds of powdered diet: standard chow (22% protein) and a HPD (38.64% protein), which consisted in standard powdered chow enriched with casein (25%) and corn starch (13%) . Fasting animals were given access to both diets in the early dark phase of the cycle, and food intake was assessed 1, 12 and 36 hours later. Food preference was determined as the percentage of consumed HPD in relation to the total amount consumed of both diets (standard chow plus HPD). The results show that maternal hyperglycemia increased offspring HPD preference in the first hour, while maternal snack intake reduced it. No significant effects were observed at 12 and 36h, indicating that offspring appetitive response to HPD was altered, but not the consummatory response. In conclusion, maternal metabolism and snack intake independently changed offspring HPD preference, without further effects by their association.

Disclosures: G.V. Silva: None. **M.G. Martins:** None. **G.P. Oliveira:** None. **A.G. Cruz:** None. **L.P. Rodrigues:** None. **A.C.I. Kiss:** None. **B.C. Woodside:** None.

Digital Abstract Session

P261. Diet, Obesity and Body Weight

Program #/Poster #: P261.03

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH-NIGMS 5R25GM082406
U54 MD007579
NIGMS/INBRE P20 GM103475
NIH-NIGMS R25GM096955

Title: Peripheral and Ventral Hippocampal Long-chain Ceramides Correlate with Depressive-like Behaviors in Rats

Authors: *L. SAMBOLIN-ESCOBALES[1], L. TIRADO-CASTRO[1], W. FONSECA-FERRER[2], D. PACHECO CRUZ[3], K. SANTOS-AVILES[1], N. HAUGHEY[4], M. COLON-ROMERO[1], A. HERNANDEZ[1], G. CHOMPRE-GONZALEZ[3], J. PORTER[1];
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Abstract: Previous studies suggest that high-fat diet (HFD), stress, and high concentrations of long-chain ceramides are linked to depression. However, it is unclear whether these factors interact to induce depressive-like behaviors. We hypothesized that combining a chronic HFD and short-term unpredictable stress (STU-stress) STU-stress would increase the concentration of long-chain ceramides in serum to increase depressive-like behavior. To test this hypothesis, adult male and female rats were exposed to 10 weeks of a HFD and four days of randomly applied stressors. Three days later, rats were subjected to sucrose grooming test (SGT) and forced swim test (FST) to assess anhedonia and depressive-like behavior. Female rats exposed to LFD or HFD showed an increased ratio of immobility to struggling only when combined with STU-stress, suggesting that stress rather than diet led to depressive-like behavior. Changes were not seen in males. At sacrifice, blood was collected to quantify ceramide concentration in serum by liquid chromatography-electrospray ionization tandem mass spectrometry. Male and female HFD exposed animals showed increased long-chain C18 and C20 ceramides and C20 ceramides were higher in females than males. This effect was amplified in animals exposed to the HFD and STU-stress. Since females exposed to HFD and STU-stress showed higher C20 ceramides levels and more depressive-like behavior, we tested whether direct infusion of C20 ceramides into the VH of rats is sufficient to increase depressive-like behavior. Male and female rats received seven infusions of C20 ceramides directly into the VH, one infusion every 48 hours. Twenty-four hours after each infusion, a sucrose preference test (SPT) was performed to assess anhedonia. Male and female rats that received C20 infusions showed less sucrose preference suggesting the development of anhedonia. In summary, our data suggest that a brief exposure to unpredictable stressor with either LFD or HFD increases peripheral C20 ceramides and depressive-like behavior only in female rats. However, local increases in C20 ceramides in the VH are sufficient to cause anhedonia in both sexes suggesting that diet and STU-stress may not have increased C20 ceramides in the brain of male rats sufficiently to induce depressive-like behaviors.

Disclosures: L. Sambolin-Escobales: None. **L. Tirado-Castro:** None. **W. Fonseca-Ferrer:** None. **D. Pacheco Cruz:** None. **K. Santos-Aviles:** None. **N. Haughey:** None. **M. Colon-Romero:** None. **A. Hernandez:** None. **G. Chompre-Gonzalez:** None. **J. Porter:** None.

Digital Abstract Session

P261. Diet, Obesity and Body Weight

Program #/Poster #: P261.04

Topic: F.07. Autonomic Regulation

Title: Age-related alterations in white matter microstructures in those with gastrointestinal symptoms

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Abstract: Gastrointestinal symptoms (GIS) such as abdominal discomfort, bloating, diarrhea and constipation are commonly found in gastrointestinal disorders such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). The onset of these symptoms usually occurs in early life and remain consistent over time. These enduring symptoms have been associated with changes in brain structure in adults experiencing various gastrointestinal disorders. However, it is unclear whether these GIS-related structural alterations in the brain are limited to adults or could be present earlier in life as well. Therefore, we investigated GIS-related changes in white matter (WM) microstructure organization in pre-adolescent (8-10 years), adolescents (12-16 years) and young adults (17-20 years). We hypothesized that those who have experienced GIS will display altered WM compared to the controls. We also hypothesized that there would be age-specific differences in the WM, as age of onset and duration of GIS has been found to play a role in impacting structural alterations. We tested these hypotheses by using structural brain images provided by the Philadelphia Neurodevelopmental Cohort (PNC) database. We conducted diffusion tensor imaging (DTI) analysis in order to objectively analyze the fractional anisotropy (FA) across the whole brain and compare between participants with GIS (N=90) and their age and sex matched controls (N=97). Our results show that all participants who experienced GIS have an altered WM microstructure organization. Specifically, in comparison to the controls, pre-adolescents with GIS displayed increased fractional anisotropy (FA) in the anterior cingulum, the superior frontal gyrus, the middle frontal gyrus, the right hippocampal cingulum and the right anterior thalamic radiation. The adolescent GIS group showed decreased FA in the left middle temporal gyrus and the cerebellar areas such as the right cerebellum crus1 and the middle cerebellar peduncle, in comparison to their control counterparts. Finally, compared to controls, the young adult GIS group displayed increased FA in the cingulum from the anterior to the posterior division, the superior frontal gyrus, the anterior thalamic radiation, the bilateral superior and inferior longitudinal fasciculus, the internal and capsule. These altered regions are a part of the somatosensory and motor pathways which are involved in pain perception and indicate a dysfunction in the gut-brain axis. In conclusion, the structural differences found in the GIS participants, especially at different ages, support the need for further research into the neurophysiological impact they might have.

Disclosures: **A. Pirwani:** None. **Z. Fang:** None. **A. Smith:** None. **G. Northoff:** None. **N. Ismail:** None.

Digital Abstract Session

P261. Diet, Obesity and Body Weight

Program #/Poster #: P261.05

Topic: F.07. Autonomic Regulation

Support: NIH Grant DK100024
NIH Grant DK120632
Pratt School of Engineering

Title: Voiding and muscle contractility dysfunction in a rat model of detrusor underactivity

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Abstract: Detrusor underactivity (DUA) is an understudied health concern with inadequate clinical management. The limited availability of animal models that exhibit the pathophysiology of DUA impedes the development of new therapeutic approaches. The current studies characterized voiding function and contractility of bladder and urethral tissues in an obese prone (OP) rat model of DUA. Eight-week old female OP and obese resistant (OR) rats were fed a 60% fat diet from 9 weeks old to end of study. At 45-65 weeks old, rats were anesthetized with urethane (1.2 g/kg sc) for acute urodynamic studies. A catheter was inserted into the bladder dome to infuse saline and measure pressure. A bipolar paddle electrode was placed between the pubic symphysis and the EUS to record EMG signals. After testing of voiding function, rats were euthanized and full thickness longitudinal bladder strips and urethral rings (proximal and middle) were harvested and mounted in chambers (DMT, Denmark). Viability was assessed with potassium chloride (120mM). Both bladder strips and urethral rings underwent escalating concentration response curves to carbachol (10^{-8}M to 10^{-5}M). Urethral rings were then exposed to caffeine ($4*10^{-2}$M) for 10 min. Bladder strips also underwent electric field stimulation (EFS) with or without atropine (10^{-7}M) or alpha,beta-methylene ATP (10^{-6}M), whereas EFS in urethral rings was in the presence or absence of dantrolene (10^{-5}M). Voiding efficiency decreased by 31% in OP rats compared to OR rats (95% CI = -41, -22, $p < 0.0001$). EUS activity during filling increased by 4.6 μV (95% CI = 2.4, 6.8, $p = 0.0011$) and EUS bursting time decreased by 1.45 s (95% CI = -2.57, -0.317, $p = 0.0175$) in OP rats. In addition, pressure at volume threshold increased in OP rats by 6.8 cmH_2O (95% CI = 2.2, 11, $p = 0.0067$). Compared to OR rats, OP rats had decreased contraction strength on exposure to carbachol in the bladder ($p = 0.0002$) and proximal urethra ($p = 0.0004$), whereas OP rats had increased contraction strength in the middle urethra ($p = 0.002$). Following EFS, OP rats had increased contractility at 32 Hz in both the proximal ($p = 0.006$) and middle ($p = 0.047$) urethra. Contractility was also increased with caffeine in the middle urethra of OP rats ($p = 0.006$). These results suggest that impaired cholinergic-dependent contractions of the bladder may contribute to poor voiding function in OP

rats. In addition, the middle urethra of OP rats appears to compensate for poor bladder contractility by increasing its activity and responsiveness. This urethral compensation may indicate an attempt to stimulate the pudendo-vesical reflex to promote bladder activation.

Disclosures: E.J. Gonzalez: None. **W.M. Grill:** None. **M.R. Odom:** None. **J.L. Hannan:** None.

Digital Abstract Session

P261. Diet, Obesity and Body Weight

Program #/Poster #: P261.06

Topic: F.07. Autonomic Regulation

Support: NSF IOS 1743673
NSF IOS 1933264

Title: First encounters: establishment of the microbiota-gut-brain axis in mice

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Abstract: At birth, the mammalian fetus exits a sterile womb and enters a world teeming with microbes. These microorganisms, primarily bacteria, colonize the gut, as well as other sites on the body, and influence immune function, behavior, and disease states. We recently reported significant differences between the brains of germ-free and conventionally colonized mice on the day of birth, but not one day earlier. This raises the questions that our current work aims to answer - when do microbes arrive in the gut, which species of bacteria are present during colonization, and what impact does this event have on the brain? We have established timed pregnancies in Swiss Webster mice, and collected the brains and colons of male and female offspring either one day before birth or after birth at 3h, 6h, 12h, 24h, postnatal day (P)3, and P7. Through quadruple-tagged fluorescent in situ hybridization and immunocytochemistry, and subsequent confocal imaging, we examined when bacteria begin to arrive in the colon and in what quantities. We have visualized bacteria in the colon as early as 3h after birth, with a stark increase in bacterial quantity between 24h and P3. Via qPCR and 16S rRNA gene sequencing we are examining which bacterial species are present and in what quantities at each time point. Finally, we used c-Fos immunohistochemistry to examine neural activation in brain regions which receive input from the vagus nerve, a primary route of gut-brain communication; these include the paraventricular nucleus of the hypothalamus, the nucleus of the solitary tract, and the arcuate nucleus of the hypothalamus. This work allows us to pinpoint exactly when the newborn gut is colonized by microbes, identify which bacterial species are present during colonization, and identify associated changes in brain activation in primary areas of interest.

Disclosures: **N.M. Ronczkowski:** None. **A. Castillo-Ruiz:** None. **B. Chassaing:** None. **N.G. Forger:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.01

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant R01DK047320
NIH Grant 1F32DK124963
NIH Grant U54MD007601

Title: Ablation of Selenoproteins in Agrp Neurons Protects Against Diet-induced Obesity in a Sexually Dimorphic Manner

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Abstract: The trace element selenium (Se) is known mainly for its antioxidant properties and is critical for proper brain function. The role of Se in regulating energy metabolism, and the sexually dimorphic nature of Se functions, however, are underappreciated, and warrant increased attention. Recent work in our lab has highlighted the importance of Se utilization in hypothalamic regulation of energy metabolism. Dietary Se is incorporated into selenoproteins in the form of the unique amino acid selenocysteine (Sec). The objective of this study was to assess the role of selenoproteins in Agouti-related peptide (Agrp)-positive neurons, an orexigenic sub-population of the hypothalamus. We generated mice with Agrp-Cre-driven deletion of selenocysteine tRNA (Trsp-Agrp KO mice), which is essential for Sec incorporation into selenoproteins, thus ablating selenoprotein synthesis in Agrp-positive neurons. The metabolic phenotype of Trsp-Agrp KO mice challenged with a high-fat diet was characterized via glucose tolerance test (i.p. injection) and the use of analytical chambers to measure food intake and respiratory metabolism. Prior to sacrifice, mice were challenged with leptin (i.p. injection) to assess neuronal leptin responsivity via immunohistochemistry and western blot. Brown adipose tissue (BAT) morphology and thermogenic protein expression were also analyzed. Female Trsp-Agrp KO mice displayed resistance to diet-induced obesity, which was accompanied by improved glucose tolerance and elevated energy expenditure levels without changes in food intake. Female Trsp-Agrp KO mice also had greater leptin sensitivity and showed signs of elevated BAT thermogenesis. Male Trsp-Agrp KO mice displayed no changes in metabolic phenotype. Loss of selenoproteins in Agrp-positive neurons of the hypothalamus promotes energy expenditure and reduces diet-induced obesity in a sexually dimorphic manner, leading to resistance to a high-fat diet in females.

Disclosures: **D. Torres:** None. **M. Pitts:** None. **L. Seale:** None. **A. Hashimoto:** None. **K. An:** None. **A. Hanato:** None. **K. Hui:** None. **M. Berry:** None. **S. Remigio:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.02

Topic: F.09. Food and Water Intake and Energy Balance

Support: Supported by gifts for research from Cain-Stanley & Co.

Title: Eating response is rapidly elicited by $GABA_A$ or $GABA_B$ receptor stimulation in the lateral septum

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Abstract: Over 40% of adults in the USA are obese and therefore identifying the neural substrates of overeating is a priority. Recent studies have highlighted the lateral septum as important in eating control mechanisms. The current study used central injections of GABA receptor agonists and antagonists to explore potential roles for $GABA_A$ and $GABA_B$ receptors within the lateral septum in the control of food intake. The study's hypotheses predicted that GABA receptors disinhibit pathways leading to feeding, therefore agonists of GABA $_A$ and $_B$ receptors would increase food intake, while antagonists would decrease food intake. We used 4 groups of satiated adult male Sprague-Dawley rats (n ≥11 subjects/group) chronically implanted with a guide cannula terminating in the lateral septum. Doses of drugs dissolved in the artificial CSF vehicle were injected through the guide cannula into the lateral septum in 0.3 µl volumes during the early portion of the light-phase. Ingestion of a palatable milk-based mash diet and water were measured 1, 2, 4 and 24 hours post injection. Each experiment had a within-subjects repeated measures design with all subjects tested in each condition in counterbalance order. Experiment 1 showed that injection of the $GABA_A$ receptor agonist, muscimol (doses: 0.1, 0.2, and 0.3 µg), into the lateral septum elicited intense, dose-dependent feeding responses (e.g. mean food intake 1 hour after the 0.3 µg dose was 6.7 g , SE = 1.8). Experiment 2 showed that pretreatment injections with the $GABA_A$ receptor antagonist, picrotoxin (doses: 0.05, 0.1, 0.2 µg), markedly reduced the muscimol-elicited feeding response, indicating mediation by $GABA_A$ receptors. Experiment 3 tested the $GABA_B$ receptor agonist, baclofen hydrochloride (doses: 0.45, 1.125, 2.25 µg), and found a similar dose-dependently elicited eating response (e.g. mean food intake 1 hour after the 2.25 µg dose was 6.9 g, SE = 1.4). Finally, experiment 4 showed that pretreatment injections with the $GABA_B$ receptor antagonist, 2-hydroxysaclofen (doses: 0.5, 1.25, 2.5 µg), substantially reduced baclofen-elicited eating, consistent with elicited feeding being mediated by $GABA_B$ receptors. In contrast, water intake was not significantly affected by any of the drugs 1 hr after injection, which provides support for GABA receptors being involved in eating but not drinking responses. These results, showing that activation of lateral septal

$GABA_A$ or $GABA_B$ receptors are capable of strongly stimulating eating behavior, suggest potential roles in feeding control neurocircuitry.

Disclosures: I. Gabriella: None. **B.G. Stanley:** None. **A. Tseng:** None. **K. Sanchez:** None. **H. Shah:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.03

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant GM127251
NIH Grant RL5GM118969, TL4GM118971, and UL1GM118970
NIH Grant R25GM069621-11

Title: Chemoarchitectural analysis of cocaine- and amphetamine-regulated transcript, alpha-melanocyte-stimulating hormone, and dopamine beta-hydroxylase: Systematized mapping within a standardized stereotaxic reference atlas.

Authors: *B. E. PINALES[1], K. J. GALVAN[2], E. J. PEREZ[2], P. A. PARADA[2], M. A. PEVETO[2], K. A. S. BURNETT[2], K. T. LORENZANA[2], A. M. KHAN[2];
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Abstract: Cocaine- and amphetamine-regulated transcript (CART) and alpha-melanocyte-stimulating hormone (αMSH) are known for their appetite-inhibiting effects. Norepinephrine is a neuromodulator involved in autonomic function, synthesized by the enzyme dopamine beta-hydroxylase (DβH). These neural messengers are distributed within several regions of the mammalian brain, yet their anatomical arrangements have not been systematically reported within a standardized reference atlas at high-spatial resolution. *Our objective is to provide high-spatial resolution maps of CART, αMSH, and* DβH within a standardized atlas (*L.W. Swanson, Brain Maps 4.0, 2018), with a primary focus on their spatial arrangements and putative colocalization.* A Nissl stain (thionin) was used for cytoarchitectural analysis in coronal-plane sections of the adult male rat brain. This facilitated the atlas level assignment of adjacent sections, fluorescently tagged with CART, αMSH, and DβH antibodies, the patterns were then mapped onto digital atlas templates. We report co-spatial relationships between CART, αMSH, and DβH within atlas levels (23-30). The immunoreactive labels were observed in the paraventricular thalamic nucleus (PVT), central and medial amygdalar nucleus (MEA; CEA), lateral hypothalamic area (LHA), arcuate (ARH), paraventricular (PVH), periventricular- (pv), and dorsomedial hypothalamic (DMH) nuclei. Both anorexigenic markers are co-localized to a high degree, with a few single-label fibers evident. CART-immunoreactive cells were observed in the ARH, PVH, zona incerta (ZI), LHA, and supraoptic nucleus (SO); however, cells expressing CART and αMSH displayed moderate co-expression within the ARH. DβH-immunoreactive fibers were present within the same subregions as the anorexigens, but with

minimal co-localization (*e.g.*, PVH, pv, ARH, LHA, and PVT). The PVT showed a dense representation for each label; however, sparse expression was seen in dorsal or ventral regions where CART and DβH were separately expressed. Putative co-localization of the three markers was in the medial and lateral aspects of the nucleus. Robust staining of CART, αMSH, and DβH was also observed in hypothalamic regions (*e.g.*, ARH, PVH, and some subregions of the LHA). Fibers for each were moderately expressed within the CEA/MEA, with a greater distribution of DβH. The aforementioned mapping of co-spatial patterns among CART-, αMSH-, and DβH-ir fibers, may aid in precision targeting using tract tracers or virally-directed methods. Moreover, the spatial arrangement of these neuromodulators within a stereotaxic reference space can aid in their further study using functional techniques.

Disclosures: **B.E. Pinales:** None. **K.J. Galvan:** None. **E.J. Perez:** None. **P.A. Parada:** None. **M.A. Peveto:** None. **K.A.S. Burnett:** None. **K.T. Lorenzana:** None. **A.M. Khan:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.04

Topic: F.09. Food and Water Intake and Energy Balance

Support: NSERC Discovery Grant RGPIN-2017-06272

Title: Viral Tracing of Axonal Projections from Tyrosine Hydroxylase-expressing Neurons of the Zona Incerta

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Abstract: Hypothalamic zona incerta (ZI) tyrosine hydroxylase (TH) neurons produce dopamine and can also signal via GABA transmission. GABA ZI neurons drive motivated behaviors like feeding, hunting, and sleep via projections to the paraventricular thalamus (PVT), periaqueductal gray (PAG), and lateral hypothalamic area (LHA), respectively. However, it is not known if these behaviors require dopamine transmission. To determine the brain regions affected by dopaminergic transmission we identified the projection sites of ZI TH neurons. We used a *Th-cre* mouse line expressing Cre recombinase in TH neurons and validated the specificity of *Th-cre* expression in *Th-cre;L10-Egfp* neurons. Dual fluorescence *in situ* hybridization on *Th-cre;L10-Egfp* brain tissue showed that Cre-mediated expression of *Gfp* mRNA colocalized in all ZI *Th* mRNA cells. Interestingly, while all *Th-cre* neurons in the rostromedial ZI were TH-immunoreactive (TH-ir), less than 20% of *Th-cre* neurons in the lateral ZI were TH-ir. To visualize ZI TH projections, we injected two male *Th-cre* mice with a Cre-dependent adeno-associated virus encoding mCherry. In light of differential TH expression in the ZI, *Th-cre* mice

were given either a focused injection (25 nl) targeting the rostromedial TH-ir neurons or a larger volume (75 nl) to encompass the lateral ZI also. About 60% of TH-ir neurons from our focused injection were dsRed-ir, while in the larger injection only 24% were dsRed-ir. High resolution spatial maps revealed that both injection cases produced a similar pattern of dsRed-ir fibers, though the larger injection produced a greater density. In the cerebral hemispheres dsRed-ir fibers were found in the lateral septum and bed nucleus of the stria terminalis. Projections to the thalamus were directed towards the rostral PVT and the nucleus of reuniens. In the hypothalamus, dsRed-ir fibers were prominent in the LHA and anterior, dorsomedial, and posterior hypothalamic nuclei but absent in the suprachiasmatic, ventromedial hypothalamic, and premammillary nuclei. Caudally, the PAG contained dense dsRed-ir fibers which spread to the superior colliculus and parabrachial nucleus. In aggregate, we observed widespread projections from ZI *Th-cre* neurons that were independent of TH expression. The pattern of projections from ZI TH neurons suggest putative dopamine targets, which also overlap with known GABAergic ZI projections.

Disclosures: **B. Bono:** None. **K. Negishi:** None. **K.S. Schumacker:** None. **D.P. Spencer:** None. **M. Ponce:** None. **E. Mejia:** None. **A.J. Hebert:** None. **M.J. Chee:** None. **A.M. Khan:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.05

Topic: F.09. Food and Water Intake and Energy Balance

Support: FAPESP 2019/01306-2
FAPESP 2019/06974-3

Title: Chronic central leptin infusion effects on food intake of male and female offspring of mild hyperglycemic rats

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Abstract: Maternal hyperglycemia during pregnancy and lactation can increase the susceptibility of the offspring to develop several metabolic disorders later in life, such as obesity and diabetes. There is growing evidence suggesting that the early programming of neuroendocrine systems could play a role in this scenario. Maternal severe diabetes is associated with central leptin resistance and lifelong disorganization of the hypothalamic pathways regulating energy expenditure and food intake. However, the effects of maternal mild hyperglycemia, which better

resembles human gestational diabetes, have not yet been investigated. In addition, most studies only explore the effects on male offspring, while females are often overlooked despite the growing evidence of sex differences on control of food intake. Therefore, the aim of the present study was to evaluate the chronic central leptin infusion effects on food intake of male and female offspring of mild hyperglycemic rats. In order to achieve this goal, pregnant Sprague-Dawley rats were divided into two experimental groups: Control (n=6) and STZ (n=6, 35 mg/kg i.p. on pregnancy day 7). Rats gave birth naturally and the litters were culled to 4 males and 4 females. On postnatal day 75 (PND 75), 2 male and 2 female offspring from each litter were used and each received a different treatment (saline or leptin). Thus, the experimental groups were formed according to the presence or absence of maternal hyperglycemia and treatment with saline or leptin, as follows: Control saline (n=6), Control leptin (n=6), STZ saline (n=6), STZ leptin (n=6). On PND 82, animals underwent surgery for cannula implantation the lateral cerebral ventricle (Alzet model 2001) which released saline 0,9% or leptin (1 microgram of leptin per day at a rate of 1 microliter/hour for 7 consecutive days). Body weight and food intake were daily monitored for 7 days before and 7 days after surgery. On PND 89, rats were killed and cannula placement was evaluated. All experimental procedures were approved by the local ethics committee (Protocol number 1134). The chronic central leptin infusion was able to decrease the food intake in offspring from all experimental groups. However, the leptin treatment affected Control and STZ offspring differently and there were also sex-related differences. In conclusion, maternal mild hyperglycemia impaired offspring sensibility to chronic central leptin infusion and males and females responded differently. More studies will be carried out to unravel the pathways involved on those changes.

Disclosures: **G.P. Oliveira:** None. **A.G. Cruz:** None. **G.V. Silva:** None. **M.G. Martins:** None. **B.C. Woodside:** None. **A.C. Kiss:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.06

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant SC1GM127251

Title: Cytoarchitecture-guided laser-capture microdissection of the arcuate hypothalamic nucleus: Developing a workflow for the standardized mapping of the sampled site in a reference atlas of the rat brain

Authors: *S. BALIVADA, K. NEGISHI, A. M. KHAN;
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Abstract: Laser-capture microdissection (LCM) of brain regions, followed by their high-throughput analysis, are well-established methods to identify the molecular constituents of a region of interest (ROI). Accurate ROI identification and dissection are the keys to connect

molecular information to ROI functionality. However, current methods lack an estimation procedure for accurately mapping the spatial location of the ROI (Khan AM, 2018. *Adv. Neurobiol*. *21*, 101). Here, we approached this problem by developing a workflow that includes cytoarchitecture-guided LCM followed by standardized mapping of the ROI using a widely used open-access rat brain reference atlas (*Brain Maps 4.0 (BM4.0)*; Swanson LW, 2018. *J. Comp. Neurol*. *526*, 935). A fresh-frozen brain collected from an adult male Sprague Dawley rat was cryosectioned at 20 µm-thickness and serially mounted onto both glass and polyethylene-napthalate-membrane (PEN-M) slides. The sections on glass, stained with thionine, were used as "guide sections" on a Leica LMD7000 for LCM of serially adjacent PEN-M sections. First, guide sections were spatially calibrated to PEN-M sections using user-defined fiducials. The arcuate hypothalamic nucleus (ARH) target ROI was identified based on its cytoarchitectural features on the guide sections, and contours delineating the ARH were drawn. These contour drawings were transferred onto the PEN-M sections, and the ROI was microdissected from those sections. LCM samples were processed for RNA using a PicoPure™ RNA isolation kit (Thermo Fisher Scientific), and RNA quality was estimated using TapeStation (Agilent). Histological analysis of the PEN-M sections used for LCM revealed that the sampled ROI was bounded by the ventromedial hypothalamic nucleus, median eminence, and third ventricle; suggesting that the dissected sample contained the ARH. Nissl-based parcellation of the guide sections, together with plane-of-section analysis, helped to identify that the sampled ROI is associated with *BM4.0* atlas levels 27-29, and the contours were mapped onto digital *BM4.0* atlas templates accordingly. Analyzed RNA samples displayed an RNA integrity number greater than 7. These initial results suggest that our workflow can be used to 1. perform reliable LCM of an ROI based on the guide section's cytoarchitecture; and, 2. map the location of the ROI within the *BM4.0* rat brain atlas. Our plans are to develop this method further by quantifying microdissection accuracy using mapped contours and validating these findings for hypothalamic ROIs that require greater precision to microdissect, such as sub-regions of the lateral hypothalamic area.

Disclosures: S. Balivada: None. **K. Negishi:** None. **A.M. Khan:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.07

Topic: F.09. Food and Water Intake and Energy Balance

Support: Startup funds from the President and Provost of the California Institute of Technology and the Biology and Biological Engineering Division of California Institute of Technology
Searle Scholars Program
Mallinckrodt Foundation
McKnight Foundation
Klingenstein-Simons Foundation
New York Stem Cell Foundation

NIH R56MH113030 and R01NS109997

Title: Decoding of thirst states in the mammalian brain

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Abstract: The motivation to drink is triggered by two distinct thirst states. Increased internal osmolality induces osmotic thirst that drives animals to drink pure water. Conversely, loss of body fluid volume induces hypovolaemic thirst, in which animals drink both water and minerals to recover blood volume and electrolyte composition. Sensory nuclei in the Lamina terminalis (LT) including the subfornical organ (SFO) and organum vasculosum of lamina terminalis (OVLT) are critical for sensing both types of thirst-inducing stimuli. However, how different thirst modalities are encoded in the brain and how the thirst need state is decoded in higher order brain centers remains unknown. Here we employed single cell RNA-seq based stimulus-to-cell-type mapping to identify cellular substrates that detect the two kinds of physiological thirst states as well as thirst need signal decoding cellular populations in downstream hypothalamic nuclei. These studies revealed diverse types of excitatory and inhibitory neuron in each sensory LT nuclei SFO and OVLT. We show that unique combinations of these neuron types are activated under osmotic and hypovolaemic stresses. These results elucidate the cellular logic that underlies the detection of distinct thirst modalities. Furthermore, optogenetic gain of function in thirst-modality-specific cell types in the SFO and OVLT recapitulated water-specific and non-specific fluid appetite caused by the two distinct dipsogenic stimuli. Furthermore, we identified neural populations downstream of SFO and OVLT that orchestrate thirst motivation and endocrine responses in response to the thirst need state. Together, these results outline a robust and rapid strategy to map out cellular substrates for motivated behaviors and reveal that thirst is a multimodal physiological state that is mediated by specific neuron types in the mammalian brain.

Disclosures: **A. Pool:** None. **T. Wang:** None. **J. Ngai:** None. **Y. Oka:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.08

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH Grant R00DK119574
NIH Grant R01DK114014
NIH Grant T32NS105607
Penn Institute for Diabetes, Obesity and Metabolism
NSF Grant DGE-1845298
Klingenstein Fund
Simons Foundation

Title: Gut-brain pathways that regulate hypothalamic hunger neurons to control feeding

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Abstract: Food intake is influenced by coordinated activity in neural circuits that integrate physiological needs. Proper feedback from the gastrointestinal (GI) tract communicating energy status is critical in maintaining homeostasis. While extensive work has identified gut-to-brain pathways that activate satiation centers in the brain, it remains unknown if the same pathways are used to disseminate nutrient status information to brain regions that promote food-seeking and consumption. To explore the gut-brain pathways that inhibit hunger circuits, we assessed neural activity in Agouti-related protein (AgRP)-expressing neurons of the arcuate nucleus of the hypothalamus. AgRP neurons are active during hunger, inhibited by food intake, and are necessary and sufficient for feeding. Importantly, AgRP neuron activity decreases rapidly during direct nutrient infusion in the GI tract, suggesting that the nutritive value of food detected in the gut regulates AgRP neuron activity. We find that the inhibition of AgRP neurons during direct infusion of food is calorie-dependent and individual macronutrients (i.e. fat, carbohydrate, protein) are each sufficient to inhibit AgRP neurons. Do different macronutrients signal to the brain via a common gut-brain pathway? We hypothesized that the vagus nerve, a major highway between the gut and the brain, is crucial for relaying information from the GI tract to AgRP neurons. To test this hypothesis, we engineered mice to express the genetically encoded calcium indicator GCaMP6s in AgRP neurons and monitored *in vivo* neural activity in mice that received a complete sub-diaphragmatic vagotomy. Vagotomy blocked the ability of intragastric fat to inhibit AgRP neuron activity. Additionally, we found that cholecystokinin (CCK), a satiation peptide which is sufficient to inhibit AgRP neurons, can no longer alter AgRP neuron activity following vagotomy. These results support a model whereby fat, through the release of CCK, stimulates vagal afferents to ultimately inhibit AgRP neurons. However, we found that a complete diet containing all macronutrients maintains the ability to inhibit AgRP neurons following vagotomy, suggesting that not all macronutrients signal to the brain exclusively via the vagus nerve. Ongoing experiments further explore the mechanisms through which distinct macronutrients relay energy status to the brain. Taken together, this work highlights multiple gut-brain pathways that facilitate the inhibition of hunger circuits during calorie consumption to regulate feeding behavior.

Disclosures: N. Goldstein: None. **J.R.E. Carty:** None. **A.D. McKnight:** None. **A.L. Alhadeff:** None. **J.N. Betley:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.09

Topic: F.07. Autonomic Regulation

Support: NIH R01 HL113270
NIH R01 HL128388

NIH R01 HL145875

Title: Altered Neuronal Activity of the Organum Vasculosum of the Lamina Terminalis Contributes to Genetic Salt-Sensitive Hypertension

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Abstract: The organum vasculosum of the lamina terminalis (OVLT) is located along the rostral wall of the third ventricle, lacks a complete blood brain barrier, and contains NaCl-sensitive neurons that regulate thirst, neuroendocrine function and arterial blood pressure (ABP). The present study assessed whether activation of OVLT neurons directly alters sympathetic nerve activity (SNA) and contributes to salt-sensitive hypertension. Male Sprague-Dawley rats (250-400 g) received an OVLT injection of rAAV9-CaMKIIa-hChR2(H134R)-mCherry (50nL, $1x10^{13}$ vg/mL, Addgene) and an optical ferrule (200um OD, Thor Labs). At 2 wks later, optogenetic excitation of OVLT neurons (5 ms pulse, 50% duty cycle, 9-12 mW) produced frequency-dependent increases in ABP (1Hz: 0±1; 5Hz: 3±1; 10Hz: 6±1; 20Hz: 9±2 mmHg; n=6, P<0.05). These pressor responses were unaffected by the vasopressin antagonist Manning Compound (10ug/kg, IV; 20Hz: 8±1 mmHg) but completely abolished by the ganglionic blocker chlorisondamine (5mg/kg, IV; 20Hz: 1±1 mmHg; P<0.05). Second, optogenetic activation of OVLT neurons (n=5) significantly increased the discharge of bulbospinal vasomotor neurons in the rostral ventrolateral medulla (Δ; 5Hz: 4±1, 10Hz: 7±2, 20Hz: 11±2Hz; P<0.05), lumbar SNA (5Hz: 113±4%; 10Hz: 120±7%; 20Hz: 129±10%; P<0.05) and renal SNA (5Hz: 108±2%; 10Hz: 114±3%; 20Hz: 134±5%; P<0.05). Third, chronic chemogenetic activation of OVLT neurons by rAAV9-hSYN-HA-hM3D(Gq)-IRES-mCherry (50nL, $1x10^{13}$ vg/mL, Addgene, n=5) and clozapine-N-oxide (3 mg/kg/day, 7 days) significantly increased fluid intake (Day 0: 52±8mL vs Day 7: 177±29mL, P<0.05) and ABP (Day 0: 89±3 vs Day 7: 103±3 mmHg, P<0.05). Ganglionic blockade decreased ABP more at Day 7 versus Day 0 (-53±4 vs -38±5 mmHg, P<0.05). Finally, Dahl-Salt-Sensitive and Dahl-salt-resistant rats were fed 0.1% or 4% NaCl diet for 3-5 wks. In vivo single-unit recordings of OVLT NaCl-sensitive neurons indicated a significantly higher discharge in Dahl-salt-sensitive rats fed 4% NaCl (4.2±1.3 Hz, n=8) versus Dahl-salt-sensitive fed 0.1% (1.9±0.4Hz, n=7) or Dahl-resistant rats fed 0.1% (1.7±0.6Hz, n=7) or 4% (1.8±1.7Hz, n=6). Inhibition of OVLT neurons by muscimol injection (5mM, 20nL) significantly decreased lumbar SNA and ABP in Dahl-salt-sensitive fed 4% NaCl (SNA: -22±4%, ABP: -12±2mmHg, n=6; Ps<0.05) but not Dahl-salt-sensitive fed 0.1% (SNA: -2±1%, ABP: -3±1mmHg, n=5) or Dahl-resistant rats fed 0.1% (SNA: -1±1%, ABP: -1±1mmHg, n=5) or 4% (SNA: -3±1%, ABP: -4±3mmHg, n=5). Altogether, these findings suggest activation of OVLT neurons increase SNA and ABP, and the elevated activity of OVLT neurons contribute to salt-sensitive hypertension.

Disclosures: S.D. Stocker: None. **K.N. Browning:** None. **W.B. Farquhar:** None. **M.M. Wenner:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.10

Topic: F.09. Food and Water Intake and Energy Balance

Support: R01DK102918
RF1AG059778

Title: Mapping of brain-wide circuits that control food intake by comparing neural activity in different need-based states and feeding behaviors

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Abstract: Neurons expressing agouti-related peptide (AgRP) in the arcuate nucleus of the hypothalamus (ARH) are critical for regulating hunger and food-seeking behavior. AgRP neurons are highly active during periods of caloric depletion and are inhibited in satiated state. While the activity of AgRP neurons is well-known to be modulated by peripheral hormones such as leptin or ghrelin, recent advances in real-time imaging of neuronal activity in freely moving animals has revealed that AgRP neurons are rapidly inhibited by sensory detection of food (Betley et al., 2015; Chen et al., 2016; Garfield et al., 2016) and that this can be potentiated by the palatability of the presented food (Garfield et al., 2016), indicating that AgRP neurons are also potently modulated by presynaptic inputs from other regions of the brain. However, the origin and identity of these presynaptic neurons remains poorly characterized. In this study, we used an unbiased approach to fluorescently label neurons brain-wide that are activated in response to periods of high metabolic need using a transgenic mouse line (Ai14;Arc^{CreERT2}) to identify neurons that are active following a fast or during presentation of food. In these mice, the immediate early gene *Arc* (which is well-defined as a molecular marker of activated neurons) drives expression of Cre recombinase following administration of 4-hydroxy-tamoxifen (4-OHT), thereby permitting the permanent labeling of activated neurons using Cre-dependent expression of the reporter tdTomato. This approach allows us to identify novel brain regions involved in the CNS response to caloric depletion and food presentation.

Disclosures: T. Ouellette: None. **K. O'Connell:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.11

Topic: F.09. Food and Water Intake and Energy Balance

Support: ADA Pathway 1-15-INI-12
LLHF Fellowship grant 2019-D-014-FEL

Title: Chemogenetic activation of olfaction results in sex-specific modulation of energy homeostasis

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Abstract: Olfactory inputs are important for hedonic evaluation of food, resulting in food choice and possible consumption. The hypothalamus is a well established center integrating internal hormonal signals originating from peripheral organs to adjust energy intake and expenditure. Whether external stimuli, such as olfactory information are processed within the hypothalamus to contribute to energy balance regulation remains unknown. Here, we employ chemogenetic manipulation of olfactory mitral cells to specifically interrogate alterations in hypothalamic activity and whole-body energy metabolism. Using mice genetically encoding the human M3 muscarinic DREADD receptor, we show that acute stimulation of Tbx-21-positive neurons caused by clozapine-n-oxide (CNO) led to a rapid rise in oxygen consumption, heat production, and an increase in brown adipose tissue temperature in females, with no observed changes in activity or food intake. Analysis of thermogenesis related gene expression in brown adipose tissue (BAT) suggests that these changes are due to an increase in creatine metabolism in BAT in females with no changes observed in males. Acute CNO delivery produced c-fos early gene activation in various regions in the brain, including main olfactory bulb, amygdala and several hypothalamic nuclei. In particular, cFos activation was visible in the female dorsomedial hypothalamus, a hypothalamic nucleus playing a role in feeding, thermoregulation and circadian regulation. Taken together, these results indicate that the mammalian brain has developed dedicated neurocircuits to integrate olfactory inputs in the control of energy balance, which present different characteristics in male and female rodents.

Disclosures: P. Jovanovic: None. **Y. Wang:** None. **E. Novinbakht:** None. **N. Morones:** None. **C.E. Riera:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.12

Topic: F.09. Food and Water Intake and Energy Balance

Title: The effect of intra-gastric nutrients on cerebral BOLD signal and striatal dopamine release in lean and obese humans before and after diet-induced weight loss

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Radiology and Biomed. Imaging, [7]Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT; [8]Dept. of Medicine,, Fleischer Inst. for Diabetes and Metabolism, Albert Einstein Col. of Med., Bronx, NY

Abstract: Objective: Nutritional signals that arise following the ingestion of nutrients play an important role in the central control of eating behavior. Animal studies have shown that in diet-induced obesity, these regulatory mechanisms are disturbed. To assess this in humans, we studied the effect of intra-gastric macronutrient infusions on neuronal activity using functional MRI.
Methods: In 28 lean and 30 obese humans, we compared the effects of intra-gastric infusions of glucose 50% (250ml, 500kcal), Intralipid® 20% (250ml, 500kcal) and water (250ml) on i) the BOLD signal of the hypothalamus, nucleus accumbens, caudate nucleus, putamen, amygdala and insula with a region-of-interest (ROI) and a explorative whole-brain voxel-wise analysis using functional MRI, and ii) striatal dopamine release using SPECT imaging. Neuroimaging was repeated in the obese group after a 3-month hypocaloric diet with a weight loss of 9.5 ± 2.8%.
Results: In the nucleus accumbens, the BOLD signal decreased after both the intra-gastric infusion of glucose and lipid in lean, but not in obese subjects. In the putamen, signal decreased after the intra-gastric glucose infusion in both lean and obese subjects, and after the intra-gastric lipid infusion in lean subjects only. In addition, a voxel-wise analysis identified several regions with a decrease in BOLD signal in lean subjects, with the most pronounced effects in the striatum and frontal pole after the intra-gastric glucose infusion and in the insula and frontal regions after the lipid infusion. This pattern of nutrient-induced decreases in BOLD signal was absent in the obese subjects. Intra-gastric glucose infusion induced striatal dopamine release in lean and obese subjects while intra-gastric lipid infusion induced dopamine release in the lean subjects only. The lack of response in neuronal activity in the obese subjects was not reversible after weight loss.
Conclusion: Using fMRI, we identified a lean response phenotype in brain neuronal activity and striatal dopamine release after intra-gastric infusion of macronutrients. In obese individuals, this neuronal response is attenuated and this is not reversed by a diet-induced weight loss of 10%. Lipid sensing in the dorsal striatum seems to be particularly reduced in obese humans. These findings imply a defect in the feedback of energy consumption to the brain in obese humans, in a taste and preference independent manner. This may contribute to the pathological eating behavior of obese humans and the frequent weight-regain after weight loss interventions.

Disclosures: K.A. Van Galen: None. **A.G. Schrantee:** None. **K.W. ter Horst:** None. **S.E. la Fleur:** None. **J. Booij:** None. **R.T. Constable:** None. **G.J. Schwartz:** None. **R.J. DiLeone:** None. **M.J. Serlie:** None.

Digital Abstract Session

P262. Ingestive Behavior: Circuits

Program #/Poster #: P262.13

Topic: F.09. Food and Water Intake and Energy Balance

Support: CBBRe Trainee Grant
USD Graduate Creative Scholarship Grant
Wesley W. Parke Research Award Endowment

Title: Water and Brain Function: Effects of Hydration Status on Transcranial Magnetic Stimulation

Authors: *S. S. CRITZER[1], T. J. BOSCH[1], K. A. FERCHO[2], L. A. BAUGH[1];
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Abstract: INTRODUCTION: Transcranial magnetic stimulation (TMS) is used to study, diagnose and treat neuropsychiatric conditions but can fail or produce variable results. TMS depends on volume conduction so should depend on brain volumes. Hydration can affect brain volumes but the effects on TMS are unknown. We aimed to characterize the effects of hydration on basic motor TMS in awake behaving humans.
METHODS: 30 participants (8M, 22F) were tested in dehydrated and rehydrated states. TMS was targeted to the left M1-HAND area for first dorsal interosseus muscle while TMS-evoked potentials (TEP) and motor evoked potentials (MEP) were recorded. Threshold, hotspot, recruitment curve, paired pulse, and cortical silent period measures were recorded. Control data were collected for age, gender, handedness, urine specific gravity, blood glucose, maximum voluntary contraction, sleep, stimulant-depressant use, and fluid intake patterns.
RESULTS: Rehydration decreased the motor threshold and shifted the motor hotspot. Significant effects on TMS measures occurred despite being re-localized and re-dosed to these new parameters. Rehydration increased short excitatory facilitation of the MEP and magnitude of specific TEP components in inhibitory paired pulse protocols, consistent with favored excitability.
DISCUSSION: Results reflect macroscopic and microscopic volume changes, including shifts in 3D positioning, decreased scalp-cortex distance bringing cortex closer to the stimulation and recording sites, astrocyte volume changes including swelling-induced glutamate release, increased excitability, and ease of activating circuits in the rehydrated brain. Paired pulse TMS showed evidence of altered neurotransmission events (glutamate/GABA-dependent) in the stimulated circuits. Differential effects of hydration were observed on the short timeframe of activity in motor circuits that produces the MEP versus the longer timeframe of activity across all stimulated circuits that produces EEG signals. Increased short excitatory facilitation reflects increased excitability and generation of larger action potential volleys reaching the muscle, whereas altered inhibitory TEP peaks reflect effects on slower electrical activity that reaches EEG electrodes.
CONCLUSIONS: Variables like osmolarity, astrocyte volume, and brain volumes can affect neurostimulation and recording. Controlling for hydration may reduce variability in research studies and improve success rates in neurostimulation treatments. Rehydration offers a mechanism to 1) macroscopically bring target cortical areas closer to the TMS coil and 2) microscopically favor excitability.

Disclosures: S.S. Critzer: None. **T.J. Bosch:** None. **K.A. Fercho:** None. **L.A. Baugh:** None.

Digital Abstract Session

P263. Ingestive Behavior: Peptide Signals

Program #/Poster #: P263.01

Topic: F.09. Food and Water Intake and Energy Balance

Support: Cancer Research Society
Medical Research Fund

Title: Expression of orexin and melanin-concentrating hormone genes in tumor-bearing mice

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Abstract: Many cancer patients present with cancer anorexia/cachexia syndrome (CACS), characterized as a severe loss of appetite and body weight. This is a leading cause of patient mortality with limited interventions available. Thus, it is important to illuminate the underlying mechanisms of CACS. It is thought that peripheral tumours can influence the energy balance circuitry in the brain, however, this process remains poorly understood. Melanin concentrating hormone (MCH) and orexin neurons are orexigenic cells that comprise the hypothalamic energy balance circuitry. Thus, it was hypothesized that the presence of a tumor alters MCH and orexin expression which contributes to cancer anorexia/cachexia. To test this, 8-week old, male C57BL/6 mice were injected subcutaneously in the hind flank with vehicle or Lewis-Lung carcinoma (LLC) cells (high dose: $1x10^6$cells or low dose: $0.5x10^6$cells). Once some mice reached an established end point, the LLC groups were sacrificed along with time-matched controls. RT-qPCR was performed to analyze the expression of MCH and orexin mRNA in the hypothalamus.

High dose LLC injections resulted in tumour growth that reached established endpoints after 14 days. In these mice, white adipose tissue (WAT) mass was reduced, while food intake (FI) and lean muscle mass remained similar to vehicle controls. Additionally, body weight (BW) gain was significantly reduced compared to controls, however there was large variability within the group. Furthermore, MCH/orexin gene expression was positively correlated with BW change. Thus, tumor-bearing mice that gained little to no weight after injection expressed near-control levels of MCH/orexin mRNA. In contrast, LLC mice with BW gain comparable to controls had elevated MCH/orexin mRNA expression. Secondly, low dose LLC injections resulted in tumours that took up to 30 days to reach the endpoint. These mice had a reduction in BW gain, WAT and FI when compared to controls. MCH gene expression was not different from that of controls and was not correlated with BW change, FI or final tumor mass. On the other hand, orexin mRNA was significantly reduced and showed a positive correlation with FI, but it was not correlated with BW change or final tumour mass.

In conclusion, our study suggests that the expression of appetite-promoting MCH and orexin in tumor-bearing mice is correlated with anorexia and weight loss. Mice with upregulated MCH and orexin genes were able to maintain BW and FI comparable to controls, whereas those that

showed signs of anorexia and weight loss did not upregulate these genes. Thus, lack of counterregulatory response by these neuropeptides to the tumor load may contribute to CACS.

Disclosures: C. Morden: None. **M. Hirasawa:** None. **M. Licursi:** None. **H. Briggs:** None.

Digital Abstract Session

P263. Ingestive Behavior: Peptide Signals

Program #/Poster #: P263.02

Topic: F.09. Food and Water Intake and Energy Balance

Title: Cart peptide mediates anorexia in zebrafish by modulating the activity of a specific telencephalic region

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[1]Indian Inst. of Sci. Educ. and Res., Pune, India; [2]Indian Inst. of Sci. Educ. and Research, Pune, Pune, India

Abstract: Of the various neuromodulatory agents found in the central nervous system, the class of peptide modulators is of particular interest in the context of innate motivated behaviours, where they have been shown to modify synaptic gain and alter intrinsic properties of the neurons to initiate or extend stable behavioural states (e.g. sleep/arousal, fear and anxiety etc.). An important behaviour where peptides have been shown to exert modulatory control is in the regulation of feeding behaviour. Although much is known about the neuropeptides involved in regulating feeding and the anatomical networks they affect, the details of the exact molecular mechanisms employed still remain poorly understood. In this study, we used pharmacological intervention coupled with behavioural monitoring and neuronal activity imaging in adult zebrafish (Danio rerio) to better understand the molecular mechanisms involved in the initiation and maintenance of Cocaine-and Amphetamine-Regulated-Transcript (CART) -mediated anorexia. We generated activity maps of the adult zebrafish brain in varying energy states and also under the influence of CART peptide. These studies found a strong correlation between high levels of the neural activity marker, phosphorylated extracellular signal-regulated kinase (pERK) in a region of the dorsal telencephalon and an associated anorexic behavioural output. We found that active N-methyl-D-aspartate (NMDA) receptor signalling is necessary for CART peptides' action, as the increase in pERK levels and reduction in feeding drive were both disrupted by blocking NMDA receptor signalling. We then tested if protein kinase A (PKA) is involved in mediating this effect. Indeed, we found that PKA activity was required for CART-induced upregulation of pERK in the dorsal telencephalic region and suppression of feeding drive. Additionally, we recorded the activity of telencephalic neurons, in ex vivo whole brain preparations, to varying strengths of excitatory stimuli. Interestingly we found that the response in this region was sensitized under conditions of CART treatment or satiety. Indicating that CART action via PKA sensitizes NMDA receptors, possibly via post-translational modification

of receptor subunits, leading to ERK activation that maintains a neural representation of sated state.

Disclosures: **A. Maduskar:** None. **D. Bodas:** None. **A. Ghose:** None. **D. Wakhloo:** None. **T. Kaniganti:** None. **N. Subhedar:** None.

Digital Abstract Session

P263. Ingestive Behavior: Peptide Signals

Program #/Poster #: P263.03

Topic: F.09. Food and Water Intake and Energy Balance

Title: Coexpression and distribution of MCH, CART and NK3R in the mouse hypothalamus

Authors: ***P. A. MILLER**, M. J. CHEE;
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Abstract: Melanin concentrating hormone (MCH) neurons are important for energy balance and sleep regulation. These neurons are exclusively expressed in the hypothalamus, but their distribution within this region is widespread. Furthermore, MCH neurons also express additional chemical messengers and can be marked by their coexpression of cocaine- and amphetamine-regulated transcript (CART) or the neurokinin 3 receptor (NK3R). Recent transcriptomic studies indicated that a third of MCH neurons coexpress both CART and NK3R, but the relative distribution of these or other MCH neurons have not been mapped to the mouse brain. In order to capture the neurochemical and neuroanatomical heterogeneity of MCH neurons, we quantified the proportion of MCH neurons that coexpress CART and/or NK3R and mapped the distribution of these cells to mouse brain atlas templates from the *Allen Reference Atlas*. We identified MCH neurons by native EGFP fluorescence (EGFP-f) produced by *Mch-cre* neurons in *Mch-cre;L10-Egfp* mice, as over 96% (979/1016) of all MCH-immunoreactive (MCH-ir) neurons coexpressed EGFP-f, though some EGFP-f neurons (64/1043) were not MCH-ir. High resolution spatial maps revealed that EGFP-f neurons may be spread over the medial hypothalamus in the anterior and dorsomedial hypothalamic nuclei and the medial zona incerta. MCH neurons distributed lateral to the fornix were found in the lateral hypothalamic area and in the extremity of the zona incerta, surrounding the ventral tip of the globus pallidus. We also performed serial immunohistochemical stains on *Mch-cre;L10-Egfp* mouse brain tissue to label NK3R-ir neurons using tyramide signal amplification followed with standard immunohistochemical staining to label CART-ir neurons. About half (49%) of all neurons counted expressed EGFP-f only and were most commonly found in the lateral hypothalamus around the globus pallidus. In contrast, 47% of EGFP-f neurons coexpressed CART and were more common in the medial hypothalamus. Of these EGFP+CART cells, half of them also coexpressed NK3R, which appeared equally distributed throughout the medial and lateral hypothalamic regions. Less than 4% of EGFP-f neurons coexpressed NK3R but not CART. In aggregate, these results indicate robust neurochemical and neuroanatomical heterogeneity of MCH neurons, and this may facilitate gene- or stereotaxic-driven approaches to isolate distinct MCH neuron subpopulations.

Disclosures: **P.A. Miller:** None. **M.J. Chee:** None.

Digital Abstract Session

P263. Ingestive Behavior: Peptide Signals

Program #/Poster #: P263.04

Topic: F.09. Food and Water Intake and Energy Balance

Support: CIHR PJT-153173

Title: Electrophysiological characterization of putative subpopulations of melanin-concentrating hormone neurons

Authors: D. R. ADEKUNLE, L. FANG, K. KATO, *M. HIRASAWA;
Div. of Biomed. Sci., Mem. Univ., St. John's, NL, Canada

Abstract: The hypothalamus is home to a heterogeneous population of neurons that are crucial in maintaining physiological and behavioural homeostasis. Melanin-concentrating hormone (MCH) neurons are essential regulators of energy and glucose homeostasis, sleep-wake behaviours, motivation, learning and memory. As MCH neurons are involved in these diverse processes, it is likely that there are specific subpopulations that might serve these distinct functions. MCH neurons occupy different areas of the hypothalamus and zona incerta (ZI), which may send projections to different parts of the central nervous system. Furthermore, neurochemical subpopulations of MCH neurons have been described: MCH neurons that co-express cocaine and amphetamine regulated transcript (CART) and those that do not. Therefore, we hypothesized that MCH neurons in distinct anatomical areas or with differential CART expression represent functional subgroups with different electrophysiological properties. To test this, whole cell patch clamp recording was performed on acute mouse brain slices in three MCH neuron-rich brain areas: medial hypothalamus (MH), lateral hypothalamus (LH) and ZI. Following recording, a subset of cells was immunohistochemically identified as MCH+/CART+ or MCH+/CART- neurons. MCH neurons in the three areas assessed had no difference in intrinsic properties such as the resting membrane potential (RMP), action potential (AP) waveform and firing activity upon positive current injections. Furthermore, these neurons in the three anatomical areas showed no electrophysiological differences when held at a subthreshold potential. In contrast, MCH+/CART- neurons had more depolarized RMP and fired APs than MCH+/CART+ neurons, suggesting that MCH+/CART- neurons are more excitable. These results suggest that MCH neurons are not electrophysiologically homogeneous. These differences in intrinsic properties, in addition to their neurochemical property and connectivity, are likely to be critical in how MCH neuron subpopulations function within the network.

Disclosures: **D.R. Adekunle:** None. **L. Fang:** None. **K. Kato:** None. **M. Hirasawa:** None.

Digital Abstract Session

P263. Ingestive Behavior: Peptide Signals

Program #/Poster #: P263.05

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH GM127251

Title: An analysis and representation in an atlas reference space of synaptic interactions between alpha-melanocyte stimulating hormone-immunoreactive axonal fibers and neurons expressing hypocretin/orexin or melanin concentrating hormone: light and electron microscopic evidence

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Abstract: Alpha-melanocyte stimulating hormone (αMSH), melanin-concentrating hormone (MCH), and hypocretin/orexin (H/O) are three major neuropeptide systems involved in the hypothalamic control of feeding and the regulation of metabolic state. MCH and H/O are neuropeptides which can be found in various sub-regions of the lateral hypothalamic area (LHA) and are known orexigenic peptides. On the other hand, αMSH is an anorexigenic peptide which is primarily expressed in the arcuate hypothalamic nucleus (ARH). Due to the opposing behavioral effects that these neuropeptides exert, along with their differential localization within the hypothalamus, there has been an interest in the field to determine whether putative appositions between αMSH-immunoreactive (-ir) axonal fibers and H/O-ir and MCH-ir somata are formed, and if so, whether these can be discerned to form synaptic relations at the electron microscopic (EM) level. Previous reports of such appositions, however, did not offer descriptions of their precise spatial distributions. Here, we report, within a standardized spatial framework, the mesoscale distributions of putative appositions between αMSH-ir fibers and H/O-ir or MCH-ir somata within the adult male rat hypothalamus from levels 23-30 of a standardized rat brain atlas (Brain Maps 4.0; Swanson, 2018, J. Comp. Neurol.). With the use of double-label immunoperoxidase histochemical staining and atlas-based mapping, we found that the LHAs had the highest density of putative appositions between αMSH-ir fibers and MCH-ir somata, with the LHAjvd and LHAd also containing high densities. Similarly, we found that the LHAs had the highest density of putative appositions between αMSH-ir fibers and H/O-ir somata, with the LHAd also having a high density. EM data from select regions also provided evidence of synaptic relationships between αMSH-ir terminals and H/O-ir and MCH-ir somata. The maps presented here will help determine regions of high likelihood of possible interactions between αMSH and H/O or MCH, and will allow for further testing to determine whether there is a clear pattern or organization of the distributions of these neuropeptides.

Disclosures: **A. Toccoli:** None. **L. Soto Arzate:** None. **V.I. Navarro:** None. **E. Peru:** None. **D. Sotelo:** None. **A. Szilvásy-Szabó:** None. **R. Yvette Magdolna:** None. **E. Farkas:** None. **A. Guevara:** None. **J. Guerra-Ruiz:** None. **K. Negishi:** None. **S. Balivada:** None. **A. Arnal:** None. **C. Fekete:** None. **A.M. Khan:** None.

Digital Abstract Session

P263. Ingestive Behavior: Peptide Signals

Program #/Poster #: P263.06

Topic: F.09. Food and Water Intake and Energy Balance

Support: CIHR PJT-153173
CIHR Doctoral Research Award GSD-167023

Title: Prostaglandin E2 signalling in MCH neurons contributes to high-fat diet-induced obesity

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Abstract: Due to the increased availability of energy-dense, high-fat foods, the incidence of obesity and non-alcoholic fatty liver disease are on the rise without effective therapeutic options.High-fat diet (HFD) induces inflammation of the brain, including the hypothalamus where the energy balance circuitry exists. Interestingly, HFD-induced hypothalamic inflammation can drive further food intake and weight gain, causing a vicious feedforward cycle. However, the molecular mechanism by which inflammation promotes positive energy balance remains poorly understood. The lateral hypothalamus (LH) is a hypothalamic nucleus that has been shown to undergo inflammation after prolonged HFD consumption. Orexigenic melanin-concentrating hormone (MCH) neurons within the LH are known for their role in promoting weight gain, fat accrual, and hepatic lipid accumulation. Given the role of MCH neurons, we hypothesized that MCH neurons mediate the hyperphagia and weight gain induced by HFD-induced hypothalamic inflammation.To test this, male rats or mice were fed a standard chow or a HFD for at least 4 weeks up to 14 weeks. Animals were then sacrificed, and in-vitro patch clamp electrophysiology was performed.We found that HFD induced a robust depolarization of MCH neurons compared to chow-fed controls. We then asked what is causing this depolarization? Prostaglandin E_2 (PGE_2) is an inflammatory mediator that is downstream of various cytokines known to be elevated after HFD feeding. Indeed, we found that PGE_2 mimicked the HFD effect while inhibition of cyclooxygenase-2, a prostanoid-synthesizing enzyme, reversed it. Further, antagonizing the PGE_2 EP2 receptor (EP2R) subtype blocks the PGE_2- and HFD-induced depolarization of MCH neurons. These results suggest that HFD increases endogenous PGE_2, which activates EP2R to depolarize these neurons. To determine the functional role of EP2R, mice lacking this receptor in MCH neurons ($MCH^{EP2R\ KO}$) and their littermate controls were fed with HFD. Preliminary results indicate that $MCH^{EP2R\ KO}$ mice gain less weight and consume fewer calories than the controls. Further, $MCH^{EP2R\ KO}$ mice tend to have less adiposity without a

difference in lean muscle mass compared to controls. Also, $MCH^{EP2R\ KO}$ mice have smaller livers than controls, and appear to be protected from liver steatosis. Together, we found that MCH neurons are activated by PGE_2-EP2R signaling during HFD feeding. This mechanism may provide a link between HFD-induced inflammation and the development of obesity and associated metabolic syndrome.

Disclosures: L. Fang: None. **M. Licursi:** None. **M. Hirasawa:** None.

Digital Abstract Session

P263. Ingestive Behavior: Peptide Signals

Program #/Poster #: P263.07

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH GM127251

Title: High spatial representation of the inputs and outputs of the lateral hypothalamic area dorsal region of the hypothalamus in the adult male rat using the common spatial framework of Brain Maps 4.0

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Abstract: The lateral hypothalamic area (LHA) was long considered to be one large monolithic structure spanning that vast majority of the hypothalamus. However, Swanson (2004; *Brain Maps 3.0*) proposed further provisional subdivisions of this larger structure which were initially based on careful reconsideration of cytoarchitecture. These provisional subdivisions, which are retained in Swanson (2018; *Brain Maps (BM) 4.0*), have since been further tested with the targeted use of tract-tracers to explore if connectional differences between subregions may offer support. These experiments, however, have thus far only covered select subdivisions of the LHA, leaving others untested. Here we explore the inputs and outputs of the lateral hypothalamic area dorsal region (LHAd) from *BM4.0* atlas levels 27-41 of the male rat in order to test whether it differs in connections from that of the lateral hypothalamic area juxtadorsal (LHAjd), lateral hypothalamic area juxtaparaventricular (LHAjp) and lateral hypothalamic area suprafornical (LHAs) regions which have previously been reported. We have employed the use of the tract-tracers cholera toxin B subunit (CTB) and *Phaseolus vulgaris*-leucoagglutinin (PHA-L) in order to visualize the inputs and outputs respectively of the LHAd. Inputs and outputs were represented with high-spatial resolution within the common spatial framework of *BM4.0* in order to bring these data into spatial registry with those that have previously been generated. Preliminary results suggest that connections of the LHAd include reciprocating inputs and outputs to regions such as the CEA, VMH, TU, RE, VTA, SUM, COM, MRNm and RR with the largest densities of these in VTA and SUM. The observed inputs to the VTA were compared

with, and were found to confirm, results from retrograde tracing studies of the VTA using the track-tracer, Fluorogold. Efferent projections from LHAd also include BLA, RE, PVT, PAG, PH, RM and RL while afferent inputs to LHAd also include LHAp, VISC, PAG, PH, SPFpm, NPC, CLl and the SUB of the hippocampus. Thus far, these preliminary results appeared to demonstrate some variations and overlap in the connectional profiles of the LHAd when compared to that of LHAjp, LHAjd and LHAs. These preliminary results offer some additional support to the hypothesis that the provisional cytoarchitectural subdivisions of the LHA have connectional differences.

Disclosures: V.I. Navarro: None. **K. Negishi:** None. **A.M. Khan:** None. **E.M. Walker:** None.

Digital Abstract Session

P263. Ingestive Behavior: Peptide Signals

Program #/Poster #: P263.08

Topic: F.09. Food and Water Intake and Energy Balance

Support: NIH GM127251

Title: Mesoscale and microscale interactions of fibers immunoreactive for alpha-melanocyte stimulating hormone and hypocretin/orexin with somata immunoreactive for neuronal nitric oxide synthase: A representation in an atlas reference space of the adult male rat, with supporting ultrastructural evidence

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Abstract: Hypocretin/orexin (H/O) and α-melanocyte-stimulating hormone (αMSH)-expressing neurons of the hypothalamus have been implicated as participants within feeding control circuits. Nitric oxide (NO) has been reported to modulate the effects of various feeding-related neuropeptides; however, this influence has not been characterized on anatomical grounds. To investigate the relationship between feeding-control circuits and NO-producing neuronal populations in the hypothalamus, the localization, distribution, and putative appositions of neuronal nitric oxide synthase (nNOS)-expressing neurons in relation to that of H/O- and αMSH-expressing neurons was examined. A combined diaminobenzidine/nickel-enhanced diaminobenzidine reaction was used to distinguish the neuronal populations in reactions involving paired combinations of antibodies against these neuropeptides. An adjacent tissue series was Nissl-stained to delineate cytoarchitectonic boundaries and assign levels according to *Brain Maps 4.0* (L.W. Swanson, 2018; *J Comp Neurol*.) αMSH-ir somata were restricted to the ARH and immediately adjacent regions. The highest αMSH-ir fiber density was within the DMHa and DMHv. H/O-ir somata in the LHAs, LHAd, and LHAjd comprised 67% of the total

average of H/O-ir somata per subject, with the densest population found within the LHAs. nNOS-ir somata were most prominent within the DMHv. H/O-ir fibers contacted nNOS-ir somata more than αMSH-ir fibers, with 18.8% of all nNOS-ir somata receiving putative appositions from H/O-ir fibers, while αMSH-ir fibers only formed putative appositions onto 15.8% of all nNOS-ir somata. These putative appositions were mainly found in regions where high colocalization of nNOS-ir somata with H/O- and αMSH-ir fibers was present. In addition to these quantitative data, we also generated high-spatial resolution representations of neuronal populations and mesoscale representations of putative appositions between key hypothalamic neuropeptides and nNOS-ir somata. These data sets allowed for the generation of high-spatial resolution aggregate density maps to visualize areas with high presence of these neuronal populations. The predictive power of these data maps has been demonstrated by the fact that we found, at the EM level, evidence of synapses between αMSH-ir fibers and nNOS-ir somata in the AHN and LHA. In sum, we have mapped interactions between feeding-related neuropeptides and nNOS-expressing neurons; these maps can aid functional investigations using techniques such as chemogenetics, optogenetics, or electrophysiology.

Disclosures: **D. Sotelo:** None. **A. Guevara:** None. **E. Peru:** None. **V.I. Navarro:** None. **A. Toccoli:** None. **A. Szilvásy-Szabó:** None. **R. Yvette Magdolna:** None. **E. Farkas:** None. **J. Guerra-Ruiz:** None. **L. Soto Arzate:** None. **K. Negishi:** None. **S. Balivada:** None. **A. Arnal:** None. **C. Fekete:** None. **A.M. Khan:** None.

Digital Abstract Session

P264. Mechanisms of Energy Consumption and Metabolism

Program #/Poster #: P264.01

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Rhythm Pharmaceuticals
NIH K12-GM111725

Title: Acute and lasting effects of setmelanotide, an MC4R agonist, on hypothalamic Pomc-deficient and high-fat-fed wildtype mice

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Abstract: Arc-*Pomc* knockout mice have a selective loss of pro-opiomelanocortin (*Pomc*) gene expression in the arcuate nucleus of the hypothalamus. Due to a lack of central endogenous melanocortin-4 receptor (MC4R) agonists these mice develop early onset obesity, and progressive insulin resistance. Setmelanotide is a melanocortin-4 receptor agonist, currently under FDA review for treatment of human POMC and LEPR deficiency obesity. Here we investigated the effects of early MC4R agonist intervention prior to obesity onset in this POMC-deficient mouse model. We tracked the impact of setmelanotide treatment in two groups of male mice: Arc-*Pomc* knockout mice, fed regular chow, and their wildtype counterparts, fed a 45%

high-fat diet. These two groups of mice were randomized into three cohorts: one treated with setmelanotide throughout the entire study, one treated with setmelanotide only for the first 4 weeks of the study and then switched to vehicle, and one cohort that received vehicle for the entire study. We serially measured body weight, food intake, body composition, glucose tolerance, insulin tolerance, and several measures of metabolism, including oxygen consumption, energy expenditure, and ambulatory activity. Among other results, at the end of the study (24 weeks of age), Arc-*Pomc* knockout mice in the chronic setmelanotide treatment group weighed significantly less and had improved glucose and insulin tolerance when compared to the vehicle group. Wildtype mice on high-fat diet, treated chronically with setmelanotide, also showed improved fasting insulin when compared to their vehicle group. However, despite the observation of beneficial effects of a single month's treatment prior to obesity onset, cessation of acute treatment resulted in a steady reversal of metabolic benefit and the return of these animals to a native phenotype. We conclude that the obesity syndrome caused by a loss of hypothalamic *Pomc* expression was completely blocked by setmelanotide treatment started before the onset of obesity and that continued treatment is required for continued metabolic benefit.

Disclosures: Z. Thompson: None. **B.P. Shah:** A. Employment/Salary (full or part-time):; Rhythm Pharmaceuticals. **A.S. Garfield:** A. Employment/Salary (full or part-time):; Rhythm Pharmaceuticals. **M.J. Low:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Rhythm Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Rhythm Pharmaceuticals.

Digital Abstract Session

P264. Mechanisms of Energy Consumption and Metabolism

Program #/Poster #: P264.02

Topic: F.04. Stress and the Brain

Support: Brain and Behavior Foundation
Whitehall Foundation
Akira Akimura Foundation

Title: Neuropeptidergic regulation of traumatic stress and metabolism via the locus coeruleus

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Abstract: Clinical observations have shown that patients with psychiatric disorders, like Post-Traumatic Stress Disorder (PTSD), may develop metabolic syndromes such as Type II Diabetes and obesity in their lifetime. Empirical evidence from both systems neuroscience and endocrinology have suggested a mechanistic link between psychological stress and metabolism.

Our laboratory has been focused on further characterizing the relationship between trauma-like stress and metabolism by investigating the role of the neuropeptide Pituitary Adenylate Cyclase-Activating Peptide (PACAP) and its receptor PAC1. PACAP and its receptor PAC1 have been known to be associated with PTSD and metabolic dysfunction. Previous findings in the laboratory have shown that PACAP innervates the brown adipose tissue (BAT), a metabolically active type of fat tissue that converts food substrates into heat energy. PACAP receptor PAC1 is also abundant in BAT. These two findings suggest a role for PACAP and PAC1 in BAT thermogenic regulation, a key aspect of energy metabolism. We also found that the locus coeruleus (LC), a major source of norepinephrine (NE) in the forebrain, is rich in PAC1 expression. LC can modulate aspects of traumatic stress and metabolism via the sympathetic pathway, which innervates the BAT and various brain areas. Our major hypothesis is that traumatic stress enhances metabolism on the short term for high energy demand, but in the long run can lead to decreased metabolism, thereby inducing metabolic syndromes. To answer our questions, we use mice (males and females aged between 3-4 months) with floxed PAC1 receptors and employ a range of techniques, including viral-mediated knockdown, the stress-enhanced fear learning (SEFL) behavioral assay for traumatic stress, immunohistochemistry, in situ hybridization, and qRT-PCR. Upon knockdown of PAC1 receptors in BAT, we found that expression of UCP1 (uncoupling protein 1), a critical BAT-specific mitochondrial protein involved in cellular respiration, increased. We have also observed that viral-mediated knockdown of PAC1 receptors in the LC led to enhanced fear expression in SEFL, decreased fat mass and enhanced expression of UCP1 acutely. After a chronic time-point, fear expression in SEFL was still enhanced, but fat mass increased. Taken together, our results indicate that PACAP/PAC1 is an important neuropeptidergic system in the sympathetic node that links the brain and the body to regulate traumatic stress and associated metabolic changes.

Disclosures: **F.A. Mohamed:** None. **S. Eleazer:** None. **K.L. Blankenship:** None. **S.K. Hart:** None. **P. Rajbhandari:** None. **A.K. Rajbhandari:** None.

Digital Abstract Session

P264. Mechanisms of Energy Consumption and Metabolism

Program #/Poster #: P264.03

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: National Institute of Mental Health
National Institute of Diabetes and Digestive and Kidney Diseases
1K01MH096175-01
Oklahoma Tobacco Research Center

Title: Restriction of dietary fat, but not carbohydrate, dampens striatalresponse to food images

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Abstract: Weight loss interventions often target restriction of either dietary carbohydrate or fat. Here, we investigated selective isocaloric reduction of dietary carbohydrate versus fat on fMRI brain activity to visual food stimuli (Clinicaltrials.gov #NCT0084604). Fifteen weight-stable adults (age=35.3±7.7 years; m=7, f=8) with obesity (BMI=35.87±4.7 kg/m^2) consented to two 14-day inpatient admissions to the NIH Clinical Center for a controlled feeding study. Each stay began with 5 days on a baseline eucaloric diet (50% carbohydrate, 35% fat, 15% protein), followed by cutting 30% of daily calories solely by reducing either carbohydrate (RC diet) or fat (RF diet) while keeping other macronutrients at baseline. Subjects completed both diet conditions in randomized crossover fashion. After approximately 4 days on each condition (baseline, RC and RF), subjects completed evening fMRI scans, 4.5 hours after their last meal, including a task requiring them to rate the pleasantness of 144 food pictures, varying from highly processed, high calorie foods to uncooked fruits and vegetables. A reward-circuit mask (including bilateral OFC and striatum) was applied for small volume correction. Mean food pleasantness did not differ across condition. Additionally, weight loss was not significantly different after 4 days on each condition. Results of a repeated measures ANOVA (3dANOVA2; AFNI) revealed an overall effect of dietary condition on reward circuit response to all food stimuli in the left putamen (96 voxels; x -19, y 9, z 6) and right putamen (32 voxels; x 25, y 5, z 10) (F(5.49); p_{uncorr}=0.005, cluster extent > 20 voxels surviving multiple comparisons using 3dClustSim at alpha=0.05). Post-hoc analyses revealed while the RC condition did not alter reward circuit response to food pictures from baseline, the RF condition caused a reduction of activity to food images bilaterally in a region spanning the left dorsal putamen and caudate (142 voxels; x -19, y 9, z 6) and right caudate (115 voxels; x 23, y 3, z -2) compared to baseline; p_{uncorr}=0.005, surviving multiple comparisons using 3dClustSim at alpha=0.05. Our results suggest that selective reduction of energy intake from dietary fat dampens reward circuit response to food images in people with obesity whereas isocaloric carbohydrate restriction had no significant effect.

Disclosures: I. Gallagher: None. **V.L. Darcey:** None. **J.A. Avery:** None. **J. Guo:** None. **A. Courville:** None. **K. Simmons:** None. **J. Inglehom:** None. **A. Martin:** None. **K.D. Hall:** None.

Digital Abstract Session

P264. Mechanisms of Energy Consumption and Metabolism

Program #/Poster #: P264.04

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: ANR-16-CE14-0026 (fat4brain)
AXA Research Grant
European Research Council

Title: Impairement of sensory cortex activation in obese mice

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Abstract: Obesity is a worldwide threat for health and is associated with major metabolic complications such as type 2 diabetes. Obesity increases the risk for ischemic stroke but the fate of hemodynamics in obese organisms is not well known. To bring further insights on the impact of obesity on sensory-evoked regulation of cerebral microcirculation, we explore relative changes in Cerebral Blood Volume (CBV) in the barrel cortex of obese and age-matched control mice at early ages during whisker stimulation using functional Ultrasound (fUS) imaging.Experiments were performed on 40 male mice divided into 2 groups, according to the time spent on high fat diet (HFD). In the first group, the mice are 2 months old and had 3 weeks of HFD (3W group, n=22), in the second group the mice are 4 months old and had 2 months of HFD (2M group, n=18). The mice were weighed, their fat measured with echoMRI and underwent 2 different metabolic tests: an oral glucose tolerance test (OGTT) and an Insulin tolerance test (ITT). The imaging sessions are fully non-invasive. The animals were anesthetized and placed in a stereotaxic frame. Ultrasound imaging acquisitions were performed in a coronal plane at Bregma – 1.70 mm, through the intact skin and skull. We measured CBV changes in the barrel cortex in response to whisker stimulation (2Hz). The acquisition was composed of a one minute baseline followed by 5 successive trials. Each trial consisted in 30 seconds of stimulation and 60 seconds of recovery. For each mouse we computed relative changes in power Doppler compared to the initial baseline. Then we averaged the 5 trials for each mouse. We also computed the correlation between stimulation pattern and the CBV time signal in each pixel of the Doppler film. As expected, both groups showed a significant weight and fat mass gain due to HFD compared to controls. For the metabolic tests, the 3W group showed normal time course responses to OGTT and ITT whereas the 2M group showed hyperglycemia in a fasted state, glucose intolerance and insulin resistance which are typical of a pre-diabetic stage. The 2M group shows an altered vascular response compared to controls (control mice CBV is 40 % higher than HFD, $p < 0.01$). Surprisingly, the 3W group also shows the same alteration of the vascular response to stimulation (40 %, $p < 0.001$). In the mean correlation map of the 3W, we observe a clear difference of activation between the HFD mice and control mice who showed an intense and spatially defined activation in the barrel cortex during stimulation, whereas the 3W HFD mice showed a less pronounced activation.fUS imaging showed an early neurovascular alteration in obese mice before the pre-diabetic stage where the mice do not have metabolic issues.

Disclosures: H. Soleimanzad: None. **C. Morisset:** None. **M. Massana:** None. **F. Pain:** None. **C. Magnan:** None. **H. Gurden:** None. **M. Tanter:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.01

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant R01MH117852

Title: Divergent midline thalamic projections to hippocampus and amygdala regulate retrieval and renewal of conditioned fear

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Abstract: The expression of memories is strongly linked to the context in which learning occurs. This is particularly relevant to fear extinction because extinction retrieval is poor outside of the extinction context, resulting in fear 'renewal.' Recent work from our laboratory indicates a key role of the thalamic nucleus reuniens (RE) in acquisition and expression of fear extinction in rats. Here we explored whether projections from the RE to the hippocampus (HPC) or basolateral amygdala (BLA) are involved in context-dependent retrieval of extinction memories. Male Long-Evans rats were infused with retrograde AAVs expressing the inhibitory opsin ArchT (AAVretro-CaMKII-ArchT-GFP) (n=9-12 per group) or control virus (AAVretro-CaMKII-GFP) (n=9-10 per group) into the HPC or BLA. Optical fibers were implanted into the RE for delivery of continuous green light (532nm). Rats underwent 5 tone-shock pairings in a distinct context, and the next day were extinguished in a different context (45 CS-only trials). Over the next two days, rats received within-subjects counterbalanced extinction retrieval tests in which light was either on or off during CS-only trials. Freezing served as the index of conditioned fear and was recorded with an automatic, unbiased motion threshold system. Inactivating RE → HPC projections during extinction increased freezing when compared to light-off responding. In animals receiving BLA AAVs, we observed that opsin was concentrated in the midline centromedial nucleus (CM), dorsal to the RE. Because midline projections to the BLA have been implicated in conditioned fear, we next examined whether CM → BLA projections are involved in fear renewal. Presentation of the extinguished CS in a novel context caused renewal, which was attenuated by inhibition of CM neurons projecting to BLA. There were no light-dependent effects in GFP rats. These data suggest a role for CM → BLA projections in promoting fear expression in novel contexts, whereas RE → HPC projections mediate fear suppression during retrieval in the extinction context. In conclusion, we have identified two distinct thalamic pathways that differentially modulate extinction retrieval and fear renewal.

Disclosures: K. Vasudevan: None. **J. Liu:** None. **K.R. Ramanathan:** None. **S. Maren:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.02

Topic: G.01. Fear and Aversive Learning and Memory

Support: Margaret Q. Landenberger Foundation

Title: Early life stress results in atypical defensive behaviors in mice

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Abstract: Defensive behaviors are a crucial aspect of survival and conservation of species. The ability to correctly develop and execute defensive strategies in response to threats is a complex process. Adverse experiences during early life significantly enhance the risk for the development of emotional and threat-related pathologies. One paradigm used to study ELS in the mouse is the limited bedding and nesting (LBN) model, in which dams and pups are placed in a low resource cage for seven days. This leads to disrupted maternal care, and results in behavioral, anatomical, and physiological changes in the brains of pups. It remains unknown how these adaptations affect naturalistic defensive responses and contribute to the development of fear-related disorders, as well as how sex may contribute to these adaptations. In order to test the effects of ELS on defensive behaviors, we utilized a Looming Disk (LD) behavioral assay. In the mouse, looming stimuli mimic the approach of aerial predators, and evoke innate, species-specific defensive behaviors such as running and freezing. Our data indicates that ELS affects typical defensive responses seen in mice. We have identified a relationship between ELS and atypical defensive responses such as darting. We also see an increase in reaction time to a threatening stimulus, followed by a decrease in the length of time spent sheltering or freezing after the looming stimulus. The results of this work indicate that fragmented and atypical responses to threatening stimuli occur as a result of ELS. These deficits may be related to changes in the brain's serotonergic (5-HT) system. Future work will examine what anatomical and physiological effects ELS has on the 5-HT system, and how this contributes to the changes in defensive behavior that we observe.

Disclosures: A.M. Polter: None. **A. Kisner:** None. **C.R. Cody:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.03

Topic: G.01. Fear and Aversive Learning and Memory

Support: MH113053
MH117791

Title: Ventral pallidum neurons dynamically signal relative threat

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Abstract: The ventral pallidum (VP) is anatomically poised to contribute to threat behavior. Recent studies report a VP population that scales firing increases to reward but decreases firing to aversive cues. Here, we tested the hypothesis that VP neurons signal relative threat through firing decreases. We recorded VP single-unit activity from male Long Evans rats (n = 14) undergoing fear discrimination consisting of cues predicting unique foot shock probabilities: danger (p = 1.00), uncertainty (p = 0.25) and safety (p = 0.00). Fear discrimination took place over a baseline of reward seeking. Rats' behavior and VP single-unit firing discriminated danger, uncertainty and safety cues. Cue-inhibited neuron-types (Low firing and Intermediate firing) dynamically signaled relative threat, decreasing firing according foot shock probability during early cue presentation, but disproportionately decreasing firing to uncertain threat as foot shock drew near. Low firing neurons increased firing to reward, consistent with a bi-directional signal for general value. Intermediate firing neurons were unresponsive to reward, revealing a specific signal for relative threat. Consistent with salience signaling, another population of VP neurons showing firing increases to cues, also increased firing to reward; cue firing reflected fear output, and relative threat that was disproportionate to foot shock probability. The results reinforce anatomy to reveal the VP as a neural source of a dynamic, relative threat signal.

Disclosures: M. Moaddab: None. **M.H. Ray:** None. **M.A. McDannald:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.04

Topic: G.01. Fear and Aversive Learning and Memory

Support: KAKENHI 19H05234
RIKEN Junior Research Associate Program

Title: Prefrontal cortical control of emotional responding through projections to the brainstem noradrenaline system

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Abstract: Trauma-related disorders including post-traumatic stress disorder (PTSD) are among the most common neuropsychiatric conditions in the world. Hyperarousal and an inability to extinguish emotional memories are characteristic symptoms of PTSD and dysregulation of the noradrenaline system has been implicated as a mechanism of PTSD pathology. The brainstem locus coeruleus (LC) is the major source of noradrenaline to the forebrain and plays important

roles in arousal, stress responses, cognition and emotional processing through its broad connectivity throughout the nervous system. Previously we demonstrated how distinct submodules within LC differentially regulate forebrain target sites in the amygdala and prefrontal cortex to control fear extinction learning. However, it is unclear how forebrain sites such as the prefrontal cortex regulate LC-noradrenaline function and whether prefrontal inputs to LC modulate emotional processing is not known.
Here we used viral optogenetic and anatomical tracing approaches in rats to elucidate the detailed prefrontal-to-LC anatomical connectivity and test whether prefrontal innervation of the LC regulates fear extinction learning. Using anterograde viral and retrograde tracing approaches, we found that the LC/pericoeruleus area receives projections from both dorsal and ventral subregions of the mPFC (dmPFC, vmPFC). Using transsynaptic rabies virus, we also found that dmPFC and vmPFC project to a mPFC-projecting subpopulation of LC, demonstrating reciprocal connectivity between these regions. We next examined whether the dmPFC and vmPFC synaptic inputs to the LC participate in extinction of auditory fear memories. To test this question, we injected archaerhopsin-T (ArchT) into either the dm- or vmPFC and inhibited axon terminals from these regions in the LC during fear extinction learning sessions. We found that inhibition of the dmPFC input to the LC enhanced extinction learning. By contrast, inhibition of the vmPFC to LC pathway impaired extinction learning. This demonstrates that distinct mPFC subregions bidirectionally regulate fear extinction learning through projections to the LC-noradrenaline system. These findings suggest that the mPFC provides top-down cognitive control of the LC noradrenergic system for flexible control of emotional processing.

Disclosures: M. Watanabe: None. **A. Uematsu:** None. **J.P. Johansen:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.05

Topic: G.01. Fear and Aversive Learning and Memory

Support: NSERC Grant 2018-04401

Title: The associative nature of fear: Learned and inherent sources of danger

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Abstract: Animals acquire knowledge about features that predict danger through classical conditioning, an important survival skill. In first order conditioning (FOC), rats are trained to associate a neutral stimulus with an inherent source of danger, such as a shock, causing fear of that conditioned stimulus (CS1). If the learned CS1 is later presented with a different 2nd neutral stimulus (CS2) in the absence of shock, the rat also becomes afraid of CS2. This is called second

order conditioning (SOC). The basolateral amygdala (BLA) encodes negative valence of FOC memories and has been implicated in SOC memories as well. However, whether other sensory areas are involved in SOC is not clear. We mapped the activation pattern of several brain regions following SOC to understand the circuitry involved in this type of learning.
Adult (3-6 months) Sprague Dawley rats of both sexes were trained to associate either an odor, tone, or context (CS1) with a shock then trained to associate another neutral cue from a different sensory modality (CS2) with CS1. cFos immunohistochemistry was employed to study activity upon reactivation of these memories across multiple brain regions including: layer II of the anterior and posterior piriform cortex (aPC/pPC), CA1 and CA3 of the dorsal and ventral hippocampus (dHipp/vHipp), the BLA, and the primary auditory cortex. In a subset of animals D-APV was infused in the BLA immediately following tone/odor SOC to block NMDARs.
Odor, context, and tone may be used interchangeably as conditioned stimuli to produce FOC and SOC in the rat. When odor is used as a CS1 in FOC or CS2 in SOC increased neural activity is seen in the BLA, pPC, and vHipp CA1. When context is used as CS1 and odor as CS2, the dHipp CA1 and vHipp CA3 are also activated. Preliminary data suggests that when tone is used as CS1 with odor as CS2, the auditory cortex is recruited instead. These results postulate there are common brain regions involved with the expression of SOC fear memories regardless of the sensory modalities of the CSs, and that ancillary regions are recruited depending on the identity of the CS1. D-APV infusion to the BLA immediately following tone/odor SOC resulted in impaired recall of the odor CS2 upon re-exposure, highlighting how NMDAR signaling in the BLA is crucial for the expression of SOC fear memory.
A traumatic event that elicits emotions which can be transferred to temporally-associated neutral stimuli has the capacity to produce aberrant negative thought patterns, potentially leading to issues with mental health. With ever increasing diagnoses of anxiety, understanding the circuitry and mechanism of second order conditioning is critical.

Disclosures: **S. Carew:** None. **T. Sepahvand:** None. **C. Crossley:** None. **A. Ghosh:** None. **S. Edwards:** None. **C. Reinhardt:** None. **Q. Yuan:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.06

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIMH grant MH-092443
NIMH grant MH-094268

Title: Dopamine-dependent rewiring of the anterior insular cortex circuit retains explicit emotional memory

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Abstract: We tend to preferentially remember emotionally salient events that are associated with the feelings of joy, sorrow, pleasure, or pain. These associative emotional memories serve as potent predictors of important events when re-encountering similar situations, therefore shaping adaptive responses for survival. Supported by recent human neuroimaging studies, the anterior insular cortex (AIC) is emerging as an important brain region in learning and memorizing emotionally salient events. Nevertheless, there has been no systematic study to elucidate a neural mechanism by which AIC plays a role in emotional learning and memory. By utilizing two complementary paradigms of fear conditioning [delay fear conditioning (DFC) and trace fear conditioning (TFC)] in conjunction with pharmacological intervention, electrophysiology, and optogenetics, we show a neuronal mechanism whereby AIC-centered neural circuits retain explicit emotional memory. Pharmacological inactivation of AIC impaired fear memory retention in TFC, but not in DFC, showing the necessity of AIC in retaining explicit fear memory. Given that potentiation of excitatory synaptic transmission represents a neurobiological substrate underlying associative learning and memory, we next measured miniature excitatory postsynaptic currents (mEPSCs) in layer V pyramidal neurons of AIC, the main output neurons of AIC. As a result, we found significant increase in both amplitude and frequency of mEPSCs. By employing retrograde tracing, we next searched for upstream brain regions that project glutamatergic axons to and therefore possibly form excitatory synaptic potentiation onto AIC neurons. Among them, we focused on the basal amygdala (BA) and dorsomedial prefrontal cortex (dmPFC) for further study because of their necessity in TFC. By using projection-specific optogenetic inhibition, we tested the necessity of BA-to-AIC and dmPFC-to-AIC pathways in explicit fear memory retention. As a result, we found that silencing either BA-to-AIC (strong effect) or dmPFC-to-AIC (modest effect) pathway during TFC abolishes explicit fear memory retention. Given that dopamine (DA) modulates synaptic plasticity in response to emotionally salient events, we next hypothesized that DA gates potentiation of BA-to-AIC and/or dmPFC-to-AIC synapses, which underlies explicit fear memory retention. Blocking DA signaling in AIC using D1R-type receptor antagonists abolished explicit fear memory retention. Taken altogether, the present study shows a potential mechanism by which DA-gated potentiation of BA-to-AIC and/or dmPFC-to-AIC synapses retains explicit emotional memory.

Disclosures: H. Namkung: None. **S. Lockhart:** None. **J.D. Chabot:** None. **B.J. Lee:** None. **A. Sawa:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.07

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant R01MH065961
NIH Grant R01MH117852

Title: Optogenetic inhibtion of basolateral amygdala principal neurons attenuates the retrieval of both recent and remote cued fear memories in rats

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Abstract: After initial encoding in the brain, memory undergoes reorganization with the passage of time. It has been demonstrated that different brain circuits underlie the retrieval of recent and remote fear memories in animal models. Despite extensive evidence implicating the basolateral amygdala (BLA) in fear memory, there are controversial results concerning the involvement of the BLA in the retrieval of remote fear memory. In this study, we show that optogenetic inhibition of BLA principal neurons reduces retrieval of both recent and remote fear memory in rats. To inhibit neuronal activity in the BLA, we expressed the red-shift microbial rhodopsin JAWS (AAV8-CaMKII-JAWS-GFP) or a control GFP (AAV8-CaMKII-GFP) in male rats. In the first experiment, rats underwent auditory fear conditioning during which a conditioned stimulus (CS; 2 kHz tone) was paired with a footshock (n = 5-6/group). Cued fear memory was measured 1 day (recent memory) and 7 days (remote) after conditioning in a neutral context. In the second experiment, male and female rats (n = 7-8/group)were conditioned using a within-subject procedure in which one conditioned stimulus (CS1; 8 kHz tone) was paired with footshock 14 days (remote memory) prior to testing, and a second CS (CS2; 2 kHz tone) was conditioned 1 day prior to testing (recent memory) in a distinct context. Retrieval testing to each CS was conducted in four counterbalanced tests (i.e., there were two tests for each CS, one with light on and the other with light off) and freezing behavior served as the index of conditional fear. In both experiments, we found that optogenetic inhibition of the BLA (red light, 635 nm, continuous illumination starting 10 sec before the first CS test trial) significantly attenuated freezing behavior to both the remotely and recently conditioned CS; rats expressing the control virus did not exhibit memory deficits. We did not observe any difference between male and female rats in the second experiment. Consistent with previous lesion studies, the present study indicates that the BLA is essential for the retrieval of both recent and remote cued fear memory.

Disclosures: J. Liu: None. **S. Maren:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.08

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant MH114961
NIH Grant GM127251
NIH Grant GM069621 (RISE)

Title: Topographic organization of neural inputs to the basolateral amygdala during postnatal development: A tract-tracing analysis of hippocampal and prefrontal cortical projection neurons

Authors: *A. PINEDA SANCHEZ[1], A. J. SANTARELLI[2], K. NEGISHI[3], L. P. MONTES[3], A. M. KHAN[4], A. M. POULOS[5];

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Abstract: Prior studies indicate that the capacity to acquire and retain contextual fear memories undergoes a protracted maturation that spans key developmental periods from infancy, juvenility, adolescence to adulthood. However, the status of context fear conditioning (CFC) neural circuits at these stages of development remains poorly characterized. Determining the ontogenetic organization of basolateral amygdalar (BLA) nucleus afferents - including those originating from the hippocampus and medial prefrontal cortex - may provide a window into the functional contributions of these projections. The BLA, comprised of anterior and posterior subregions, extends ~3.3 mm rostrocaudally. We iontophoretically infused the retrograde tracer, Fluorogold, into rostral and caudal portions of the BLA of 44 male rats at postnatal days (PND) 16, 21, 32 and 87. Following four days to allow for retrograde transport, brains from perfused rats were sectioned to identify retrogradely labeled Fluorogold neurons in the anterior cingulate dorsal area dorsal part (ACAd), prelimbic (PL) and infralimbic (ILA) areas of the medial prefrontal cortex, and the ventral part of Field CA1 (CA1v) of the hippocampal region. Fluorogold injections in rostral (*AP* -2.0 to -2.7 mm from ß) and caudal (*AP* -3.25 to -3.90 mm from ß) portions of the BLA resulted in retrograde labeling of neurons in all regions and ages examined. Our results revealed that the PL undergoes significant growth from juvenility to adulthood that largely results from injections within the rostral portion of the BLA. In contrast, the ILA underwent significant pruning from juvenility to adulthood, that resulted from ipsilateral injections within rostral BLA. The ACAd underwent significant pruning from juvenility to adulthood irrespective of the BLA injection site. The CA1v underwent significant growth from juvenility to adulthood that was also irrespective of BLA injection site. Control experiments involving injection of the anterograde tracer, *Phaseolus vulgaris* leucoagglutinin (PHA-L), into the PL confirmed the existence of monosynaptic projections from the PL to the BLAa and BLAp. These results provide insight into developmental differences in the connectivity patterns of key limbic circuits during development, which may account for the differential activation patterns we have previously reported for these animals in these regions.

Disclosures: A. Pineda Sanchez: None. **A.J. Santarelli:** None. **K. Negishi:** None. **L.P. Montes:** None. **A.M. Khan:** None. **A.M. Poulos:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.09

Topic: G.01. Fear and Aversive Learning and Memory

Title: Time alone following initial acquisition is sufficient to make signaled active avoidance dependent on the retrosplenial cortex

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Abstract: Signaled active avoidance (SAA) behavior involves a multistage learning process supported by a shifting neural substrate. While recent work has begun to explore the circuitry by which the avoidance response is initially acquired and expressed, less is known about the mechanisms underlying the long-term maintenance of the response. In particular, it is unknown whether these mechanisms are brought online by continued training following acquisition, or whether time alone is sufficient to recruit them, suggesting a role for a systems consolidation-like process. Because it plays a known role in the long-term maintenance of aversive memory, we hypothesized that the retrosplenial cortex (RSC) is necessary for the expression of avoidance after substantial training. In an initial experiment, rats received intra-retrosplenial infusions of an AAV containing the gene construct for either the inhibitory hM4Di DREADD or GFP on a CamKII promoter to ensure expression in pyramidal neurons. Following recovery, subjects received training in an SAA paradigm in which they learned to shuttle across a divided chamber during a tone in order to avoid a foot shock. To compare the effects of retrosplenial inactivation across different phases of SAA maintenance, four days of initial acquisition were followed by two sessions of SAA preceded by CNO or vehicle in a counterbalanced order. Subjects then underwent two additional days of SAA training without drug treatment prior to two final sessions that were again preceded by counterbalanced administration of CNO or vehicle. While CNO inactivation of the retrosplenial cortex had no effect on the avoidance response following the initial four days of training, a robust decrement was observed at the latter time point, after subjects had undergone at least eight daily sessions of SAA. Then, in a subsequent experiment, we set out to determine if RSC is recruited by the passage of time or by continued training following initial acquisition. Rats expressing hM4Di in RSC pyramidal neurons received four days of SAA training followed either by two test sessions identical to those described above or by four days of time off in the homecage prior to two test sessions. CNO inactivation of RSC following the time off period caused a significant decrement in the avoidance response, while inactivation immediately following the initial four days of training had no effect. Thus, our data confirm that RSC plays a role in the long-term maintenance of the avoidance response, and that RSC is recruited to SAA by a systems consolidation mechanism and not by continued training following initial acquisition of the response.

Disclosures: **M.R. Kreitlow:** None. **L. Keppler:** None. **J.M. Moscarello:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.10

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH R01MH117852
NIH R01MH117852S01

Title: Chemogenetic inhibition of basolateral amygdala neurons projecting to the infralimbic cortex rescues stress-induced impairment in fear extinction in male, but not female, rats

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Abstract: Stress exposure prior to extinction learning results in an impairment in long-term extinction memory. For example, extinction training conducted shortly after fear conditioning results in diminished extinction retention, known as the 'immediate extinction deficit' (IED). Underlying this impairment is perturbed neural activity in a variety of extinction-relevant circuits. In particular, footshock stress causes a lasting increase in firing rate in the basolateral amygdala (BLA) and a decrease in firing rate in the infralimbic (IL) cortex, a region critical for extinction learning and retention. We hypothesize that BLA projections to the medial prefrontal cortex underlie suppression of IL neuronal activity and mediate the IED . To examine this, we infused male and female rats (n = 8 per group) with a retrograde CAV expressing Cre recombinase (CAV2-Cre) into the IL and a Cre-dependent AAV expressing an inhibitory designer receptor exclusively activated by designer drugs (DREAADs) into the BLA (AAV-DIO-CaMKII-hM4di-mCherry). After recovery from surgery, animals received a systemic injection of either clozapine-N-oxide (CNO; 5 mg/kg, i.p.) or vehicle immediately prior to a standard 5-trial auditory fear conditioning procedure; this was followed after 15-min by a 45-trial extinction session. Animals were then tested for extinction retention 48 hours later. CNO administration did not affect freezing behavior during the conditioning or extinction sessions. During retention testing, however, male, but not female, rats treated with CNO displayed significantly lower levels of freezing compared to rats treated with the vehicle. These results suggest that silencing IL projectors in the BLA rescues the IED in male rats. This is consistent with our hypothesis that stress-induced BLA activation dampens IL activity to impair extinction.

Disclosures: **A.N. Binette:** None. **O.W. Miles:** None. **K.R. Ramanathan:** None. **S. Maren:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.11

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH/NIMH R01MH106617
NARSAD Grant

Title: Prefrontal projections to the basolateral amygdala mediate safety learning

Authors: *E. KORZUS, J. PASTORE, T. W. BAILEY, J. MAYER, J. H. SPEIGEL, III; Psychology, Univ. of California Riverside, Riverside, CA

Abstract: This research focuses on developing a basic understanding of the circuit-level mechanisms governing behavior. While the prelimbic (PL) and infralimbic (IL) subdivisions of the medial prefrontal cortex (mPFC) are recognized for their excitatory and inhibitory effects on the fear circuit, respectively, the mechanisms driving fear discrimination remains unclear. Our previous research demonstrated that fear discrimination learning is mediated by prefrontal mechanisms. We showed that differential fear conditioning sparks neuronal ensembles associated with the inhibition of generalized fear in both the prelimbic and infralimbic cortices. In addition, real-time recordings from large neuronal population acquired from PL using head-mounted miniature microscopes across learning on a fear discrimination task revealed distinct PL neuronal assemblies, which show safety learning-triggered quantitative changes. Current research evaluated the role of PL projections to the basolateral amygdala (BLA) in differential fear conditioning. Considerable evidence indicates that coactivator of transcription and histone acetyltransferase cAMP response element binding protein (CREB) binding protein (CBP) is critically required for normal neural function and long-term memory consolidation. CBPΔHAT mutant has no intrinsic acetyltransferase activity due to its inability to interact with a donor of acetyl group, acetyl-CoA but retains all protein-protein interaction domains. When expressed acutely in adult excitatory neurons, CBPΔHAT functions as a specific blocker of long-term memory consolidation without disrupting information acquisition or short-term memory (Korzus et al. 2004). Our current data showed that expression of the selective inhibitor of long-term memory consolidation CBPΔHAT via a viral vector in PL neurons projecting to BLA results in severe deficits in differential fear conditioning but not in fear conditioning. We found decreased levels of histone acetylation and decreased induced-levels of activity marker cfos protein expression in neurons expressing CBPΔHAT. However, induced-levels of CREB phosphorylation were spared in the CBPΔHAT mutant mice indicating that upstream signals triggering the induced gene expression program remain intact in neurons expressing CBPΔHAT. Thus, CBPΔHAT mutant protein interferes with the epigenetic regulation of gene expression via negative modulation of histone acetylation resulting in circuit and behavioral deficits. Fear is known to be difficult to control and understanding the neural mechanisms that underlie the appropriate balancing of fear responses has clinical implications.

Disclosures: E. Korzus: None. **J. Pastore:** None. **T.W. Bailey:** None. **J. Mayer:** None. **J.H. Speigel:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.12

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant R15MH118705

Title: Dissociation between the nucleus accumbens subregions in associative fear processing

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Abstract: The nucleus accumbens (NAcc), consisting of core (NAcC) and shell (NAcS) sub-regions, has primarily been studied as a locus mediating the effects of drug reward and addiction. However, there is ample evidence that this region is also involved in regulating aversive behavior, but the neurobiology is poorly understood. In our studies, we sought to elucidate the precise functions of the two sub-regions in regulating cued fear expression and extinction. We first examined expression of Arc as an indicator of neuronal activity during associative fear processing. Groups of mice were exposed to several control conditions and normal cued fear training, and then tested for fear expression or extinction. Arc expression was restricted to the NAcC during fear expression, whereas it was restricted to the NAcS during fear extinction. Next, mice were stereotaxically implanted with cannulae directed at either the NAcC or NAcS. We infused lidocaine into the NAcC or NAcS 5 mins prior to a fear expression test which also served as extinction training. Fear expression was not influenced by lidocaine infusions into the NAcS, however, extinction memory was disrupted during an extinction retention test. These data indicate that the NAcS is important for the consolidation of fear extinction. In contrast, mice with local infusions of lidocaine into the NAcC showed attenuated fear expression that was not due to their ability to freeze, suggesting that the core sub-region is involved in fear memory recall. To understand the unique roles of the NAcS and NAcC in fear processing, mice were infused with AIDA, an mGluR1 antagonist within the NAcS or NAcC prior to extinction training. Inactivating mGluR1s within the NAcS resulted in disrupted extinction memory but had no effect on fear expression or within-session extinction. There was no effect on fear processing when mGluR1s were blocked in the NAcC. These data suggest that mGluR1 signaling within the NAcS is critical for the consolidation of extinction, but not for expression or extinction learning itself. We next infused a MEK inhibitor into the NAcS post-extinction, which disrupted extinction retention. These data indicate that mGluR1 through ERK signaling specifically within the NAcS is critically important for the consolidation of extinction memory. These findings suggest that the NAc may be considered a critical region involved in regulating both fear recall and extinction memory depending on the subregion. It's dual role in appetitive and aversive regulation likely underlies the high co-morbidity between anxiety disorders and substance use disorders.

Disclosures: S. Dutta: None. **A.M. Jasnow:** None. **J. Beaver:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.13

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01 MH113007

Title: Oxytocin selectively excites interneurons and inhibits output neurons of the bed nucleus of the stria terminalis (BNST)

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Abstract: ***Abstract*** The dorsolateral bed nucleus of the stria terminalis ($BNST_{DL}$) has high expression of oxytocin (OT) receptors (OTR), which were shown to modulate fear and anxiety. The $BNST_{DL}$ contains GABA-ergic neurons classified based on intrinsic membrane properties into three major types. Using *in vitro* patch-clamp recordings in male rats, we demonstrate that OT selectively excites Type I neurons in an OTR-dependent manner as revealed by a significant depolarization of the resting membrane potential (from -62.39 ± 0.84 mV to -57.94 ± 1.39 mV, $P = 0.0013$, paired *t*-test), increased input resistance (from 170.98 ± 8.6 MΩ to 233.64 ± 18.6 MΩ, $P = 0.0085$), reduced rheobase (from 21.96 ± 2.29 pA to 17.32 ± 2.26 pA, $P = 0.0023$), reduced fast (from 8.63 ± 0.89 mV to 7.47 ± 0.77 mV, $P = 0.0062$) and medium spike afterhyperpolarization (from 10.69 ± 0.61 mV to 8.45 ± 0.72 mV, $P = 0.0001$), reduced threshold and latency to a first spike
(from - 38.38 ± 0.68 mV to -39.75 ± 0.88 mV, $P = 0.0346$), as well as a left shift in the spike frequency/current relationship. OT also increased spontaneous firing rate of Type I neurons recorded in a cell-attached mode (from 0.88 ± 0.66 Hz to 2.19 ± 0.93 Hz, $P = 0.0242$). As Type I neurons are putative $BNST_{DL}$ interneurons, we next recorded inhibitory synaptic transmission in all three types of neurons. We demonstrate that OT increases the frequency (from 1.03 ± 0.20 Hz to 2.64 ± 0.47 Hz, $P = 0.0033$), but not amplitude ($P = 0.9205$) of spontaneous inhibitory post-synaptic currents (sIPSCs) selectively in Type II neurons, an effect abolished by the presence of an OTR antagonist or tetrodotoxin. In addition, OT reduced spontaneous firing rate in Type II neurons (from 1.85 ± 0.74 Hz to 1.15 ± 0.69 Hz, $P = 0.0525$), without changing the amplitude of evoked IPSCs (eIPSCs, $P = 0.2459$, paired *t*-test) in these neurons. These results suggest an indirect effect of OT in Type II neurons, which is mediated via OT-induced increase in firing of Type I interneurons. In contrast, In Type III neurons, OT reduced the amplitude but not frequency, of both sIPSCs ($P = 0.0314$) and eIPSCs ($P = 0.0105$), suggesting a direct postsynaptic inhibitory effect. As Type II and Type III neurons were shown projection neurons of the $BNST_{DL}$, these results present a model of fine-tuned modulation of intrinsic $BNST_{DL}$ neurocircuitry by OT, which selectively excites Type I interneurons and inhibits Type II and Type III output neurons, via an indirect and direct mechanism, respectively.
Grant: National Institute of Mental Health R01 MH113007

Disclosures: W. Francesconi: None. **F. Berton:** None. **V. Olivera-Pasilio:** None. **J. Dabrowska:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.14

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH R01MH065961

Title: The role of the nucleus reuniens in coordinating prefrontal-hippocampal synchrony during the expression of fear and extinction memories.

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Abstract: The nucleus reuniens of the thalamus (RE) is a small midline structure interconnecting the medial prefrontal cortex (mPFC) and hippocampus (HPC) that has been proposed to coordinate mPFC-HPC interactions. One mechanism by which the RE may facilitate mPFC-HPC communication is by the coordination of neural oscillations. Recent work has revealed that the expression of fear and extinction memories results in distinct 4 and 8 Hz network oscillations in the amygdala and mPFC. Additionally, RE inactivation impairs extinction retrieval and also mPFC-HPC synchrony during working memory tasks. We thus hypothesize that the RE may participate in these network oscillations and facilitate mPFC-HPC communication during fear and extinction memory retrieval. Using auditory fear conditioning and extinction, we show in male rats that the RE displays distinct 4 and 8 Hz oscillations that correlate with freezing behavior (n=4). In an additional experiment, we show the mPFC and HPC display similar 4 and 8 Hz oscillations (n=7), and pharmacological inactivation of the RE impairs 8 Hz mPFC-HPC coherence and results in a shift in spectral power towards the freezing-related 4 Hz oscillation (n=4). These data extend our current understanding of fear and extinction network dynamics.

Disclosures: M. Totty: None. **K. Ramanathan:** None. **S. Maren:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.15

Topic: G.01. Fear and Aversive Learning and Memory

Support: Center for Inclusive Education

Title: Subthreshold fear conditioning produces a rapidly developing neural mechanism that primes subsequent learning

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Abstract: Learning results in various forms of neuronal plasticity that provide a lasting representation of past events, and understanding the mechanisms supporting lasting memories has been a primary pursuit of the neurobiological study of memory. However, learning also alters the capacity for future learning, an observation that likely reflects its adaptive significance. In the laboratory we can study this essential property of memory by assessing how prior experience

alters the capacity for subsequent learning. Previous studies have indicated that while a single weak fear conditioning trial is insufficient to support long-term memory, it can facilitate future learning such that another trial delivered within a protracted time window results in a robust memory. Here, we sought to determine whether or not manipulating neural activity in the basolateral amygdala (BLA) using designer receptors exclusively activated by designer drugs (DREADDs) during or after the initial learning trial would affect the ability of the initial trial to facilitate subsequent learning. Our results show that inhibiting the BLA prior to the first trial prevented the ability of that trial to facilitate learning when a second trial was presented the next day. Inhibition of the BLA immediately after the first trial using DREADDs was not effective, nor was pharmacological inhibition of protein kinase A or the mitogen activated protein kinase. These findings indicate that the neural mechanisms that permit an initial subthreshold fear conditioning trial to alter later learning develop rapidly and do not appear to require a typical post-learning consolidation period.

Disclosures: K.E. Cole: None. **J. Lee:** None. **R.G. Parsons:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.16

Topic: G.01. Fear and Aversive Learning and Memory

Support: Grant-in-Aid for JSPS Research Fellows (201610802)

Title: A sensorimotor neural circuit for transforming aversive experiences into emotional memories

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Abstract: Innately aversive experiences induce immediate escape and autonomic reactions, accompanied by persistent emotional memories. A common view is that external sensory properties of aversive experiences instruct memory formation; while competing views suggest that the bodily reactions to unpleasant events engage an emotional state that produces associative memories. The neural mechanisms that transduce aversive events into emotional states which regulate learning and behavior are unclear. Here we identify a brainstem-cuneiform (CnF) circuit which conveys both aversive external-sensory and aversive internal-motor information to the lateral and basal amygdala (LA/B), a brain region which stores emotional memory, to set an aversive sensorimotor state which instructs associative fear memory and enhances defensive behaviors. Using viral tracing and synaptic connectivity approaches, we found that glutamatergic CnF neurons project to and make synaptic connections with LA/B neurons. Rabies tracing studies revealed that this ascending CnF-to-LA/B receives monosynaptic inputs from spinal cord and brain regions which participate in aversive-sensory and aversive-motor functions. Notably,

LA/B neurons encode an aversive sensorimotor state during innately aversive experiences and both the sensory and motor components of this state representation are conveyed through the CnF-to-LA/B pathway. Finally, optogenetic perturbation experiments showed that during aversive events the CnF-LA/B pathway instructs aversive emotional memory formation and modulate the intensity of ongoing escape behaviors. Our findings reveal a brainstem circuit that enables aversive sensorimotor state encoding in amygdala networks and functions to produce emotional memories and enhance defensive behavioral reactions. This mechanism may explain the individual scaling of emotional memory strength by the magnitude of bodily reactions to trauma.

Disclosures: L. Yeh: None. **Y. Kasuga:** None. **J.P. Johansen:** None. **Y. Ishizu:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.17

Topic: G.01. Fear and Aversive Learning and Memory

Title: The neural circuitry of conditioned nausea in rats

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Abstract: Chemotherapy-induced nausea and vomiting is one of the most troubling and difficult to manage side effects in cancer patients. Twenty-five to fifty % of patients undergoing chemotherapy will develop anticipatory nausea and vomiting (ANV), in which symptoms occur in anticipation of treatment. ANV is triggered by environmental cues and shows little response to traditional antiemetic therapy, suggesting that unique neural pathways mediate this response. ANV decreases quality of life and can lead to early cessation of treatment. Understanding the underlying neural mechanisms of this disorder is critical to the development of novel therapeutic interventions. The purpose of the present study was to identify brain areas activated during ANV. We used a rat model of ANV, by pairing a novel context with the emetic drug lithium chloride (LiCl) to produce conditioned nausea behaviors in the LiCl-paired environment alone. Male and female rats were treated with NaCl or LiCl (96 mg/kg) on alternate days for 4 treatments (day 1,3,5,7). On Day 9, they were placed in the context and we measured gaping, an analogue of human vomiting. To identify brain regions associated with acute LiCl and ANV, we measured c-fos activation by immunochemical staining. Behavioral results were analyzed by 2-way ANOVA (sex x treatment) and c-fos activation by 3-way repeated measure ANOVA of cell numbers containing c-fos expression (sex x treatment x area as repeated measure). Both male and female rats exhibited gaping behavior on test day, but males exhibited more gaping behavior than females ($p < 0.0244$)
Acute LiCl and ANV activated different brain areas differentially ($F = 11.4$, $p < 0.0001$ for area

x treatment). Acute LiCl activated multiple brain regions including the supraoptic nucleus of the hypothalamus, central nucleus of the amygdala (CeA), nucleus of the solitary tract (NTS) and area postrema (AP), that were not activated during ANV. ANV activated c-fos expression in the frontal cortex and insula of males, and the VTA of both sexes, indicating areas unique to the conditioned response. These data suggest that therapies such as ondansetron which target the area postrema are not effective in ANV because it is not a site activated during ANV response. Further studies aimed at characterizing the cell types that are activated in the conditioned nausea response will help identify novel therapeutic targets for the treatment of this condition, improving both quality of life and outcomes for patients undergoing chemotherapy.

Disclosures: S. Sette: None. **N. Hernandez:** None. **J. Murphy:** None. **S. Zimmerman:** None. **Z. Reavis:** None. **A. Bernanke:** None. **C.M. Kuhn:** None.

Digital Abstract Session

P265. Neural Systems of Fear and Defensive Behavior

Program #/Poster #: P265.18

Topic: G.01. Fear and Aversive Learning and Memory

Support: UM support for Jacek Debiec
Nathan Horrell is supported by UM's IRACDA program (NIH K12-GM111725)

Title: Effects of electroshock treatment on functional connectivity of brain regions underlying fear memories in rats: a graph theoretical approach

Authors: *N. D. HORRELL[1], A. M. WHITE[1,2,3], K. BONEFAS[3,4], Y. NAKAMURA[4], B. SAVARD[1], M. PATEL[1], M. SHOW[1], X. AN[6], S. IWASE[4,3], M. ZOCHOWSKI[3,5], J. DEBIEC[1,2,3];
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Abstract: Background: Although electroconvulsive therapy (ECT) has been successfully used for decades in the treatment of severe psychiatric disorders, little is known about the underlying brain mechanisms. One of the major risks of ECT that significantly limits its use is an overall memory impairment. Nevertheless, the mechanisms of ECT-dependent memory impairments are not well understood. Here, using a rat model of fear conditioning, we characterize the effects of electroshocks on functional connectivity of brain regions that may underly fear memories.
Methods: All experiments were conducted using Sprague-Dawley rats. On two consecutive days before fear conditioning, subjects were habituated for 10 minutes in the conditioning chamber. On the day of fear conditioning, subjects learned to associate a neutral sound (1-5kHz, 50-80 dB; the conditioned stimulus [CS]) with an electric foot shock (1-s, 0.5-mA; the unconditioned stimulus) delivered through a grid floor. 24 hours after fear conditioning, subjects were returned to the conditioning chamber and exposed to the CS tone a single time to reactivate the fear

memory. Immediately after fear memory reactivation, subjects received either an electroconvulsive shock under anesthesia ($n = 4$) or a sham procedure ($n = 4$). 24 hours later, all subjects were returned to the conditioning chamber and exposed to the CS tone 5 times; subjects were then perfused, and the brain was collected. c-Fos, an immediate early gene commonly used as a measurement of neural activity, was quantified in regions of interest. **Results**: We present preliminary data on the functional connectivity of regions of interest based on c-Fos expression analyzed using graph theory from animals that received an electroshock or a sham procedure: correlation matrices, network visualizations, participation coefficients, and measurements of centrality (i.e., betweenness and degree centrality).

Disclosures: N.D. Horrell: None. **A.M. White:** None. **K. Bonefas:** None. **Y. Nakamura:** None. **B. Savard:** None. **M. Patel:** None. **M. Show:** None. **X. An:** None. **S. Iwase:** None. **M. Zochowski:** None. **J. Debiec:** None.

Digital Abstract Session

P266. Fear Learning and Memory

Program #/Poster #: P266.01

Topic: G.01. Fear and Aversive Learning and Memory

Title: Hypnotic suggestions provide evidence for a causal role of affect sharing in driving observational fear learning

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Abstract: In humans and other social species, fears of and knowledge about what is dangerous and should be avoided are often learned through observing others in distressing situations rather than through one's own experiences, termed observational fear learning. Our aim was to assess the causal role of affect sharing - an important aspect of empathy that describes the ability to partially re-experience what and how another person is feeling - in observational fear learning. N=39 healthy students screened for above-average hypnotic suggestibility (30 female, 9 male, age range 18-24 years) completed an observational Pavlovian fear conditioning paradigm, consisting of a learning stage followed by a test stage. In the learning stage, the participant watched videos of another person - the demonstrator - responding with distress when receiving electric shocks (unconditioned stimulus) paired with a predictive color cue (conditioned stimulus; CS+; a second color served as CS-). In the test stage, an increased skin conductance response (SCR) to the CS+ compared to the CS- in the absence of the demonstrator indexed observational fear learning. Each participant completed this paradigm under two different hypnotic suggestions, which were administered before each learning stage to induce high or low affect sharing with the demonstrator, following a counterbalanced within-subject design. Each suggestion was revoked (so-called "cancellation") after the learning stage to bring the participants back to their habitual level of affect sharing before the start of the test stage. In the

learning stage, high affect sharing resulted in stronger unconditioned skin conductance responses, increased eye gaze toward the demonstrator's face, and higher self-reported unpleasantness while witnessing the demonstrator's distress. In the test stage, participants showed a stronger conditioned fear response (SCR) when they had learned under high, compared to low, affect sharing. These findings demonstrate that affect sharing is causally involved in driving observational fear learning, and thus advance our understanding of the role of empathy in social learning.

Disclosures: **A. Müllner-Huber:** None. **L. Anton-Boicuk:** None. **E. Pronizius:** None. **L. Lengersdorff:** None. **A. Olsson:** None. **C. Lamm:** None.

Digital Abstract Session

P266. Fear Learning and Memory

Program #/Poster #: P266.02

Topic: G.01. Fear and Aversive Learning and Memory

Support: NSERC

Title: More Than Reduplication: Dissociable Electrophysiological markers of First and Higher Order Conditioning

Authors: ***P. DHAMIJA**[1,2], A. WONG[1,2], A. GILBOA[1,2];
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Abstract: Background: Compared to the vast literature exploring the neurobiology of first order conditioning (FOC), relatively little attention is devoted to high order conditioning (HOC), partly because of the misconception that both rely on equivalent stimulus-stimulus associative neural mechanisms. Recent rodent research has demonstrated dissociable FOC and HOC neural substrates, with the hippocampus being required for HOC associations formation and maintenance. In this study, we aimed to determine if this held true for humans with two predictions: (1) The electrophysiological responses of FOC and HOC are spatially and temporally distinct and (2) The hippocampus is involved in higher order associations. Methods: Middle aged adults (40 - 65 years, n = 14) underwent aversive auditory FOC using an aversive burst of white noise as the unconditioned stimuli (US+/US-) and subsequent HOC. Four initially neutral tones were used for FOC (CS+/CS-) and subsequent HOC (HO+/HO-) stimuli. Behavioural and neural responses were indexed by pupil dilation and electroencephalogram responses respectively.Results: Pupillometry demonstrated robust acquisition of FOC and HOC as demonstrated by enlarged pupil size to CS+/HO+ compared to CS-/HO-. Interestingly, while responses to CS+ were extinguished during testing, HO+ responses were maintained suggesting post-learning independence. Evoked response potentials for both CS and HO tones revealed typical cortical auditory components including a clear P1, N2 and P3 at roughly 50 ms, 150 ms, and 250 ms respectively. When comparing CS+ and CS- stimuli, there was a significantly greater

P3b peaking bilaterally over frontoparietal electrodes in response to CS+ tones. By contrast, HO+ was significantly larger as early as the N2 component over central-parietal electrodes as well as greater modulation of P3 over occipitoparietal and N3 over frontal electrodes,. Using source estimation algorithms, medial prefrontal and insula were identified as the source for the significant P3b component in response to CS+, whereas the hippocampus was identified as a major source of neuronal activity for the significant N2 component in response to SO+. Discussion: This experiment demonstrates that first order and higher order conditioning associations have both dissociable patterns of electrophysiological response and sources of activity. Furthermore, it suggests that HOC does not rely on stimulus-stimulus associative neural mechanisms and might instead rely on higher order hippocampally supported associative neural mechanisms potentially involving stimulus-value expectation associations.

Disclosures: P. Dhamija: None. **A. Wong:** None. **A. Gilboa:** None.

Digital Abstract Session

P266. Fear Learning and Memory

Program #/Poster #: P266.03

Topic: G.01. Fear and Aversive Learning and Memory

Support: American Psychological Foundation COGDOP Award
NIH Grant MH121772

Title: The Relationship between Individual Variation in Fear Extinction and Activation of Infralimbic Cortex Afferents

Authors: *A. S. RUSSO, K. COLE, R. G. PARSONS;
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Abstract: Pavlovian fear conditioning occurs when an individual learns to associate an aversive, unconditioned stimulus (US) with a neutral, conditioned stimulus (CS) such that the individual expresses a conditioned fear response (CR) when presented with the CS alone. Extinction is the diminished expression of the CR in response to the CS as a result of multiple presentations of the CS alone. Because it is known that patients diagnosed with posttraumatic stress disorder (PTSD) display poor ability to extinguish conditioned fear, it is of interest to understand which brain circuits are activated during successful extinction, and what goes wrong when extinction is not successful. Previous work has implicated activation of the infralimbic cortex (IL) as important during extinction, yet knowledge about the activity of this brain area in subpopulations of subjects that vary in their ability to extinguish fear responses is lacking. Results from earlier work have shown that there is elevated activation of mitogen-activated protein kinase (MAPK), a protein important for fear and extinction learning, in the IL of rats which display good extinction compared to rats which display poor extinction. However, the cause of IL-hypoactivation in poor-extinguishers remains elusive. It is possible that deficient activation of cells which send projections to the IL is responsible for the lack of IL activity seen in poor-extinguishing

individuals. Using a projection-targeted activity mapping approach, we assessed activation of cells in a range of brain areas which send projections to the IL in rats which display good and poor extinction recall following an extinction recall session and control rats which had either been exposed to a fear recall session or remained in their home cages. AAV-CAG-GFP (Addgene) works as an effective retrograde tracer, producing robust and consistent labeling in areas such as the ventral hippocampus (vHPC), basolateral amygdala (BLA), paraventricular thalamus (PVT), claustrum (CLA), and prelimbic cortex (PL) after being infused in the IL. Furthermore, preliminary results suggest that rats which undergo extinction recall show more activation of cells in the vHPC and PVT compared to control groups. Further analysis will determine whether rats displaying individual differences in extinction recall differ in activation of IL-projecting cells from the aforementioned brain areas. Ultimately, completion of this project may implicate areas which could be targeted for pharmacological treatment of PTSD and help us understand what makes some individuals particularly susceptible to PTSD.

Disclosures: **A.S. Russo:** None. **K. Cole:** None. **R.G. Parsons:** None.

Digital Abstract Session

P267. Cellular and Molecular Mechanisms of Reward

Program #/Poster #: P267.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH DK113170
BrightFocus Foundation
NIH DA047785
NIH AA027172
NIH DA043361

Title: Autophagy regulates cocaine reward behaviors via Becn2 in the dopaminergic system

Authors: Y.-J. KIM[1], Q. KONG[2], K. KURAMOTO[1], M. HUANG[1], H. Y. MELTZER[3], M. XU[2], ***C. HE**[1];
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Abstract: Drug abuse is one of the foremost public health problems of our time. Cocaine is a widely abused drug worldwide, and produces a variety of behaviors, including psychomotor stimulation, reward, craving, and relapse. The mechanisms that underlie cocaine-induced disorders are unresolved and effective treatments are lacking. Here we report the discovery that an autophagy-related protein Becn2 is a novel regulator of cocaine reward behaviors. Global or dopamine (DA) neuron-specific mutations in Becn2 protect mice from cocaine-stimulated locomotion and reward behaviors, as well as cocaine-induced DA accumulation and signaling, due to an increase in presynaptic dopamine receptor 2 (D2R) in DA neurons, an autoreceptor that inhibits DA release. DA neuron-specific re-expression of wild-type Becn2, or D2R antagonism,

in the global Becn2 mutant mice rescues their cocaine-induced behaviors. We further found that Becn2 regulates D2R trafficking, degradation, and cocaine-induced behaviors via interacting with a D2R-bound adaptor protein GASP1. In addition, we found that inactivating Becn2 by upstream autophagy inhibitors stabilizes striatal presynaptic D2R, reduces DA release, and prevents the physiological and behavioral responsiveness to cocaine in normal mice. Thus, we demonstrate that the autophagy protein Becn2 is essential for cocaine psychomotor stimulation and reward through the regulation of DA signaling, and targeting Becn2 by upstream autophagy inhibitors is a potential strategy for preventing cocaine-induced behaviors.

Disclosures: Y. Kim: None. **C. He:** None. **M. Xu:** None. **H.Y. Meltzer:** None. **Q. Kong:** None. **M. Huang:** None. **K. Kuramoto:** None.

Digital Abstract Session

P267. Cellular and Molecular Mechanisms of Reward

Program #/Poster #: P267.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant DA047433
Boettcher Foundation Webb-Waring Award
NARSAD Young Investigator Award

Title: A Role for VTA glutamate and GABA co-releasing neurons in consummatory reward

Authors: *D. MCGOVERN[1], E. PREVOST[1], A. PHILLIPS[2], D. H. ROOT[1];
[1]Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO; [2]Neurobio., Univ. of California Irvine, Irvine, CA

Abstract: Within the last 10 years a unique population of neurons that express the cellular machinery to vesicularly package and release both glutamate and GABA has been identified within the brain's reward center, the ventral tegmental area (VTA). The contribution of these co-releasing neurons to motivated behavior is unclear. These experiments utilized a genetically modified mouse line that expresses **cre** recombinase in neurons with the vesicular glutamate transporter (VGLUT2) and **flp** recombinase in neurons with the vesicular GABA transporter (VGaT). Infusing intersectional viral constructs to record and manipulate **cre and flp** positive neurons allowed for selective monitoring and perturbation of exclusively glutamate and GABA co-releasing neurons within the VTA. Population level changes in intracellular calcium were recorded using a **cre and flp** dependent GCamP6m and fiber photometry to determine the neuronal response to reward by glutamate/GABA co-expressing neurons. Neuronal activity was measured from these neurons when mice consumed various rewarding solutions (fat, sucrose, and saccharine).
Results indicate that although mice display a distinct behavioral preference for fat, neuronal activity is greater for sweeter solutions (sucrose and saccharin) and neuronal activity was blunted by prior feeding. To determine the role of this activity in reward, we injected either a **cre and flp**

dependent inhibitory opsin (iC++) or excitatory channelrhodopsin2 in the VTA to optogenetically silence or excite glutamate/GABA co-expressing neurons during reward consumption. Glutamate/GABA silencing reduced consumption for certain solutions. Optogenetic activation of these neurons selectively increased preference for sweet solutions. Together, these results suggest that VTA glutamate/GABA neurons significantly impact consummatory reward behavior.

Disclosures: D. McGovern: None. **D.H. Root:** None. **A. Phillips:** None. **E. Prevost:** None.

Digital Abstract Session

P267. Cellular and Molecular Mechanisms of Reward

Program #/Poster #: P267.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: OD011132
NIH 5T32NS096050-23
MH117103

Title: The medial orbitofrontal cortex controls value-based action through neurotrophin systems and interactions with the ventral hippocampus

Authors: *E. P. WOON, S. L. GOURLEY;
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Abstract: Value-based decision making, referring to selecting actions based on the value of prospective outcomes, relies on the medial orbitofrontal cortex (MO), yet molecular mechanisms are unclear. One candidate is the Brain-derived Neurotrophic Factor receptor, tyrosine/tropomyosin receptor kinase B (TrkB). Here, we trained mice to respond for 2 distinct food outcomes. We next reduced the value of one outcome using conditioned taste aversion (CTA), then characterized decision-making strategies. We administered a TrkB antagonist during new memory encoding (during the CTA procedure) or during a period of memory retrieval (the choice test). Inhibiting TrkB during the encoding, but not retrieval, of new memories regarding food value impaired the ability of mice to preferentially respond for high-value foods. Overexpression of an inactive isoform of TrkB, *Trkb.t1*, selectively in the MO had the same consequences, particularly when outcomes were unobservable and had to be envisioned. What inputs to the MO might be needed for value memory encoding? We found that ventral hippocampal (vHC)→MO projections are necessary for value memory encoding, serving to transfer value information across contexts (*i.e.*, from the CTA context to the testing context). Overall, these patterns suggest that neurotrophin activity in the MO is necessary for value-based action, as are inputs from the vHC to the MO. Future experiments will determine whether functional vHC MO interactions require TrkB presence.

Disclosures: E.P. Woon: None. **S.L. Gourley:** None.

Digital Abstract Session

P267. Cellular and Molecular Mechanisms of Reward

Program #/Poster #: P267.04

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant 5T32DK098107-04
NIH Grant R01DA038613
NIH Grant R01MH106500

Title: Mitochondria-related nuclear gene expression in reward-related brain regions and blood mitochondrial copy number after high fat diet in male and female C57BL/6 mice

Authors: *C. A. CALARCO[1], S. M. KEPPETIPOLA[1], M. LOBO[2];
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Abstract: Rates of obesity have been steadily on the rise nationally, garnering public health concern. Both diet composition and overall weight impact many aspects of reward related behavior, and obesity is frequently comorbid with both altered reward processing and depression. Diet-induced obesity is associated with several cellular and molecular changes, including altered cellular metabolism and mitochondrial function, impacting neuronal and circuit function. Mitochondrial function in the context of diet-induced obesity has been extensively studied in muscle tissue, pancreas, and other body systems, but less work has been done characterizing the impact of high fat diet (HFD) on mitochondria in the brain, specifically in reward-related brain regions. Mitochondrial function has been shown to play an important role in both cellular activity and structural plasticity. Further, altered mitochondrial function can be sufficient to alter behavior. Here we examine how varying lengths of HFD exposure impact mitochondria-related nuclear gene expression in both male and female C57BL/6 mice and how peripheral changes in blood mitochondria correlate with brain-region-specific gene expression.
Male and female mice were group housed and exposed to HFD (45% calories from fat) or standard lab chow for 24hr, 1wk, 1mo or 3mo. Body weight and food intake were measured, and both brain tissue punches and trunk blood were collected on the final day of exposure. We used quantitative real time PCR (qPCR) to investigate the expression of several nuclear genes involved in mitochondrial function in the nucleus accumbens, dorsal striatum, lateral hypothalamus, and ventral tegmental area. HFD increased body weight in male and female mice at the 24hr and 3mo time points. Weight was also significantly increased in male mice at 1mo. Altered gene expression was observed for all the genes examined, although the patterns of increased or decreased gene expression at different time points in different brain regions were unique between sexes. Interestingly, in both sexes, more genes were significantly altered at the earlier time points (24hr and 1wk) than the later time points (1mo, 3mo), indicating rapid changes that normalize over time. DNA was extracted from whole blood and the relative expression of mitochondrial to nuclear DNA, measured via qPCR, was used as a proxy for mitochondrial copy number. These findings represent an initial probe into mitochondrial molecular mediators in reward-related brain regions after HFD and further studies are needed to

probe how these changes in gene expression correspond to behavioral and cellular changes associated with HFD.

Disclosures: C.A. Calarco: None. **S.M. Keppetipola:** None. **M. Lobo:** None.

Digital Abstract Session

P267. Cellular and Molecular Mechanisms of Reward

Program #/Poster #: P267.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIMH R01MH106500
NIDA R01DA038613

Title: Rhoa overexpression in nucleus accumbens d1-msns lowers motivation for sucrose in male mice and enhances sucrose seeking in female mice.

Authors: *S. L. COLE, M. E. FOX, R. CHANDRA, M. K. LOBO;
Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: RhoA is a GTPase involved in the regulation cytoskeletal, neuronal morphology, and dendritic arborization. Previous research from our lab has demonstrated that enhanced RhoA in nucleus accumbens D1-dopamine receptor expressing medium spiny neurons (D1-MSNs) leads to enhanced dendritic atrophy in promotes depressive-like behaviors. However, whether RhoA impairs or enhances natural reward seeking behavior has been left unexplored. Here, we infused male and female C57BL/6J mice with a retrograde virus expressing Cre recombinase into the ventral tegmental area (VTA) and a Cre-dependent viral vector in NAc to overexpress RhoA or control EYFP in NAc D1-MSNs, and examined whether RhoA regulation impacts operant responding for sucrose pellets. To parse whether enhanced RhoA expression NAc-VTA projecting MSNs/D1-MSNs affects reward learning or motivation, we utilized multiple schedules of reinforcement in both food restricted and *ad lib* fed conditions. Mice were able to choose between 1) a nose poke port producing a light cue or 2) a second nose poke port producing a light cue and sucrose delivery into a food cup. In food restricted conditions (up to 85% of baseline bodyweight), RhoA overexpression produced no alterations in acquisition of sucrose operant behavior and consumption at either FR1 or FR3 schedules of reinforcement , progressive ratio responding or in seeking for sucrose pellets under extinction conditions. However, in *ad lib* fed mice, trending results suggest that at an FR3 schedule of reinforcement, males with enhanced RhoA show lower responding relative to EYFP controls and females with enhanced RhoA show a slight enhancement in responding. Interestingly, RhoA overexpression decreased operant responding during progressive ratio testing in males, but not females. Finally, in seeking tests, where operant responding produces light cues but no sucrose delivery, RhoA overexpression decreased persistent seeking for sucrose reward in males. Conversely, female mice with RhoA overexpression in D1-MSNs showed an enhancement in responding vs female EYFP controls. Thus, enhanced motivation for sucrose in a food restricted state prevents

behavioral effects of enhanced RhoA. Collectively, these preliminary data suggest that RhoA plays a role in motivation for sucrose reward in a sex-dependent manner.

Disclosures: S.L. Cole: None. **M.E. Fox:** None. **R. Chandra:** None. **M.K. Lobo:** None.

Digital Abstract Session

P267. Cellular and Molecular Mechanisms of Reward

Program #/Poster #: P267.06

Topic: G.02. Reward and Appetitive Learning and Memory

Support: University of Utah Health Sciences
University of Utah College of Pharmacy
The ALSAM Foundation

Title: Differences Between Adolescent and Young Adult Rats In Development Of Habitual Control Over Behavior And Immediate Early Gene Expression In Dorsal Striatum

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Abstract: Addiction is thought to reflect habitual control over drug-related behaviors driven by neuroplastic changes in dorsolateral striatum (DLS). Earlier age of initial drug use correlates with propensity for addiction, suggesting that plastic processes in DLS may differ as a function of age. We examined development of habitual control over appetitive instrumental behavior in adolescent vs. young adult rats and, also, expression of *Arc* and *zif268* in DLS and dorsomedial striatum (DMS). Adolescent (post-natal day (PND) 28; n=8) and young adult (~PND105; n=8), male Long-Evans rats were trained to lever press for food reward on random-interval schedules of reinforcement. Upon completion of training, they were tested for goal-directed vs. habitual control over the instrumental behavior using a sensory-specific devaluation procedure. Rats were then sacrificed immediately after extinction testing under devalued conditions, and the brains processed for *Arc* and *Zif268* mRNA expression via RNAScope. Imaging and data analysis were conducted with the experimenters blinded to the treatment group of the animals. All rats acquired lever press responding over the days of training ($p=0.0005$), with PND105 rats pressing more across days of training than PND28 rats ($p=0.05$). Similarly, both ages showed decreased behavior under the devalued test condition ($p=0.01$). PND28 rats pressed significantly more under both valued and devalued conditions than did PND105 rats ($p<0.04$). The ratio of responding under the devalued condition to that under the valued condition, also was significantly higher in PND28 (0.9 ± 0.3) vs. PND105 rats (0.3 ± 0.1; $p<0.03$). There were no differences between the two ages in the number of cells expressing *zif268* mRNA expression in DMS ($p=0.4$) or DLS ($p=0.1$). However, there were significantly greater numbers of *Arc* mRNA-positive cells in DLS ($p<0.04$) and DMS ($p<0.005$) of PND28 vs. PND105 rats ($p<0.04$). Further, the number of cells with *Arc* mRNA expression in DLS was significantly correlated with lever pressing under the devalued condition immediately prior to sacrifice ($r^2=0.33$,

p<0.02), whereas the number of *Arc*-positive cells in DMS (p=0.2) and the number of *Zif268*-positive cells in DMS (p=0.7) and DLS (p=0.7) were not. These data suggest that adolescent rats transition to habitual control over instrumental behavior more readily than do young adult rats. Further, given that correlations between *Arc*mRNA expression in a brain region and behavioral performance reflect task-relevant encoding processes occurring in that brain region, the data suggest that *Arc*-mediated plasticity may contribute to formation of habitual control over behavior.

Disclosures: G. Haws: None. **C. Rice:** None. **J. Aguilar:** None. **T. Moore:** None. **K.A. Keefe:** None.

Digital Abstract Session

P267. Cellular and Molecular Mechanisms of Reward

Program #/Poster #: P267.07

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R01-DA-042057

Title: Fentanyl induces divergent behavioral effects in female and male rats but reduces inflammatory markers uniformly

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Abstract: The synthetic opioid fentanyl has received considerable public attention due to its role in the opioid epidemic and overdose deaths; however, the effects of fentanyl on addiction-related behaviors is not well understood, particularly between sexes. Furthermore, many naturally occurring and semi-synthetic opioids have been shown to possess pro-inflammatory effects which are thought to contribute to the rewarding effects of opioids, but the inflammatory effects of fentanyl are unknown. Thus, the purpose of this study is to assess the sex effects and inflammatory changes following fentanyl administration in male and female rats. Adult female and male Sprague-Dawley rats were subjected to acute or repeated fentanyl hydrochloride administration. Locomotor activity (LMA) and behavioral sensitization in an open field (OF) paradigm and contextual-reward behavior in a conditioned place preference (CPP) paradigm were assessed. In the OF paradigm, animals were given a single (acute) or repeated (once daily for 8 days) fentanyl (20 µg/kg; subcutaneous) injections. On days 1 and 8 of the experiment, LMA in the OF field arena was measured. Brain regions encompassing the mesolimbic circuit were probed for the inflammatory marker (interleukin 6, IL-6) analysis by western blot. In the CPP paradigm, animals were subjected to either 4 or 16 µg/kg fentanyl injections to assess context-associated reward. In the OF paradigm, females, but not males, showed elevated LMA in response to acute and repeated fentanyl exposure (20 µg /kg). In contrast, males showed greater behavioral sensitization only after one week of daily fentanyl administration. Furthermore,

fentanyl administration was shown to lead to decreased inflammatory cytokine (IL-6) levels in regions of the mesolimbic circuit (prefrontal cortex, infralimbic cortex, and nucleus accumbens) in both sexes. In the CPP paradigm, both sexes preferred the contextual cues associated with fentanyl; however, females preferred the 16 μg /kg fentanyl dose, while males preferred both 4 and 16 μg /kg doses. Our results suggest that males and females exhibit divergent behaviors to fentanyl. Females were more acutely responsive albeit lacking a robust behavioral sensitization to daily or alternating fentanyl administration. Males showed more contextual preference than females for a low dose of fentanyl in the CPP paradigm. The uniform decrease in inflammatory load across sexes suggests that the locomotor activating and contextual-rewarding of fentanyl are not dependent on IL-6 based inflammatory response.

Disclosures: **S.A. Perrine:** None. **S. Kallakuri:** None. **A. Gaulden:** None. **N. Burson:** None. **N. Sadik:** None. **I. Ghosh:** None. **S. Khan:** None. **S. Brummelte:** None.

Digital Abstract Session

P268. Neuropharmacology: Decision Making, Catecholamines, Subcortical Circuitry

Program #/Poster #: P268.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH R01MH102456
NSF DGE-1848739

Title: Role of vasopressin signaling in the ventral pallidum in the sex-specific regulation of social play behavior in juvenile male and female rats

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Abstract: Social play is predominantly displayed by juveniles of many mammalian species, including rats and humans, and engagement in social play helps develop social competence throughout life. Children diagnosed with autism spectrum disorder, a sex-biased neurodevelopmental disorder, show decreased involvement in social play, thus emphasizing the need to better understand the neural mechanisms underlying social play in both sexes. We recently showed that vasopressin (AVP) acting in the lateral septum (LS) regulates social play behavior in juvenile rats and that AVP acting in the ventral pallidum (VP) regulates sociosexual motivation in adult rats, with AVP modulating both behaviors in a sex-specific manner. However, whether AVP signaling in the VP has a role in the sex-specific regulation of juvenile social behaviors, like social play, is unknown. Here, we hypothesized that, similar to the LS, AVP in the VP regulates social play in a sex-specific manner. First, in order to examine the organization of the AVP system in the VP in juvenile rats, we examined AVP-immunoreactive (-ir) fiber density and AVP 1a receptor (V1aR) binding density across the anterior to posterior extent of the VP. We found a robust sex difference in AVP-ir fiber and V1aR binding density in

the VP, with denser AVP-ir fibers and denser V1aR binding in juvenile males compared to females. Next, we wanted to determine the effects of pharmacological manipulation of AVP signaling in the VP on social play behavior. Using a specific V1aR antagonist, we found that V1aR blockade in the VP increased social play duration in juvenile male rats but decreased social play duration in juvenile female rats compared to subjects of the same sex who received vehicle infusions. The opposite effect was found when administering synthetic AVP into the VP, with decreased social play duration in juvenile males and increased social play duration in juvenile females compared to subjects of the same sex who received vehicle infusions. These findings demonstrate that, similar to the LS, AVP signaling in the VP inhibits social play in males while it facilitates social play in females. Finally, to start addressing the underlying sex-specific mechanisms, we are currently determining whether social play exposure alters the activation of V1aR-expressing VP neurons in sex-specific ways. Together, these findings start to elucidate the involvement of AVP signaling across a network of brain regions in the sex-specific regulation of social play behavior in juvenile rats.

Disclosures: J.D.A. Lee: None. **C.J. Reppucci:** None. **S.M. Bowden:** None. **E.D.M. Huez:** None. **R. Bredewold:** None. **A.H. Veenema:** None.

Digital Abstract Session

P268. Neuropharmacology: Decision Making, Catecholamines, Subcortical Circuitry

Program #/Poster #: P268.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NWO Vici Award 453-14-005 to RC

Title: Establishing the link between spontaneous eye blink rate and striatal dopamine synthesis capacity

Authors: R. VAN DEN BOSCH[1], J. MAATTA[1], L. BARREIRO FERNANDEZ[2], L. HOFMANS[1], D. PAPADOPETRAKI[1], ***R. COOLS**[1];
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Abstract: Spontaneous eye blink rate (sEBR) has been described in the literature as an index of dopamine transmission in the striatum (Jongkees et al 2016). Work with nonhuman primates has shown that sEBR predicts dopamine levels (Taylor et al., 1999) and dopamine D2 receptor availability (Groman et al., 2014) and work with human volunteers has demonstrated a link with (dopamine drug effects on) reinforcement learning (Slagter et al., 2015). However, there is no direct evidence for a link between dopamine levels and spontaneous EBR in humans. One aim of the present large pharmacological PET study with 100 young healthy volunteers was to establish this link. Specifically, we investigated whether individual differences in sEBR, quantified using electrooculography at rest, can be predicted from striatal dopamine synthesis capacity, measured with [^{18}F]-FDOPA PET. Preliminary analyses suggest a positive association between sEBR

(blinks per minute) and individual variation in ventral striatal dopamine synthesis capacity (see Figure), but additional analyses are required to substantiate this observation. We will additionally explore whether sEBR also predicts individual differences in common dopamine drug effects on reinforcement learning task and associated neural signals in frontostriatal circuitry, measured on separate sessions in the context of this same study. *References:* Groman at al. (2014). Journal of Neuroscience, 34(43), 14443-14454; Jongkees & Colzato (2016). Neurosci Biobehav Rev, 71, 58-82; Slagter et al (2015). Neuropsychologia, 71, 126-132; Taylor et al (1999). Experimental Neurology, 158, 214-220.

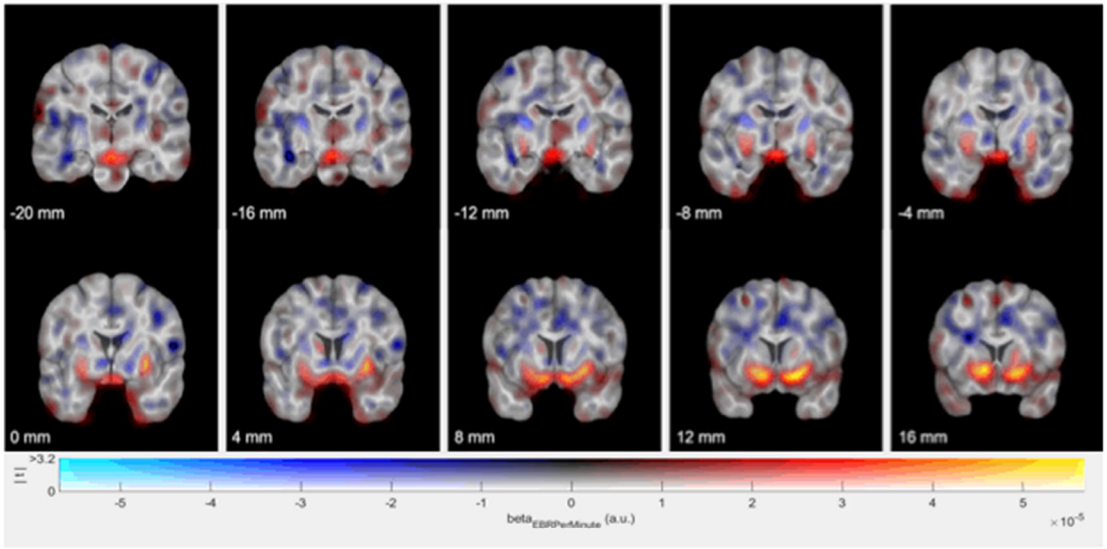

Figure *Voxels showing a positive (red) or negative (blue) regression coefficient on blinks per minute in 92 participants (scored by two independent raters, Cronbach α = 0.98). The z coordinates correspond to the standard MNI brain. Neuroimaging data are plotted using a procedure introduced by Allen et al. (Allen et al., 2012) and implemented by Zandbelt (Zandbelt, 2017).*

Disclosures: **R. van den Bosch:** None. **J. Maatta:** None. **L. Barreiro Fernandez:** A. Employment/Salary (full or part-time):; F.Hoffman-LaRocheLtd. **L. Hofmans:** None. **D. Papadopetraki:** None. **R. Cools:** None.

Digital Abstract Session

P268. Neuropharmacology: Decision Making, Catecholamines, Subcortical Circuitry

Program #/Poster #: P268.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NWO Vici Award 453-14-005 to RC

Title: Striatal dopamine synthesis capacity predicts methylphenidate effect on striatal and prefrontal reward and punishment learning

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Abstract: The psychostimulant methylphenidate is widely used by both patients and healthy people for its enhancing effects on a range of cognitive functions. However, the neural locus of its actions on cognition remains unclear. The effects may reflect direct action on dopamine and noradrenaline transmission in the prefrontal cortex, or modulation of dopamine in the striatum. We tested the hypothesis that methylphenidate acts on the striatum to influence the gating of prefrontal cortical representations in proportion to their association with reward vs punishment. We used fMRI to assess the neural locus of methylphenidate's effects on a reversal-learning task, as a function of variation in striatal dopamine synthesis capacity, measured with [18F]DOPA PET, across 88 healthy participants. Participants predicted whether a stimulus was associated with a reward or punishment outcome, and updated predictions when an unexpected reward/punishment outcome signaled a reversal of contingencies. Outcomes were not contingent on the response, but only signaled the currently relevant stimulus-outcome mapping. We compared methylphenidate's effects with those of the selective dopamine D2/3-receptor antagonist sulpiride to address neurochemical selectivity to dopamine.
Both methylphenidate and sulpiride boosted reward vs punishment-based reversal accuracy to a greater degree in participants with higher striatal dopamine synthesis capacity. Consistent with this behavioral effect, the higher synthesis participants showed greater methylphenidate-induced increases in lateral orbitofrontal BOLD signal, specifically to unexpected rewards vs punishments. Trial-level orbitofrontal BOLD estimates predicted the striatal dopamine-dependent effect of methylphenidate on reversal accuracy. Methylphenidate also increased valence-nonspecific prediction error signals in the striatum and stimulus-specific visual association cortex, but this effect was greater in participants with lower dopamine synthesis. These results reveal a double dissociation: methylphenidate boosted reward vs punishment prediction learning (signals) while attenuating valence-nonspecific striatal prediction error signals to a greater degree in higher-dopamine participants. We hypothesize that these orbitofrontal and striatal effects reflect modulation of a model-based, task-appropriate outcome prediction strategy and a model-free, task-inappropriate winstay-loseshift strategy, respectively. These results concur with the working hypothesis that drugs like methylphenidate counteract biased competition between distinct frontal and striatal learning strategies.

Disclosures: R. Van Den Bosch: None. **B. Lambregts:** None. **J. Määttä:** None. **L. Hofmans:** None. **D. Papadopetraki:** None. **A. Westbrook:** None. **R. Verkes:** None. **J. Booij:** None. **R. Cools:** None.

Digital Abstract Session

P268. Neuropharmacology: Decision Making, Catecholamines, Subcortical Circuitry

Program #/Poster #: P268.04

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant T32 DA007268
NIH Grant R01 DK106188

Title: Input specific enhancements in excitatory transmission in the nucleus accumbens core following junk-food diet consumption

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Abstract: Alterations in brain reward circuits, including the Nucleus Accumbens (NAc), contribute to over-eating and craving that drives the development and persistence of obesity in humans. In rats, cue-triggered food-seeking is enhanced in obesity-prone rats and this behavior is mediated by calcium-permeable AMPA receptors (CP-AMPARs) in the NAc. AMPA-type glutamate receptors provide the main source of excitation to the NAc, a small portion of which are CP-AMPARs under basal conditions. Interestingly, when placed on a sugary, fatty junk-food diet, a rapid and persistent increase in both NAc CP-AMPAR synaptic transmission and surface expression is observed in male obesity-prone, but not obesity-resistant rats. While increases in CP-AMPAR surface expression are observed following consumption of the junk-food diet, increases in synaptic transmission only emerge following a 24-hour deprivation period in which junk-food is removed and replaced with standard chow. While this demonstrates junk-food induced enhancement in glutamatergic transmission in the NAc, it does not provide any information about the specific inputs affected. The NAc receives glutamatergic input from a wide variety of brain regions, including direct connections from the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA). Whether junk-food induced increases in CP-AMPAR transmission are input specific is unknown. To differentiate these inputs, we used whole-cell patch clamp recordings in combination with a viral optogenetic strategy to selectively record responses in either mPFC-to-NAc or BLA-to-NAc inputs. Following stereotaxic injection of pAAV-CamKII-Chronos-GFP into either the mPFC or BLA, we prepare ex vivo brain slices containing the NAc and then record optically-evoked AMPAR currents in medium spiny neurons using whole-cell patch-clamp electrophysiology. The contribution of CP-AMPARs to overall AMPAR transmission was determined using naspm (200 μM), a CP-AMPAR selective antagonist. We found that eating junk-food (10 days) enhanced CP-AMPAR transmission in mPFC-to-NAc inputs, whereas preliminary results in BLA-to-NAc inputs show no change in CP-AMPARs. Thus, initial results suggest that junk-food induced enhancements in CP-AMPARs occur preferentially at mPFC inputs. Therefore, junk-food consumption may alter mPFC function, but not BLA function, in a manner that may promote CP-AMPAR insertion. Others have shown alterations in the mPFC following high-fat diet consumption, including both changes

in perineuronal nets and spine density, suggesting that changes in NAc transmission may be downstream of these effects.

Disclosures: T.L. Fetterly: None. **C.R. Ferrario:** None.

Digital Abstract Session

P268. Neuropharmacology: Decision Making, Catecholamines, Subcortical Circuitry

Program #/Poster #: P268.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: CIHR project grant to Catharine Winstanley (PJT-162312)
UBC Department of Psychiatry salary support to Tristan Hynes
NSERC USRA Award to Chloe Chernoff

Title: Noradrenergic ADHD medications improve decision making and impulse control in male and female rats when rewards are paired with salient audiovisual cues

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Abstract: The flashing lights and sounds of modern casinos are alluring and may contribute to theaddictive nature of gambling. Such cues can profoundly influence the noradrenaline (NA)system, suggesting a viable therapeutic target for gambling disorder (GD). While there issubstantial evidence to support the involvement of NA in the impulsive symptoms of GD, itsfunction in mediating the “pro-addictive” impact of cues is less understood. We wished toinvestigate the role of NA in our rodent assay of risky decision making and impulsivity, the cuedrat gambling task (crGT). Given the pronounced sex differences in addiction disorders and themonoaminergic regulation of behaviour, we also prioritised evaluating noradrenergic drugs inboth sexes. Female and male rats were trained to stability on the crGT and then givenintraperitoneal injections of the noradrenaline reuptake inhibitor atomoxetine, the α2A receptoragonist guanfacine, the beta receptor antagonist propranolol, and the α2 receptor antagonistyohimbine. Atomoxetine dose-dependently improved decision making score irrespective of sexor risk-preference. Guanfacine selectively enhanced decision making in risk-preferring malesand optimal-performing females. Propranolol and yohimbine had no effect on decision making.Atomoxetine and guanfacine reduced premature responses, while yohimbine bi-phasicallyaffected this index of motor impulsivity. These results support the hypothesis that NA is animportant neuromodulator of the cue-induced deficits in decision making observed in laboratory-based gambling paradigms, and suggest that NAergic drugs like atomoxetine and guanfacinemay be useful in treating GD.

Disclosures: C.S. Chernoff: None. **T.J. Hynes:** None. **C.A. Winstanley:** None.

Digital Abstract Session

P268. Neuropharmacology: Decision Making, Catecholamines, Subcortical Circuitry

Program #/Poster #: P268.06

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R01DK106188
NIH Grant T32 DA7281

Title: Effects of Junk-Food Diet on NAc Glutamatergic Transmission and Intrinsic Excitability in Feamles

Authors: *A. M. NIETO[1], A. M. FRANCE[1], H. PAPACOSTAS QUINTANILLA[1], Y. ALONSO-CARABALLO[3], C. R. FERRARIO[1,2];
[1]Dept. of Pharmacol., [2]Psychology Dept., Univ. of Michigan, Ann Arbor, MI; [3]McLean Hosp., Harvard Med. Sch., Bellmont, MA

Abstract: The nucleus accumbens (NAc) plays critical roles in motivated behaviors, including food-seeking in response to Pavlovian cues. Eating diets high in fats and sugars (i.e., junk-food) produces alterations in NAc function in males and enhances cue-triggered approach. However, few studies have examined the effects of junk-foods on NAc function in females. Activity of medium spiny neurons (MSN), the major output neurons of the NAc, is influenced by excitatory glutamatergic transmission and by intrinsic properties of MSNs that govern their firing. The goal of the studies presented here was to examine the effects of junk-food on NAc excitatory transmission and MSN intrinsic excitability in females. Obesity-prone female rats were given a junk-food diet (10 days) followed by a return to ad lib standard chow for either 24-72 hours (short junk food deprivation) or 14-16 days (long junk-food deprivation) to examine persistence of effects. Controls remained on standard chow throughout. Whole-cell patch clamp recordings from ex vivo brain slices were used to measure effects of junk-food on intrinsic excitability and AMPA receptor-mediated transmission. The estrus cycle was monitored throughout and slices were prepared when females were in the metestrus/diestrus phase of the cycle because this is when food intake and cue-triggered motivation for food are highest. Junk-food consumption followed by a short deprivation period did not alter MSN intrinsic excitability compared to chow fed rats. However, this did increase the amplitude of spontaneous excitatory post-synaptic current (sEPSC) in these same cells. In addition, preliminary data suggest that this effect is not found after long junk-food deprivation. These effects are in striking contrast to effects in males given the same junk-food diet exposure, where junk-food reduces MSN excitability, and increases AMPAR transmission, with both effects persisting throughout prolonged junk-food deprivation. Thus, females appear to be protected from the long-lasting effects of junk-food consumption compared to males. Future studies will investigate the effect of junk-food on D1- and D2-type MSNs as well as how sex hormones may influence the effects of junk-food on NAc plasticity in females.

Disclosures: **A.M. Nieto:** None. **A.M. France:** None. **H. Papacostas Quintanilla:** None. **Y. Alonso-Caraballo:** None. **C.R. Ferrario:** None.

Digital Abstract Session

P268. Neuropharmacology: Decision Making, Catecholamines, Subcortical Circuitry

Program #/Poster #: P268.07

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01-DK106188
R01-DK115526

Title: Effects of intra-NAc insulin on motivation for food

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Abstract: Insulin receptors are expressed throughout the adult brain, and insulin from the periphery has widespread distribution in the central nervous system. In humans and rodents, actions of insulin in the brain decrease food intake. In addition to these behavioral effects, recent studies have shown that insulin can affect dopamine and glutamate transmission within the nucleus acumbens (NAc). The NAc plays critical roles in food-seeking and feeding, behaviors that are mediated by both dopamine and glutamate. However, the effect of insulin on cue-triggered food-seeking (i.e., "craving" that drives the development of obesity) or motivation for food mediated by the NAc were unknown. Therefore, the current study examined the effects of intra-NAc insulin on motivation for food and cue-triggered food-seeking in adult male Sprague Dawley rats by assessing break point using a progressive ratio schedule and Pavlovian conditioned approach, respectively. Direct infusion of 60 uM insulin (0.5 uL/min) into the NAc did not affect conditioned approach behavior compared to vehicle infusion (ACSF). In contrast, intra-NAc insulin significantly reduced break point compared to vehicle infusion. In addition, home cage food intake was measured for 1 hour following testing for cue-induced food seeking and break point. On both testing days, insulin reduced home cage food intake, suggesting that the absence of effects on conditioned approach are not due to ineffective infusion in these rats. Taken together, these data show that insulin within the NAc does not alter the incentive-motivational properties of a food cue, but instead reduces the motivation to obtain food and food intake. This indicates a dissociation between the regulation of the motivation to consume food vs food seeking by insulin. This not only has implications for the regulation of food-seeking and eating, but may also be important in the dysregulation of feeding that accompanies obesity. Finally, ongoing studies to determine the role of insulin receptors and insulin like growth factor receptors (which can also be activated by insulin) will shed light on the specific mechanisms mediating these effects of intra-NAc insulin on motivation for food.

Disclosures: **J.E. Finnell:** None. **C.R. Ferrario:** None.

Digital Abstract Session

P268. Neuropharmacology: Decision Making, Catecholamines, Subcortical Circuitry

Program #/Poster #: P268.08

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NWO Vici Award 453-14-005 to RC
NIMH F32MH115600 to AW
NIMH R01 MH080066 to MJF

Title: Striatal dopamine mediates relative contributions of reinforcement learning and working memory during task learning

Authors: *A. WESTBROOK[1,2], R. VAN DEN BOSCH[2], J. MÄÄTTÄ[2], D. PAPADOPETRAKI[2], L. HOFMANS[2], R.-J. VERKES[3], M. FRANK[1,4], R. COOLS[2,3];
[1]Brown Univ., Providence, RI; [2]Donders Inst. for Brain, Cognition and Behaviour, Nijmegen, Netherlands; [3]Psychiatry, Radboud Univ. Med. Ctr., Nijmegen, Netherlands; [4]Carney Inst. for Brain Sci., Providence, RI

Abstract: Learning a new task involves slow, incremental reinforcement learning and fast, flexible, albeit capacity-limited working memory. In simpler learning tasks, working memory plays a bigger role: maintaining recent information and supporting predictions. In more complex tasks - especially those exceeding working memory capacity - people rely relatively more on reinforcement learning. Striatal dopamine has been implicated in both working memory and reinforcement learning processes, raising the prospect that striatal dopamine might mediate the relative contributions of each to task learning.
To test this hypothesis, 100 healthy young adult humans were recruited to perform the Reinforcement Learning Working Memory task (RLWM; Collins and Frank, 2012 Eur J Neurosci; 2018 PNAS), after taking either the dopamine reuptake inhibitor methylphenidate, the D2-antagonist sulpiride, or placebo. We further used [18F]-fluorodopa PET imaging to quantify participants' dopamine synthesis capacity. As expected, we find that participants relied relatively more on reinforcement learning for more demanding task levels, and relatively more on working memory on less demanding levels.
We also find that striatal dopamine influenced both the efficacy and relative contributions of these learning mechanisms. Specifically, both higher dopamine synthesis capacity, and methylphenidate versus placebo sped learning, while sulpiride slowed learning. The specific pattern of correct and incorrect responses suggests that these effects reflect modulation of distinct strategies. Methylphenidate boosted the efficacy of reinforcement learning, while sulpiride attenuated the efficacy of working memory Furthermore, individuals with higher dopamine synthesis capacity had more stable working memory contents over time and relied on working memory to a greater extent, while relying less on reinforcement learning. In addition, we found that while participants treated more demanding task levels as more effort-costly, methylphenidate blunted this effect, consistent with our prior work showing that methylphenidate can boost cognitive motivation by making people less sensitive to effort costs.

These findings demonstrate that striatal dopamine alters the relative reliance on working memory or reinforcement learning for task learning. Future pharmacological-imaging work is required to isolate the neural locus of the dissociable effects of dopamine synthesis capacity, methylphenidate, and sulpiride.

Disclosures: A. Westbrook: None. **R. van den Bosch:** None. **J. Määttä:** None. **D. Papadopetraki:** None. **L. Hofmans:** None. **M. Frank:** None. **R. Cools:** None. **R. Verkes:** None.

Digital Abstract Session

P268. Neuropharmacology: Decision Making, Catecholamines, Subcortical Circuitry

Program #/Poster #: P268.09

Topic: G.02. Reward and Appetitive Learning and Memory

Support: CIHR

Title: Investigating the effects of ropinirole on risk preference in female rats

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Abstract: Ropinirole is a D2/3 receptor agonist drug commonly prescribed to Parkinson's disease (PD) patients to alleviate motor symptoms. However, a significant minority of patients who take this drug go on to develop gambling disorder or other impulse control disorders (ICDs). Previous research using rodent models of gambling has indicated that chronic ropinirole treatment increases risky decision making and risk preference regardless of baseline preference for risk. This research has largely been focused on males, as the incidence of PD and ICDs are higher in males. The effects of chronic ropinirole administration on risky decision making in females has yet to be established. Accordingly, the object of the present experiment was to determine whether chronic ropinirole administration increases risk preference in female rats during acquisition of the rat gambling task (rGT). A cohort of 48 female Long-Evans rats (4 months old) were trained on the cued version of the rGT which is a rodent adaptation of the Iowa Gambling task commonly used in humans. In both tasks, optimal behaviour is achieved by preferentially selecting the two low-risk, low-reward options over the high risk, high reward options. In the rGT, selection of the low-risk, low-reward options results in fewer time-out penalties and more sugar-pellet rewards earned overall. The presence of reward-paired audiovisual cues in the cued version of the task reliably increases the number of risk-preferring rats. Ropinirole was delivered via osmotic mini pump at 0(saline), 2.5 or 5mg/kg per day (n=16 per group). Mini pumps were surgically implanted prior to training on the rGT and allowed for slow, continuous release of drug over a period of 28 days. Following two weeks of drug administration, results do not show the expected increase in risky decision making previously observed in male rats. Results from this experiment offer further insight into the role of

dopaminergic transmission in decision making involving risk and uncertainty and provide evidence for potential sex differences in the effects of ropinirole on risky decision making.

Disclosures: H. Brodie: None. **B. Russell:** None. **C. Winstanley:** None.

Digital Abstract Session

P269. Human Reward and Appetitive Learning and Memory: Adversity and Value-Based Decision Making

Program #/Poster #: P269.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: COBRE Grant

Title: Childhood Adversity Diminishes Confidence in Value-Based Decisions

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Abstract: Title: Childhood Adversity Diminishes Confidence in Value-Based Decisions
Authors: TS Mullins, DF Aragon, J Romero, K Rogge-Obando, E Eversole, VD Costa, & J Hogeveen
Abstract: Human development benefits from a highly stable reinforcement environment. Individuals exposed to a significant number of adverse childhood experiences (ACEs) are likely to come from households with highly unstable reward environments, which is likely to have a deleterious impact on the development of confidence during value-based decision making. While it is well established that ACEs drive elevated risk for psychopathology in adulthood, the mechanisms driving this risk remain unclear. Here, we test the hypothesis that adults with prior exposure to ACEs have decreased confidence in value-based decisions, and we investigated value-based decision confidence N=38 adult participants with varying exposure to ACEs (range: 0-10 on the ACEs Checklist) completed a reinforcement learning task. Individuals also rated the confidence associated with each decision prior to feedback. Optimal task performance was modeled as a Partially Observable Markov Decision Process (POMDP), which generated trial-by-trial estimates of the immediate expected value (IEV) associated with each choice option. Typically, we expect increases in IEV (a more rewarding stimulus) to be accompanied by increases in confidence in the choice. We evaluated the potential moderating effect of ACEs on the relationship between decision confidence and IEV. Across the sample, there was a significant association between trialwise confidence and IEV. Specifically, participants were more confident when making the choice option had a higher predicted IEV. Critically, ACEs moderated this association: individuals who experienced two or more ACEs in childhood had a reduced association between confidence and IEV relative to individuals with minimal exposure to ACEs during childhood, despite having intact IEV learning. These results provide evidence for

a potential cognitive mechanisms underlying impaired reward-guided decision making in individuals with high exposure to ACEs. Individuals with higher ACEs may not feel confident in learned reward probabilities, likely due to experienced instabilities in the early reward environment. This disruption may persist when reward probabilities are more predictable, which may increase vulnerability to developing psychopathology related to reward-based decision making.

Disclosures: T. Mullins: None. **D.F. Aragon:** None. **J. Romero:** None. **K. Rogge-Obando:** None. **E. Eversole:** None. **J. Hogeveen:** None. **V.D. Costa:** None.

Digital Abstract Session

P270. Impulsive Behavior and Inhibitory Control: Animal Models

Program #/Poster #: P270.01

Topic: H.04. Executive Functions

Support: MOST 109-2420-H-004-021

Title: Individual differences in the impulsive action on two timing-related operant behaviors are correlated in rats

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Abstract: Operant behavior performed on a differential reinforcement of low-rate-response (DRL) task has been used to characterize impulsive action and further to probe the trait impulsivity involved in this domain of impulsive behavior. Like DRL behavior, the operant behavior maintained on a fixed-interval (FI) schedule requires a timing process in the reinforcement contingency. However, behavioral inhibition is required in the performance of DRL behavior, whereas it is not in the FI behavior. To date, little is known whether there is inter-individual variability in FI behavior as compared to DRL behavior. In the present study, a cohort of rats (n=34) were first trained on a FI 30 sec (FI 30-s) schedule for 21 days. With the measure of post-reinforcement pause (PRP), the subjects were classified into three groups as high- (n=9), intermediate- (n=16), and low- (n=9) impulsivity by quartile. Subsequently, these rats were subjected to acquire a DRL 10-s and then a DRL 20-s task (each for 5 days), which measurement of the response efficiency was used to delineate the individual difference in this task. The results showed individual differences existed in FI 30-s behavior as revealed by significant differences between three groups in the 6th to 10th 3-sec bins of the inter-response-time distribution curve within a 30 sec interval. Moreover, the mean PRP was significantly lower in the high-impulsive group than the low-impulsive one. The DRL data were separately analyzed and confirmed the individual differences manifested in either DRL 10-s or DRL 20-s task by showing the significant between-group difference on the response efficiency. Namely, a lower efficiency ratio appeared in the high-impulsive group and vice versa for the low-impulsive group. Correlational

analysis was further conducted to determine the behavioral traits that might be associated with the FI and DRL tasks. Surprisingly, there was a significant positive correlation between the PRP of FI 30-s and the response efficiency of DRL 10-s, and so to the DRL 20-s task. In summary, the trait impulsivity observed in the FI and DRL tasks may be attributed to the behavioral component of timing similarly involved in these two tasks.

Disclosures: Y. Yang: None. **S. Chen:** None. **N. Yen:** None. **R. Liao:** None.

Digital Abstract Session

P270. Impulsive Behavior and Inhibitory Control: Animal Models

Program #/Poster #: P270.02

Topic: H.04. Executive Functions

Support: NIH Grant DA041708
NIH Grant DA047610
NIH Grant GM103436

Title: Early-life risperidone increases preference for small, immediate rewards in adulthood.

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Abstract: Antipsychotic drugs are given to children to alleviate various psychiatric disorders even though there is little research on their impact on brain development and behavior. This study determined if early-life administration of risperidone, the most widely prescribed antipsychotic drug in children, led to changes in impulsive behavior, as assessed through delay discounting, during adulthood. Long-Evans rats were administered vehicle or risperidone (3.0 mg/kg, sc) daily for a four-week period beginning on postnatal day 14 (n = 14 rats per drug, 7 females and 7 males). Four weeks later, rats were acclimated and trained in operant chambers and thereafter blindly assessed in a delay discounting task for five weeks. Rats received one pellet immediately if they chose one lever or four pellets after delays of 5, 10, 20, or 40 seconds if they chose the other lever. Delays increased across blocks of trials within each daily session. During the second week of testing, there was a significant difference between the rats that received risperidone and the rats that received the vehicle treatment. Rats that received risperidone were more likely to choose the small immediate reward during the 40 second delay trials. There were no sex differences in choice behavior in the task. These results indicate that risperidone administration during development can lead to more impulsive behavior during adulthood. Such outcomes raise concerns about the long-term effects of risperidone administration on disorders linked with poor impulse control such as substance abuse.

Disclosures: S.A. Steffen: None. **T. Downnen:** None. **K. Dolezal:** None. **K. Thompson:** None. **N. Nail:** None. **J.R. Yates:** None. **M.E. Bardgett:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.01

Topic: G.03. Motivation

Support: R00 DA042895
T32 DA007234

Title: Parallel and serial dopamine circuit control of conditioned behavior during Pavlovian learning

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Abstract: Environmental cues, through Pavlovian learning, become conditioned stimuli that guide animals toward the acquisition of rewards (for example, food) by invigorating and directing seeking behavior. We have previously shown that brief optogenetic excitation of dopamine neurons, in temporal association with visual sensory cues, can instantiate those cues as conditioned stimuli that evoke conditioned movements. It remains unclear 1) how dopamine-neuron mediated, cue-evoked behavior is signaled by dopamine release downstream in striatal subregions and 2) how subregional signals evolve across stages of learning. Here, we made use of a genetically encoded dopamine biosensor (dLight) to monitor dopamine signaling simultaneously in multiple striatal regions with fiber photometry, while tracking detailed movement features, during optogenetic Pavlovian cue conditioning of VTA or SNc dopamine neurons. Our results demonstrate a serial pattern of cue-evoked dopamine signaling across ventral to dorsal striatal subregions that correlates with different features of cue-evoked behavior. Cues paired with optogenetic activation of VTA dopamine neurons evoked dopamine release in the nucleus accumbens core relatively early in training, when behavioral responses were slower and directed toward the cue. This pattern evolved as conditioning progressed. Late in training, cue-evoked signals emerged in the dorsolateral striatum, as movement patterns became more vigorous and not directed at the cue. Cues paired with optogenetic activation of SNc dopamine neurons did not reliably evoke dopamine release in the dorsolateral striatum, even though those cues spurred vigorous movements. Together our studies show dissociable, parallel functions for ventral and dorsal striatal dopamine signaling in guiding versus invigorating behaviors. Further, they suggest that large-scale coordination of signaling across ventral-to-dorsal striatal dopamine networks emerges during Pavlovian learning to coordinate behavioral diversity.

Disclosures: L. Engel: None. **A. Wolff:** None. **S. Sinha:** None. **V. Collins:** None. **B. Saunders:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.02

Topic: G.03. Motivation

Support: NIH Grant F31DA046136
NIH Grant R01DA035943
NIH Grant R00DA042895

Title: Hierarchical regulation of mesolimbic dopamine and striatal encoding of reward-paired cues governs behavioral flexibility

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Abstract: The ability to resolve uncertainty surrounding reward-associated cues is essential for the proper organization and generation of reward-seeking. Dopamine neurons are reported to be sensitive to the likelihood of reinforcement yet how dopamine neurons, dopamine release, and the activity of their striatal targets are related to the coding and resolution of uncertainty is unclear. Here we make use of pharmacology, *in vivo* electrophysiology, calcium imaging, and optogenetics to determine the contributions of the mesolimbic dopamine system to the hierarchical organization of reward-seeking. Long-evans rats were trained to discriminate when a conditioned stimulus would be followed by sucrose reward by exploiting the prior and non-overlapping presentation of a separate discrete stimulus, an occasion setter. Only when the occasion setter's presentation preceded the conditioned stimulus did the conditioned stimulus predict sucrose delivery. We found that either reversible inactivation or dopamine antagonism within the nucleus accumbens prevented rats from properly estimating when the conditioned stimulus would be rewarded. Optogenetic inhibition of dopamine neurons in TH-Cre transgenic rats during either the conditioned stimulus or the occasion setter prevented rats from utilizing the occasion setting cue to increase their reward-seeking when appropriate. We recorded single neurons within the nucleus accumbens (n=235) and observed that the magnitude of conditioned-stimulus evoked inhibitions were greater when the conditioned stimulus would be followed by reward, than when it would not. Moreover, we observed a population of neurons in the nucleus accumbens that dynamically altered their firing to the conditioned stimulus, being excited when this cue was not predictive and inhibited when it would predict reward. We monitored dopamine release in the nucleus accumbens making use of fiber photometry and the fluorescent dopamine sensor dLight 1.3b. Dopamine release tracked the presentation of the occasion setter and dynamically scaled when the conditioned stimulus would or would not predict reward predicated on the prior presence or absence of the occasion setting cue. Together these results reveal a mechanism for dopamine neurons and the effects of dopamine release in the nucleus accumbens to dynamically control striatal encoding of ambiguous reward-predictive cues and appropriately generate reward-seeking.

Disclosures: **K.M. Fraser:** None. **V. Collins:** None. **F. Pat:** None. **P. Janak:** None. **B.T. Saunders:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.03

Topic: G.03. Motivation

Support: NIH Grant F32 MH122192
NIH Grant R01 DA036612

Title: Mesoaccumbal glutamate/dopamine projections require glutamate release to promote positive reinforcement

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Abstract: Ventral tegmental area (VTA) projections to the Nucleus Accumbens medial shell (NAc) drive reward-related motivation and positive reinforcement. Although primarily composed of dopamine neurons, a subset of mesoaccumbal dopamine projections also express the type 2 vesicular glutamate transporter (VGLUT2) and release glutamate at NAc terminals. Optogenetic stimulation of VGLUT2-expressing VTA neurons promotes positive reinforcement, showing characteristics distinct from typical dopamine-mediated reinforcement such as preference for shorter bursts of stimulation rather than longer ones. Further, concomitant dopamine release by VGLUT2-expressing neurons is not required for positive reinforcement, suggesting glutamate release is primarily responsible for this distinct signature of positive reinforcement. Here we asked whether glutamate release by VTA glutamate neurons, including those that co-release dopamine (or GABA), is necessary for promoting positive reinforcement. We expressed Cre-dependent Channelrhodopsin (ChR2) in VTA of male and female VGLUT2-Cre mice, in combination with either a Cre-dependent CRISPR/Cas9 AAV designed to induce indel mutations in *Slc17a6* (VGLUT2) or a control AAV. We found that disruption of VTA VGLUT2 effectively eliminated optogenetically triggered excitatory postsynaptic currents in NAc. Furthermore, disruption of glutamate release from VTA neurons abolished optogenetic self-stimulation in both nose-poke self-stimulation and real-time place tasks. Operant reward seeking for natural food rewards was left intact, suggesting disruption of VTA glutamate does not interfere with basic reward learning. Our results suggest that VTA glutamate neurons mediate positive reinforcement, which contributes to a more complex understanding of mesoaccumbal circuitry involved in addiction and other motivational disorders.

Disclosures: **S.M. Warlow:** None. **V. Zell:** None. **A.C. Hunker:** None. **L.S. Zweifel:** None. **T.S. Hnasko:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.04

Topic: G.03. Motivation

Support: K99-DA045765
R01-DA006214-25S1
Rutgers BHI Pilot Grant

Title: Orexin (hypocretin) input to ventral tegmental area modulates palatable food-seeking behavior in female rats with a history of obesity and binge-like eating

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Abstract: Introduction: Binge eating disorder (BED) is characterized by recurrent episodes of palatable food consumption with a concomitant perceived loss of control, and often presents comorbidly with obesity. Studies using animal models of addiction implicate projections of hypothalamic orexin (hypocretin) cells to ventral tegmental area (VTA) in regulating reward behavior; this signaling is hypothesized to be primarily mediated via actions at orexin-1 receptor (Ox1R). Here, we assessed the role of this orexin-VTA pathway, as well as VTA Ox1R signaling, in pathological food-seeking behaviors using a rat model of BED. **Methods**: Lean (chow, ad libitum) and obese (high fat diet; HFD; 45% fat, ad libitum) female Long-Evans rats were given access to sweetened fat (vegetable shortening/10% sucrose) for 30 minutes twice a week over 4 weeks. Rats were next tested for motivation for palatable food (sucrose pellets) on a behavioral economics assay. In Group 1 (n=32; 16/group), brain tissue was collected and processed for orexin immunoreactivity in VTA. In Group 2, rats received intra-VTA microinjections of a retrograde AAV containing either an orexin shRNA (n=9) or a control scrambled shRNA (n=8), before being exposed to the binge paradigm and tested for food motivation as above. In Group 3 (n=24), Ox1R was knocked down in one hemisphere of VTA, and TH neurons were silenced in the other. Rats were then exposed to the binge paradigm and tested for food motivation as above. **Results**: Obese, but not lean rats exhibited enhanced motivation for sucrose post-binge. This was associated with a corresponding increase in orexin terminal density in VTA in these rats. Rats that received intra-VTA orexin shRNA failed to escalate their binge intake and did not exhibit a post-binge increase in food demand. Contralateral knockdown of Ox1R and silencing of DA neurons in VTA blocked the enhancement of food motivation following binge; this was not observed in rats that received unilateral injections of either virus. **Conclusions**: These data indicate that orexin signaling in VTA, specifically at Ox1R, contributes to enhanced food-seeking behavior in obese rats with past binge experience. Together, these data highlight the orexin system as a potential therapeutic target for BED.

Disclosures: **J.B. Mehr:** None. **S. Liu:** None. **K. Fernandopulle:** None. **S. O'Connor:** None. **H.E. Bowrey:** None. **N.T. Bello:** None. **G. Aston-Jones:** None. **M.H. James:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.05

Topic: G.03. Motivation

Support: NSERC DG -343012 / DAS-04060

Title: Optogenetic Stimulation of Lateral Hypothalamic Orexin/Dynorphin Inputs to the Ventral Tegmental Area Potentiates Mesolimbic Dopamine and Promotes Reward-related Behaviours

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Abstract: Reward-related processes are key to species survival. These processes rely heavily on mesolimbic dopamine (DA) neurotransmission to direct motivated behaviours to acquire rewards. One important neuromodulatory source to the ventral tegmental area (VTA) dopamine neurons is the lateral hypothalamic neuropeptide orexin-containing (Ox) neurons. Ox in the VTA is necessary for numerous reward-related processes including conditioned behaviours. Further, exogenous application of Ox increases the firing rate of VTA DA neurons projecting to the nucleus accumbens, a key node in the brain's reward circuitry. However, the impact of endogenous Ox on mesolimbic DA neurotransmission and reward related behaviours are not known. Furthermore, Ox is co-released with the inhibitory neuropeptide dynorphin (Dyn; $LH_{Ox/Dyn}$). The contributions of endogenous $LH_{ox/dyn}$ to modulation of mesolimbic DA and motivated behaviour is not fully understood. As such, we infused male and female Ox-cre mice with cre-dependent channelrhodopsin or control (mCherry) virus. Next, we implanted optical fibres targeting the VTA. We found that optical stimulation of $LH_{Ox/Dyn}$ in the VTA in one half of a 2-chamber box produced real-time and conditioned place preference. This effect was attenuated by antagonism of Ox receptor 1 (OxR1). We next assessed the involvement of optical stimulation of $LH_{Ox/Dyn}$ in the VTA in a Pavlovian food conditioning task. We found that stimulation of $LH_{Ox/Dyn}$ terminals in the VTA increased cue-directed motivated behaviour but did not produce conditioned reinforcement. Finally, we used anaesthetized fast-scan cyclic voltammetry combined with optical stimulation of $LH_{Ox/Dyn}$ in the VTA to show that Ox release in the VTA potentiates electrically evoked mesolimbic DA. This effect was attenuated by antagonism of OxR1. Together these findings demonstrate a robust role for Ox in the VTA in modulating mesolimbic DA neurotransmission and in sculpting various reward-related behaviours. These findings provide significant support for examining the role of $LH_{Ox/Dyn}$ in states of aberrant motivation, such as addiction.

Disclosures: **C.S. Thomas:** None. **M. Rana:** None. **A. Mohammadkhani:** None. **M. Qiao:** None. **S.L. Borgland:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.06

Topic: G.03. Motivation

Support: NSERC DG -343012 / DAS-04060

Title: Projection target defined effects of endogenous orexin and dynorphin co release on activity of ventral tegmental area dopamine neurons

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Abstract: Dopamine neurons in the ventral tegmental area (VTA) respond to motivationally relevant cues and are key targets of addictive drugs; circuit-specific signaling of these neurons drives different aspects of motivated behavior. Orexins (ox; also known as hypocretin) and dynorphin (dyn) are co-expressed lateral hypothalamic (LH) neuropeptides that project to VTA. While LHox promotes drug-seeking behavior, dynorphin inhibits drug-seeking behavior. Furthermore, these peptides have opposing effects on the firing activity of VTA dopamine neurons. Previous work in our lab demonstrated that exogenously application of ox and dyn, modulate non-overlapping VTA dopaminergic projections. Specifically, exogenous application of orexin potentiates firing of dopaminergic neurons that project to the nucleus accumbens (DA-NAc), but not the basolateral amygdala (DA-BLA), whereas dynorphin inhibits firing in subpopulations of dopamine neurons in both projections. However, it is unknown if dynorphin inhibition of these circuits in opposition to LHox is driven by the LHox/dyn input, rather than other sources. This study sought to determine the effects of endogenous LHox/dyn release on DA-BLA and DA-NAc VTA neurons in mice. We expressed channel rhodopsin2 selectively in LHox/dyn neurons and photostimulated terminals in the VTA while recording VTA neuronal firing using combination of circuit tracing and patch clamp electrophysiology. VTA dopamine neurons were labeled with biocytin during recordings and posthoc imaged for tyrosine hydroxylase expression. We showed a diverse response of LHox/dyn photostimulation on dopamine neuronal firing rate. Photostimulation of LHox/dyn inputs in the VTA inhibited firing of the majority of DA-BLA neurons. However, photostimulation of LHox/dyn inputs in the VTA both increased and reduced firing of dopamine neurons that project to either the lateral or medial nucleus accumbens shell (DA-lAcbSh, DA-mAchSh). SB334687, an ox1 receptor inhibitor or NorBNI, a kappa receptor inhibitor reversed the potentiation or inhibition of firing, respectively. Furthermore, these effects on firing were not driven by synaptic glutamate or GABA release. Our findings provide evidence that LHox/dyn corelease may tune the output of the VTA by simultaneously inhibiting and activating different VTA projection neurons that are distinct in their electrophysiological properties and contribute to different aspects of reward seeking.

Disclosures: **S.L. Borgland:** None. **A. Mohammadkhani:** None. **M. Qiao:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.07

Topic: G.03. Motivation

Support: CIHR
BBRF
DFG

Title: Hippocampal neurogenesispromotes effortful responding but does not regulate effort-based choice

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Abstract: One brain region that has been implicated in the pathology of depression is the hippocampus. Interestingly, in humans, it is the brain region that is capable of generating new neurons throughout life. To date, little work has thoroughly examined how a reduction in new neurons modulates the hippocampal network during behavior and which of the complex behavioral traits of depression are caused by reductions in neurogenesis. One of the hallmarks of depression is a lack of motivation and energy to pursue goals. When patients are given a choice, they are more likely to choose rewards that require less effort to obtain, even if they are less valuable. In addition to discounting effortful rewards, patients also work less to obtain rewards.To determine the role of hippocampal neurogenesis in reward choices based on effort, we ran neurogenesis-deficient rats in an effort-discounting operant task. We tested transgenic rats (hGFAP-TK), in which drug-treatment can deplete actively dividing neural progenitors in a time- and region-specific manner and consequently stop the production of new neurons. Rats were faced with a choice between a low reward (2 sugar pellets) that was associated with a low effort (1 lever press), and a high reward (4 sugar pellets) that was associated with a higher effort (2, 5, 10 or 20 lever presses across blocks). As the amount of required effort increased, WT and TK rats chose the high reward less often, with no difference between genotypes. However, upon making a choice, TK rats lever-pressed at a slower rate than WT rats. Other behavioral measures were comparable between WT and TK rats suggesting similar levels of activity and motivation to perform the task. Moreover, untreated WT and TK rats did not differ in effort discounting or lever pressing behavior. In summary, while we found that neurogenesis did not regulate effort-based choice, it did contribute to effortful responding. Once a choice had been made, TK rats lever-pressed at a lower rate to obtain the rewards. This implicates neurogenesis in aspects of response vigor, and fits with recent findings that TK rats also obtain fewer rewards and press at a lower rate in a progressive ratio paradigm.

Disclosures: **D.R. Seib:** None. **O. Princz-Lebel:** None. **E. Chahley:** None. **S.B. Floresco:** None. **J.S. Snyder:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.08

Topic: G.03. Motivation

Support: Ontario Graduate Scholarship
Canadian Institutes of Health Research Grant 156070

Title: Ventral CA3 and CA1 projections to the lateral septum differentially regulate approach-avoidance conflict resolution

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Abstract: The ventral hippocampus is thought to be one of the primary regulators of approach-avoidance conflict resolution in rodents. Previous work has shown that the ventral CA3 (vCA3) subfield mediates the avoidance of stimuli that simultaneously predict both positive and negative outcomes, while the ventral CA1 (vCA1) directs approach towards motivationally conflicting stimuli and suppresses approach towards motivationally neutral stimuli. The caudodorsal lateral septum (LScd) and rostral lateral septum (LSr) are primary targets of extrinsic vCA3 and vCA1 projections respectively, and we posited that these distinct pathways may mediate the previously reported divergent control of approach-avoidance conflict resolution. We therefore transfected the glutamatergic cells of either the vCA3 or vCA1 in male Long-Evans rats with the inhibitory DREADD AAV8-CAMKII-hM4Di-mCherry and cannulated over the LS for direct drug (Clozapine-N-Oxide) delivery. Control animals received the same manipulations with EGFP-tagged AAV8 infused into the hippocampal subfields. Animals were trained to associate distinct visuo-tactile cues with either sucrose reward, aversive footshock, or no outcome (neutral) in a Y-maze. Following successful acquisition, animals underwent a "conflict test", in which they chose between exploring an arm with combined appetitive-aversive cues and a neutral cued arm. Chemogenetic inhibition of the vCA3-LScd pathway potentiated approach towards the conflict stimulus alone, while inhibition of the vCA1-LSr pathway potentiated approach towards both conflict and neutral stimuli compared to controls. The same manipulation in both groups had no effect on preference for the appetitive or aversive cues individually. Furthermore neither pathway was found to affect animals' ability to detect novel stimuli, change the amount of food consumed in a familiar environment, or increase locomotion alone, suggesting that the pathways that regulate the Y-maze task are specifically engaged during motivational conflict. These findings are consistent with the idea that the vCA3-LScd circuit facilities avoidance during cued approach-avoidance conflict, while the vCA1-LSr pathway is important for suppressing non-specific approach responses during the same situations.

Disclosures: **D.C.M. Yeates:** None. **R. Ito:** None. **A.C.H. Lee:** None. **D. Leavitt:** None. **S. Sujanthan:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.09

Topic: G.03. Motivation

Support: NIH grant R00-MH105549
NIH grant R01-MH120136
NARSAD Young Investigator
Rising STARs Award from UT System
CIHR grant MOP-89758

Title: Crf neurons in a paraventricular thalamic circuit regulate food-approach vs. threat-avoidance conflict

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Abstract: Balancing food-seeking with threat-avoidance behaviors is crucial for animals to survive, but which neural circuits regulate this motivational conflict remain largely unknown. To answer this question, we designed an ethologically relevant "approach-food vs. avoid-predator threat" conflict test in which rats need to overcome their fear of predator odor to reach food. Animals were initially trained to press a lever for sucrose in the presence of an audiovisual cue. During the conflict test, cat saliva was positioned in the food area adjacent to the lever. Rats exhibited robust defensive behaviors and a clear suppression in food-seeking responses in the presence of the predator odor. Using *in situ* hybridization, immunohistochemistry and *in vivo* single-unit recordings from photoidentified cell-types, we identified a subpopulation of neurons in the anterior portion of the paraventricular thalamic nucleus (aPVT) which express the stress neuropeptide corticotrophin-releasing factor (CRF) and are preferentially recruited during conflict. Chemogenetic inactivation of $aPVT^{CRF}$ neurons during conflict reduced defensive responses and restored food-seeking behavior, but had no effect when the predator odor or the food-seeking tasks were carried out independently. Using both anterograde and retrograde viral tracing methods, we characterized the anatomical connectivity between $aPVT^{CRF}$ neurons and brain regions that are implicated in the regulation of food seeking and defensive responses. We observed that $aPVT^{CRF}$ neurons project densely to the nucleus accumbens (NAc), and optogenetic activation of the $aPVT^{CRF}$-NAc pathway recapitulated the predator odor-induced food-seeking suppression and avoidance responses by mediating target-dependent synaptic transmission in the NAc. In addition, we identified the ventromedial hypothalamus (VMH) as a critical input to $aPVT^{CRF}$ neurons, and demonstrated that aPVT-projecting VMH neurons are

activated by predator odor and necessary for the expression of defensive responses during conflict. Together, our findings describe a subpopulation of neurons in a hypothalamic-thalamostriatal circuit that suppresses reward-seeking behavior under the competing demands of avoiding threats.

Disclosures: **X.O. Zhang:** None. **D.S. Engelke:** None. **J.J. O'Malley:** None. **J.A. Fernandez-Leon:** None. **S. Li:** None. **G.J. Kirouac:** None. **M. Beierlein:** None. **F.H. Do Monte:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.10

Topic: G.03. Motivation

Support: Whitehall Fellowship to DF
BRSFG-2017-02 to DF
R21MH114178 to DF
NSF1754831 to DF
NARSAD 2018 Young Investigator Award to EGA
NIH Grant T32 GM 007377 to AFD
Dean's Distinguished Graduate Fellowship to AFD

Title: Cerebellar modulation of nucleus accumbens activity

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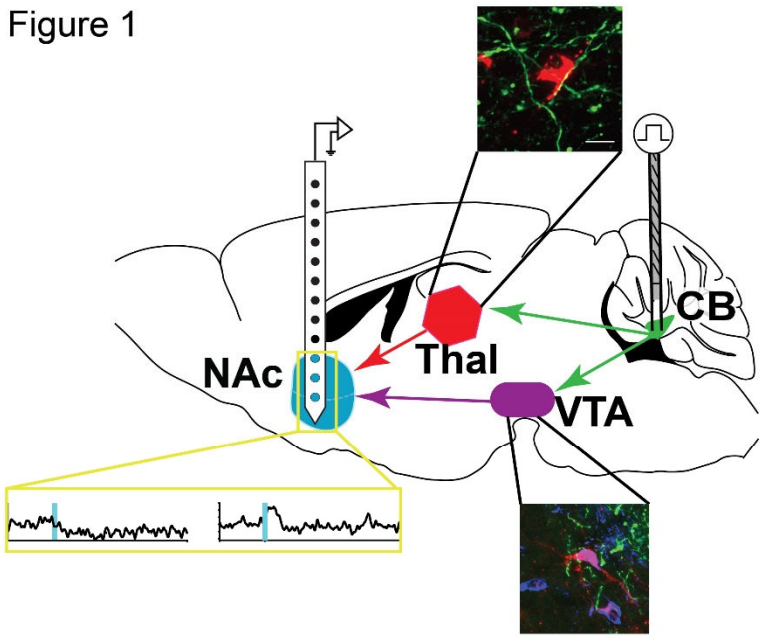
Abstract: The cerebellum (CB) utilizes input integration and feedforward processing to perform complex computations. This complexity has not been elucidated for CB non-motor functions, including cognitive and emotional processing as well as reward-driven behavior and learning. We propose that the role of the CB in limbic processes is derived in part from functional connectivity with the nucleus accumbens (NAc). Here we paired in vivo electrophysiological recordings of NAc spiking activity with microstimulation of the CB nuclei to provide the first evidence of a functional CB-NAc connection. Our data suggest that CB stimulation has both excitatory and inhibitory effects on NAc spiking activity, but differentially influences the two NAc subregions, the medial shell (NAc_{Med}) and core (NAc_{core}). CB stimulation evoked inhibitory responses more often than excitatory ones in the NAc_{Med} ($p<0.0001$). The NAc_{Core}, on the other hand, showed significantly greater probability of excitatory responses than Nac_{Med} ($p<0.05$). We also found latency differences between response types, and between NAc subregions. Excitatory responses displayed significantly shorter onset latencies than inhibitory ones ($p<0.05$) and responses in the NAc_{Core} appeared more quickly than responses in the NAc_{Med} ($p<0.05$). After confirming the localized nature of our stimulation, we investigated the presence of any

topographical organization of this connection. We found no evidence of localized clusters in the CB nuclei that would elicit the same response type in the NAc, nor any CB response clustering in NAc. Finally, we investigated the circuitry underlying CB-NAc connectivity using anatomical tracing. We identified two regions to be involved in the disynaptic CB-NAc connection: the ventral tegmental area and the intralaminar nuclei of the thalamus, both of which contain cells that receive input from the CB and project to the NAc. The results of these experiments expound upon the involvement of the CB in higher-order functions such as motivation, reward learning, and emotional processing.

Figure 1



Disclosures: **A.F. D'Ambra:** None. **S. Jung:** None. **S. Ganesan:** None. **E.G. Antzoulatos:** None. **D. Fioravante:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.11

Topic: G.03. Motivation

Support: ACB Department Funding through Brody School of Medicine at ECU

Title: Nurr1 deficiency shortens free running period, enhances photo-entrainment, and disrupts circadian cycling of the dopamine neuron phenotype

Authors: *H. PARTINGTON, J. M. NUTTER, S. BARKER, J. EELLS;
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Abstract: Neurological and neuropsychiatric disorders , including addiction, schizophrenia, and Parkinson's disease, involve dysfunction in midbrain dopamine (DA) neurotransmission and severity of disease symptoms and progression are associated with disrupted circadian rhythms. The nuclear transcription factor Nurr1, essential for DA neuron (DAN) development, survival, and maintenance, is also known to interact with circadian rhythm regulating clock gene proteins. Inthe Nurr1-null heterozygous (+/-) mice, a Nurr1 deficient model which reproduces some of the alterations in DA function found in schizophrenia and Parkinson's disease, we measured, using wheel-running activity, the free running period (tau) and photoperiod entrainment. Because Nurr1 has are role in regulating the dopamine phenotype, we also measured the circadian fluctuations in the number of DANs using tyrosine hydroxylase immunofluorescence. In Nurr1 +/- mice, tau was significantly shorter and entrainment to a 6h earlier shift in the dark cycle was faster. The Nurr1 wild-type (+/+) mice cycled DAN numbers across time, with a significantly greater number (~2-fold increase) of DANs at zeitgeber time (ZT) 0 than ZT12. The +/- mice, however, did not cycle the dopamine phenotype, as no differences in DAN numbers were observed between ZT0 and ZT12. Additionally, the +/- mice had significantly fewer DANs at ZT0 but not at ZT12 as compared to +/+ mice. Based these data, circadian fluctuations in DA transmission requires normal Nurr1 function. A better understanding is needed of the mechanisms regulating dopamine neurotransmission across the circadian cycle and how this is altered in circadian rhythm and DA neurotransmission associated disorders.

Disclosures: H. Partington: None. **J. Eells:** None. **J.M. Nutter:** None. **S. Barker:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.12

Topic: G.03. Motivation

Title: Accumbal cholinergic interneurons are critical in the influence of cues over risk taking

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Abstract: Many theories of gambling disorder posit a critical role of cues. The audiovisual cues in casinos are thought to promote riskier play, and this phenomenon can be modeled using the rat gambling task. Rats are given a choice between large, unlikely rewards (risky) and smaller, more certain ones (optimal). When casino-like cues are introduced, more rats choose the risky options. We theorize that the phasic dopamine release evoked by these cues cause compensatory changes in the dopamine system to promote risk taking. Cholinergic interneurons in the nucleus accumbens core (NAc) are critical titrators of NAc dopamine release. Cholinergic interneurons are tonically active and maintain steady levels of NAc dopamine. When motivationally relevant cues are presented, cholinergic interneurons pause, and thus the tonic stream of dopamine stops, giving way to the influence of the cue-evoked phasic dopamine we theorize to potentiate risk taking. Little is known of how cholinergic interneurons act in the NAc to affect dopamine release and orchestrate behaviour, but due to their critical role in regulating dopamine release, they are a potential treatment target for addictive disorders. We therefore asked the question as to whether NAc cholinergic interneurons could be targeted to manipulate gambling-like behaviour, hypothesizing that reducing accumbal CIN signaling should increase the influence of reward cues, thereby increasing risk taking over time. Their excitation should promote an optimal strategy. To test this, we used DREADDs in combination with ChAT-cre transgenic rats to gain bidirectional chemogenetic control over the NAc cholinergic interneurons in female and male rats. We then administered clozapine-N-oxide prior to each daily training session, to either inhibit or enhance NAc cholinergic interneuron activity. In rats that underwent inhibition, a longitudinal increase in risk taking was observed, while excitation resulted in a reduction. Interestingly, the inhibition effect was observed only in females and the excitation effect only in males -- this may be due to baseline differences in dopamine neurobiology across the sexes. These results support our hypothesis, suggesting that NAc cholinergic interneurons may be leveraged in the treatment disorders, and furthermore point to biological sex as an important consideration.

Disclosures: **A.C. Li:** None. **T.J. Hynes:** None. **K. Hrelja:** None. **L. Calderhead:** None. **C.S. Chernoff:** None. **C.A. Winstanley:** None.

Digital Abstract Session

P271. Motivation, Reward, and Subcortical Circuits

Program #/Poster #: P271.13

Topic: G.03. Motivation

Support: NIH Grant R01DA044960
NSF GRFP

Title: Electrophysiological characterization of medium spiny neurons in the nucleus accumbens of sign- and goal-trackers, a model of individual variation in incentive learning

Authors: *C. E. MARÍA-RÍOS[1], G. G. MURPHY[2], J. D. MORROW[3];
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Abstract: Pavlovian conditioned approach behavior in rats can be used to identify individual differences in sensitivity to reward cues, which is an identified risk factor for addiction. "Sign-tracker" rats (ST) will approach and interact with a reward cue, indicating that the cue itself acquires incentive value for them. In contrast, "goal-tracker" rats (GT) direct their behavior away from the cue and towards the site of impending food reward, indicating that they are using the cue solely as a predictor of the reward. As expected, ST are more susceptible to cue-induced 'relapse' of drugs of abuse when compared to GT. ST and GT exhibit different cue- and reward-evoked patterns of activity in the nucleus accumbens (NAc) during Pavlovian learning, but it is not known how these patterns arise or contribute to the ST/GT phenotype. Using whole-cell patch-clamp recordings, we explored whether differences in the intrinsic neuronal properties and in baseline excitatory inputs of NAc medium spiny neurons (MSNs) within the shell and core subregions contribute to the ST/GT phenotype. Consistent with previous literature, we found regional differences between the core and shell sub-regions. Cells in the shell exhibited lower cell capacitance and higher input resistance when compared to those in the core. We found no significant differences between ST and GT in either passive or active MSNs excitability properties. In addition, we found no significant differences in mEPSCs amplitude or frequency between ST and GT. Our findings suggest that the ST/GT phenotype does not correlate with intrinsic excitability properties of MSNs in the NAc, nor with their overall baseline excitatory synaptic properties. This suggests that the distinct activity profiles of ST and GT may result from circuit-level differences in input from other regions to the NAc. Continued work in this area will shed light on the neurobiological basis of increased susceptibility to cue-driven psychopathologies.

Disclosures: C.E. María-Ríos: None. **J.D. Morrow:** None. **G.G. Murphy:** None.

Digital Abstract Session

P272. Motivation: Cortical Neurocircuitry Animals To Humans

Program #/Poster #: P272.01

Topic: G.03. Motivation

Support: Undergraduate Research Award, Miami University
Dean's Scholarship Award, Miami University

Title: Contributions of the mesolimbic dopamine pathway in reversal learning

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Abstract: Cognitive flexibility, which can be measured with reversal learning, is the ability of an individual to alter behavior in response to the changing of environmental conditions. Past studies

have found that dopamine (DA) is released in the nucleus accumbens (NAc) during reversal learning (Radke et al., 2019, Klanker et al., 2017). The present study sought to examine the effects of manipulating DA release in the NAc on reversal learning using Designer Receptors Exclusively Activated by Designer Drugs (DREADD). AAVrg-hSyn-DIO-hM3Dq-mCherry (Addgene) was injected into the NAc of male and female DAT-Cre mice. Mice first learned to master a basic task of discriminating between an active vs. inactive nose poke in an operant box while responding for a food reward. Once the criterion for discrimination was met, the active and inactive nose-poke holes were switched (i.e., reversal). Mice were administered the ligand clozapine-n-oxide (CNO, 1.0 mg/kg) 15 min before the first reversal session, exciting DA neurons projecting to the NAc. There were no differences between Cre- (=control) and Cre+ (=experimental) mice in correct or incorrect responses following administration of CNO during the first reversal. Following this session, the animals were trained to criterion (defined by a consistent 85% correct response rate), and the correct and incorrect nose pokes were reversed again. In this second reversal, the Cre+ animals that expressed the DREADD receptor showed a significantly greater number of incorrect responses on the side that was not reinforced (i.e., errors) compared to the Cre- animals that did not express the DREADD receptor. In a second experiment, mice with partial genetic deletion of the dopamine transporter (DAT-KO mice) were tested in the reversal learning paradigm. We hypothesized that increased DA concentrations in heterozygous DAT-KO mice would affect reversal learning in a manner similar to chemogenetic activation of DA neurons. Preliminary data suggest that DAT gene deletion does not significantly affect reversal learning. These data clarify the important role that dopamine in the NAc plays in reward learning and behavioral flexibility.

Disclosures: B. Arnold: None. **S. Monroe:** None. **E. Sneddon:** None. **A. Radke:** None.

Digital Abstract Session

P272. Motivation: Cortical Neurocircuitry Animals To Humans

Program #/Poster #: P272.02

Topic: G.03. Motivation

Support: CIHR Grant 156070

Title: Optogenetic inhibition of the rat perirhinal cortex disrupts object-based approach-avoidance conflict processing

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Abstract: Extant research has implicated the ventral hippocampus (vHPC) in the detection and resolution of learned approach-avoidance conflict (AAC), whereby rats are simultaneously presented with stimuli previously associated with competing motivational valences. Emerging findings from our group have implicated the human perirhinal cortex (PRC), a brain region crucial in representing item/object information, in the resolution of AAC when discrete ‘object’,

rather than contextual 'scene' stimuli were used. Currently, learned AAC paradigms in rats have only used contextual 'visuo-tactile' panels spanning a Y-maze arm as stimuli, and thus the present study sought to examine to role of the rat PRC in object-based AAC. Adult male Long-Evans rats surgically received bilateral infusions of either the inhibitory opsin ArchT (AAV1-CaMKIIa-ArchT-GFP) or control GFP (AAV8-CaMKIIa-GFP) followed by optic fiber implantation, and were then trained to associate 3 sets of object-pairs (2 feature-unique objects) with either reward, punishment, or no outcome in a novel, object-based AAC task. Rats traversed a narrow runway apparatus to allow incidental exploration of two objects, towards a goal compartment in which animals received the outcomes associated with the object pair (sucrose, shock or no outcome). Upon successful object-outcome learning, rats completed 2 'conflict tests' (with and without optogenetic inhibition of the PRC) in which the object-pairs contained one appetitive and one aversive object, and one 'neutral' test. PRC inhibition throughout the conflict test session, but not neutral test session, resulted in a robust increase in the time spent in the goal box, compared with laser-off sessions, and with laser-on GFP-control animals. Subsequent control experiments determined that the increase in 'approach' behaviour in ArchT animals was not driven by impairments in object memory or novelty processing. Rats also completed the vHPC-dependent cued AAC Y-maze task, upon which it was found that PRC inhibition did not impact conflict behaviour. These findings indicate that normally, the rat PRC promotes avoidance behaviour for AAC decisions represented by object-cues, akin to the role of the vCA3 of the HPC in contextual AAC decisions. Thus, AAC is a process that may be mediated by heterogeneous brain regions based on the type of stimulus representation.

Disclosures: S.S. Dhawan: None. **A.C.H. Lee:** None. **R. Ito:** None.

Digital Abstract Session

P272. Motivation: Cortical Neurocircuitry Animals To Humans

Program #/Poster #: P272.03

Topic: G.03. Motivation

Support: NIH grant F31 MH112372
NIH grant EY13079
NIH grant R25GM097634
NSF grant DBI-1460880
NYU Research Challenge Fund
NYU Dean's Dissertation Fellowship

Title: Prefrontal corticostriatal cells drive food restriction evoked running and receive fewer inhibitory contacts than neighboring pyramidal cells projecting elsewhere

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Abstract: Food restriction (FR) evokes running, which may promote adaptive foraging in times of food scarcity, but can become lethal if energy expenditure exceeds caloric availability. Here, we demonstrate that chemogenetic activation of either the general medial prefrontal cortex (mPFC) pyramidal cell population, or the subpopulation projecting to dorsal striatum (DS) drives running specifically during hours preceding limited food availability, and not during *ad libitum* food availability. Conversely, suppression of mPFC pyramidal cells generally, or targeting mPFC-to-DS cells, reduced wheel running specifically during FR and not during *ad libitum* food access. Post-mortem c-Fos analysis and electron microscopy of mPFC layer 5 revealed distinguishing characteristics of mPFC-to-DS cells, when compared to neighboring non-DS projecting pyramidal cells: 1) greater recruitment of GABAergic activity and 2) less axo-somatic GABAergic innervation. Together, these attributes position the mPFC-to-DS subset of pyramidal cells to dominate mPFC excitatory outflow, particularly during FR, revealing a specific and causal role for mPFC-to-DS control of the decision to run during food scarcity. Individual differences in GABAergic activity correlate with running response to further support this interpretation. FR enhancement of PFC-to-DS activity may influence neural circuits both in studies using FR to motivate animal behavior and in human conditions hallmarked by FR.

Disclosures: **A.N. Santiago:** None. **C.J. Aoki:** None. **M. Du:** None. **E. Makowicz:** None.

Digital Abstract Session

P272. Motivation: Cortical Neurocircuitry Animals To Humans

Program #/Poster #: P272.04

Topic: G.03. Motivation

Title: Neuroanatomical evidence supporting the role of the ventrolateral prefrontal cortex in the salience network

Authors: ***L. R. TRAMBAIOLLI**[1], S. HABER[2];
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Abstract: Introduction: Salience detection is the attentional process of identifying elements that stand out from neighbor events. Human neuroimaging studies report the existence of a salience network cortically anchored by the dorsal anterior cingulate cortex (dACC) and the fronto-insular cortex (FIC). One open question is the involvement of the ventrolateral prefrontal cortex (vlPFC) in this network. Due to the spatial limitation of neuroimaging technologies, portions of the vlPFC are often labeled as part of the FIC. However, this question can be addressed using the precision of neuroanatomic tracing methods to evaluate vlPFC connections in NHP. This study was designed to quantify the afferent connections from the dACC and FIC to the vlPFC to determine whether there is a sublocation within the vlPFC specifically related to the salience network. **Method:** We injected retrograde tracers in the right vlPFC of macaque monkeys: five injections in area 47/12, two in area 45, and one in area 44. For each injection, we quantified cells in 22 areas of the frontal cortex and six areas of the insular cortex. The connectivity strength between each cortical area and the vlPFC sublocation was measured as a

ratio between the number of cells in this area by the total number of labeled cells. To ensure that our results are different from chance, we performed 10^6 permutations of random labeling of cells in each injection. Then, we statistically compared the real labeling with the distribution of random labels.**Results:** All connectivity charts presented significant differences from the chance level ($p<0.01$). The two injections in the caudal portion of area 47/12 stood apart from the rest of the vlPFC with respect to inputs from the two salience detection nodes (FIC and dACC). After identifying the cluster of cells in these nodes, we placed two new tracer injections in the cluster location. Using anterograde tracing, we found both clusters projecting axon terminals to a similar location in caudal area 47/12, equivalent to our original injection sites.**Discussion:** Here, we identified a specific location within the vlPFC, specifically in caudal area 47/12, which is structurally linked to the central nodes of the salience network. The involvement of the vlPFC in this network has not been clear due to methodological issues from resting-state fMRI, such as echoplanar distortions. Thus, this is the first study to formally identify a portion of the vlPFC that is connected to the salience network. The next step of our research will be to translate these findings to functional imaging in NHP and humans.

Disclosures: L.R. Trambaiolli: None. **S. Haber:** None.

Digital Abstract Session

P272. Motivation: Cortical Neurocircuitry Animals To Humans

Program #/Poster #: P272.05

Topic: G.03. Motivation

Support: Internationale Stichting Alzheimer Onderzoek (now Alzheimer Nederland), grant number: 15040)
Ammodo KNAW award 2017

Title: Does amyloid-β plaque accumulation correlate with cognitive effort costs? An exploratory study

Authors: *D. PAPADOPETRAKI[1], A. WESTBROOK[2], J. THOMAS[3], L. J. MENTINK[3], C. B. HOLROYD [4], J. CLAASSEN[3], R. COOLS[1];
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Abstract: Sustained attention in a cognitively demanding task is known to incur performance decrements and sensations of fatigue. These cognitive effort costs bias people to avoid mentally demanding tasks, even at the expense of potential rewards. The precise nature of the costs remains elusive, with proposed theories including resource depletion, motivational, as well as metabolic accounts. A promising metabolic proposal is the waste-disposal hypothesis (Holroyd, 2015), which holds that control is treated as costly because it results in the accumulation of

amyloid beta (Aß) in the interstitial fluid (ISF) as a by-product of cortical noradrenaline signaling. By this account, the rate of Aß accumulation underlies the phenomenology of cognitive fatigue, which is then remedied by Aß clearance during sleep. The waste disposal hypothesis predicts that long term sleep deprivation results in detrimental Aß accumulation and enhanced effort costs.

We investigated the relationship between brain Aß levels measured with ^{18}F-flutemetamol PET-CT and cognitive motivation in a population experiencing chronic sleep disruption (maritime pilots, N=19) (Thomas et al., 2020). ^{18}F-flutemetamol is a validated Aß tracer that binds to Aß plaques, comparable to ^{11}C-Pittsburgh compound B. To assess cognitive motivation, we administered a classic working memory task, the N-back task, and a subsequent cognitive effort discounting task to quantify the subjective value assigned to executing the effortful tasks. Our preliminary results do not provide evidence for a relationship between Aß levels and the value of cognitive effort.

We do observe a trend towards a positive link between response latency during N-back task performance and Aß concentration in the temporal cortex. Further analyses and future PET-CT or biomarker studies with larger sample sizes are required to definitively test the waste disposal hypothesis.

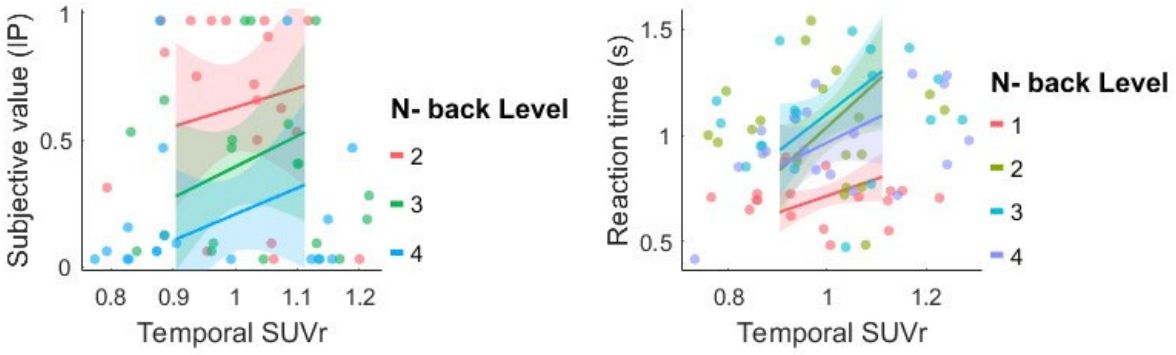

Figure 1. Left: No significant correlation between SUVr (standard uptake value ratio) and subjective value. Right: Relationship between latency during the N-back task and temporal SUVr.

Disclosures: D. Papadopetraki: None. **A. Westbrook:** None. **J. Thomas:** None. **L.J. Mentink:** None. **C.B. Holroyd :** None. **J. Claassen:** None. **R. Cools:** None.

Digital Abstract Session

P272. Motivation: Cortical Neurocircuitry Animals To Humans

Program #/Poster #: P272.06

Topic: G.03. Motivation

Title: A within-subject experiments assess the effect of Loving Kindness Meditation (LKM) A pilot EEG study

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Abstract: Background The need for social connection is a fundamental human motive. It has become much explicit that the feeling of social connection can confer mental and physical health benefits. However, in many different cultures, societal changes are leading to the growth of social distrust and alienation. As LKM characterizes a soft and slow affection, it may be associated with a slow-wave generation in the brain. Neuroimaging studies have demonstrated that LKM practice induces neural activation in the prefrontal cortex (PFC), anterior cingulate cortices (ACC), and insula. LKM may also affect subcortical brain activities and autonomic nervous system which additionally affect cardiac activities. Advance in neuroscience techniques makes it possible for its application to be used in LKM practice, for example, to give neurofeedback or to measure its progress. However, the effect of LKM training on brain modulation, especially empathy neuro-marker is still unknow. The purpose of this study aims to investigate the effect of Loving Kindness Meditation (LKM) on empathy modulation and to identify potential EEG neuro-marker. **Method:** In this study, a 46-year-old male meditation novice participant was recruited for this single-subject EEG experiment. There were consecutive 10-session LKM training under the instruction of a teacher with more than 20 years' experience of meditation. 128-chanel resting EEG signals were collected during each training session. The data were processed by EEGlab based on Matlab. Spectrum analysis was carried out to calculate the EEG power in different power bands (delta 1-4Hz, Theta 4-8 Hz, alpha 8-12 Hz, Beta 12-24 Hz, Gamma 24-40Hz). Self-reports of Loving Kindness Compassion Scale was also collected at the beginning and end of the 10-session training. **Result:** Investigation has been partially done on the change of EEG related to LKM training, and the alpha wave is supposed to be the LKM neuro-marker in the resting EEG. A significant difference in EEG alpha wave is noticed between the first 5-session and the last 5-session training ($p < 0.05$), while delta wave difference is marginal. Additionally, the Self-report of Loving Kindness Compassion Scale has improved. **Conclusion:** This study shows the long-term modulation effect of LKM training on alpha wave, which potentially could be an LKM neuromarker. Nevertheless, more training data and connectivity analysis may be needed to identify the neuromarker. The result of this study not only establishes a starting point to investigate the effect of LKM, from the perspective of neurological mechanism; but also provides a neural marker of LKM training for future neurofeedback training.

Disclosures: G. Wong: None. **S. Rui:** None. **J. Gao:** None.

Digital Abstract Session

P272. Motivation: Cortical Neurocircuitry Animals To Humans

Program #/Poster #: P272.07

Topic: G.03. Motivation

Support: ERC grant COREFEAR

Title: A role for cerebral cortex in the suppression of innate defensive behavior

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Abstract: The cerebral cortex is involved in the control of cognition and the processing of learned information and it appears to have a role in the adaptation of behavior in response to unpredictable circumstances. In addition, the cortex may have a role in the regulation of innate responses since rodents, cats or primates with surgical removal or accidental destruction of cortical regions show excessive irritability, aggression and rage elicited by threatening stimuli. However, it remains unclear whether cortex has an acute role in suppressing innate threat responses because the imprecision and chronic nature of these lesions leaves open the possibility that compensatory processes may underlie some of these phenotypes. In the present study we used pharmacogenetic inhibition to precisely, rapidly and reversibly suppress cortical pyramidal neuron function and examine its contribution to defensive behaviors elicited by a variety of innately aversive stimuli. Inhibition of cortex caused an increase of defensive responses elicited by an aggressive conspecific, a novel prey, and a physically stressful stimulus. These findings are consistent with a role of cortex in the acute inhibition of innate defensive behaviors.

Disclosures: S. Natale: None. **M. Masferrer:** None. **S. Deivasigamani:** None. **C. Gross:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.01

Topic: G.03. Motivation

Support: NIH Grant R01DA039062

Title: Sex differences in activation of reward-related brain circuitry during juvenile rat social play

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Abstract: Rough-and-tumble play is a highly rewarding social behavior conserved across mammalian species, including humans, and involves dopaminergic (DA) cells in the ventral tegmental area (VTA) that project to the nucleus accumbens (NAc). Juvenile males engage in more frequent and intense play than females, but contrary to expectation we have previously shown that engaging in play resulted in greater neuronal activation in the NAc of juvenile female rats compared to males, as measured by immediate early gene expression (Egr1). We hypothesize that, despite playing less, juvenile females experience greater motivation to acquire play. Therefore, we predicted that play specifically activates DA receptor-expressing cells in the

NAc and likewise DAergic cells in the VTA of females. To test this, we phenotyped play-active cells in these regions using RNAscope. There was no sex difference in the total number of Egr1+ cells in the VTA, however we found that 67% of Egr1+ cells colocalized with tyrosine hydroxylase (TH), a marker of DA cells, in females compared to only 52% in males. In NAc, we found a greater number of Egr1+ cells across the core and shell subregions in females compared to males, and further found that all Egr1+ cells in both sexes expressed either one or both DA receptors D1 (Drd1a) and D2 (Drd2). In the core specifically, 54.7% of D1+ cells and 61.2% of D2+ cells coexpressed Egr1 in females compared to 39.9% and 37.2% in males. These findings demonstrate that play activates a greater proportion of putative DAergic cells in the female VTA, which is mirrored by increased activation of DA receptor-expressing cells in the NAc. To directly test the motivation to engage in play behavior, we adapted the operant social preference task (Borland et al., Journal of Neuroscience Methods 2017) for juvenile rats. In this task, animals must push through a transparent, perforated one-way swinging door in order to physically interact with a sex- and age-matched play partner. We found that both males and females readily acquire this task, making an average of nearly 30 entries per 3 minute session when housed in isolation. When the weight of the door is progressively increased after each successful entry, juveniles will push through a weight of up to 200g (~200% body weight) to access a playmate. We found no sex differences in any measure of this task, suggesting a different mechanism controls the appetitive versus consummatory components of juvenile social behavior. Future directions will examine DA transmission in NAc during the social motivation task and play behavior.

Disclosures: S.E. Ashton: None. **J.W. Vanryzin:** None. **M.M. McCarthy:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.02

Topic: G.03. Motivation

Support: NIDA IRP

Title: Effect of access duration on operant responding for social interaction with a peer in male and female rats

Authors: *J. J. CHOW, J. M. CHABOT, M. VENNIRO, Y. SHAHAM;
IRP/NIDA/NIH, Baltimore, MD

Abstract: Background: We recently developed an operant model of social reward and choice where rats lever-press for access to social interaction with a peer. We reported that rats strongly prefer the social reward over opioid and psychostimulant drugs. Here we further characterized the operant social reward by determining the effect of access duration on operant responding for social interaction under fixed and progressive ratio reinforcement schedules. **Methods:** We

trained male and female rats (n=16; 8 females) to lever press for social interaction (60 s access for 1-h/day; 10 days, fixed ratio 1 to 5 schedule). Next, we assessed operant responding at various access durations (3.75 to 240 s, 1-h/day; 2 days per duration). Next, we examined the effect of duration of social interaction (15, 30, and 60 s; 3 days per duration) on progressive ratio responding. **Results:** Operant responding was higher for 3.75 to 60 s of social interaction than for 120 and 240 s. A demand analysis of the data showed that unrestrained consumption (Q_0) was 2194 and the rate of decline in consumption (a) was 4.375e-5. Progressive ratio responding was similar for 15, 30, and 60 s of social interaction. Operant responding for social reward was similar in males and females. **Conclusions:** Results indicate that rats will decrease operant responding for longer durations of social interaction and confirm our previous results that social interaction with a peer serves as an operant reward to rats.

Disclosures: J.J. Chow: None. **J.M. Chabot:** None. **Y. Shaham:** None. **M. Venniro:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.03

Topic: G.03. Motivation

Support: R01MH119422

Title: Maternal immune activation leads to sex specific effects on social-decision making and insular cortex CRF function

Authors: *N. S. RIEGER, J. P. CHRISTIANSON;
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Abstract: Prenatal infection increases risk for neuropsychiatric disorders such as autism in offspring. In the rodent maternal immune activation (MIA) paradigm, prenatal administration of the viral mimic Polyionsinic:polycytidylic acid (PolyI:C) allows for investigation of developmental consequences of gestational sickness on offspring social behavior and neural circuit function. MIA alters cortical development, stress response, and leads to altered sociability, therefore, we hypothesized that MIA would lead to changes in social decision-making in a rat social affective preference (SAP) task. In this task, test rats are exposed to both a stressed (via 2 mild foot shocks) and naive to stress same-sex conspecific and the amount of time they spend investigating these conspecifics is recorded. Under control conditions both male and female test rats show a preference for stressed juveniles but avoid stressed adult conspecifics. We found that, following 0.5 mg/kg PolyI:C dose on E12, male adult offspring (P50) had altered social decision making compared to control rats, showing no preference for either stressed juveniles or naive adults. Female adult MIA offspring, however, maintained their preference for stressed juveniles but lost their preference for naive adults. These results indicate that males show a global change in SAP based social decision-making while females show a more subtle

change in social decision making that is age and MIA dependent. Behavior in the SAP test depends upon insular cortex corticotropin-releasing factor (CRF) and is related to an increased synaptic efficacy in control males but not females. As such, we followed up this experiment by testing for changes in field excitatory postsynaptic potentials (fEPSP) using a perforated multiple electrode array. Bath application of CRF (300 nM) led to sex-specific changes in synaptic efficacy such that male MIA offspring were not affected by CRF while female MIA offspring exhibited greater sensitivity to CRF compared to sham littermates. Taken together, these results show that prenatal PolyI:C affects male and female offspring social decision making differently and this may be due, at least in part, to a loss of insular sensitivity to CRF in males and a gain of insular sensitivity to CRF in females.

Disclosures: N.S. Rieger: None. **J.P. Christianson:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.04

Topic:

Support: R01 MH121603

Title: Vasopressin in the bed nucleus of the stria terminalis regulates social behavior in male and female mice

Authors: *N. RIGNEY, G. J. DE VRIES, A. PETRULIS;
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Abstract: The neuropeptide arginine-vasopressin (AVP) has long been implicated in the regulation of social behavior and communication, but the source of AVP release relevant for behavior has not been precisely determined. Ablations of the sexually dimorphic (greater in males than females) AVP-expressing cells within the bed nucleus of the stria terminalis (BNST) result in sex-different effects on social behavior. However, it is unknown if vasopressin itself, or other neuroactive substances present in these cells, is responsible for sex-dependent effects on social and communicative behaviors. To test the role of AVP specifically, we used a shRNA viral construct (or scrambled control virus) to effectively knockdown AVP gene expression within the BNST of wild-type male and female mice and evaluate any subsequent changes in anxiety-like behaviors (elevated plus maze) or social behaviors (social investigation, ultrasonic vocalizations, scent marking, copulation, aggression). Knockdown of BNST AVP in males strongly reduced investigation of male competitors as well as aggressive signaling (tail rattling), while not altering attack initiation. In females, BNST AVP knockdown reduced female sex behavior. These results point to differential involvement of BNST AVP in social behavior in the two sexes, which may contribute to sex differences of social communication disorders.

Disclosures: N. Rigney: None. **G.J. De Vries:** None. **A. Petrulis:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.05

Topic: G.03. Motivation

Support: DFG Grant SCHW 559/15-1

Title: Anterior cingulate cortex activity correlates with approach behavior induced by repeated playback of 50-kHz ultrasonic vocalizations and the impact of social factors in rats

Authors: *A. BERZ[1,2], C. PASQUINI DE SOUZA[3], M. BRUNTSCH[1], M. K. SCHAFER[4,2], S. STEINMÜLLER[1], M. WÖHR[5,1,2], R. K. SCHWARTING[1];
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Abstract: Rats, which are highly social animals, use several routes of communication including vocal ones. Most of these are ultrasonic vocalizations (USV), which are known to serve situation-dependent socio-affective signals. In appetitive situations, for example juvenile play, 50-kHz USV occur, which are thought to reflect the sender's positive affective state and which support contact among conspecifics. Playback studies have shown that 50-kHz USV leads to a pronounced approach, especially in juvenile recipients. In contrast, a reduced approach to such playback is observed when the same playback is repeated days after the first one. Since the reasons for this memory-dependent habituation phenomenon are largely unknown, we asked whether it is due to the fact that playback is usually not followed by a social consequence. We tested three groups of male, juvenile Wistar rats, which, immediately after the first playback session, were exposed either to an empty cage, an unfamiliar male conspecific or returned back to their housing group cage. Unexpectedly, all three groups showed similar levels of habituation in the playback retest. After this retest, their brains were analyzed using c-fos immunohistochemistry focusing on the cingulate cortex, since it is involved in the processing of 50-kHz USV and behavioral responses to them. In parallel to the behavioral results, we found no group differences in the number of c-fos positive cells, but when considering individual levels irrespective of group assignment, we found a strong positive correlation between approach scores in the retest and cell numbers in the left cingulate cortex. These results do not support the hypothesis that habituation to repeated 50-kHz USV playback is due to the lack of social consequences after the first playback but indicate that the cingulate cortex is involved in the residual approach in the retest.

Disclosures: **A. Berz:** None. **C. Pasquini de Souza:** None. **M. Bruntsch:** None. **M.K. Schafer:** None. **S. Steinmüller:** None. **M. Wöhr:** None. **R.K. Schwarting:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.06

Topic: G.03. Motivation

Support: Genentech Fellowship
George A. and Marjorie H. Anderson Fellowship

Title: An amygdalo-cortical circuit for cortical integration of multisensory social cues

Authors: *A. C. NOWLAN[1], A. CORONA[1], C. C. KELAHAN[2], S. D. SHEA[1];
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Abstract: Many species, including mice, communicate using multisensory signals to coordinate social behaviors that are essential for survival (e.g. maternal care of offspring). Pup retrieval behavior requires caregivers to successfully detect both ultrasonic distress vocalizations and pup odors to retrieve isolated pups back to the nest. The onset of retrieval behavior coincides with dramatic changes in the auditory cortex of both mothers and 'surrogates' (virgin females cohoused with a mother and her pups). Interestingly, the presentation of volatile pup odors modifies the response profiles of auditory cortical (AC) neurons in caregiving mothers and surrogates, but not in naïve virgins. Currently, there is no known direct anatomical conduit between the olfactory periphery and AC that would allow this modulation to occur. Here we show that there is a projection from the basal amygdala (BA) to the AC that influences AC encoding of pup-related stimuli and may improve pup retrieval behavior. First, using fiber photometry to record population activity, we show that these amygdala-cortical projection neurons are sensitive to odors, including pup odor, and that they are primarily active while the surrogate searches for the isolated pup. Second, we find that optogenetic activation of the circuit during the presentation of auditory stimuli evokes complex changes to AC neuronal activity. Specifically, we found that optogenetic activation of the circuit preferentially suppressed auditory responses in naive females, but enhanced auditory responses in surrogates. Based on the amygdala's known role in valence processing, we hypothesize that the BA->AC circuit may attribute additional emotional salience to the representation of pup stimuli within the AC. Together these data suggest a bottom-up mechanism by which olfactory cues shape cortical auditory representations via the BA to drive appropriate social behavior.

Disclosures: **A.C. Nowlan:** None. **A. Corona:** None. **C.C. Kelahan:** None. **S.D. Shea:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.07

Topic: G.03. Motivation

Support: MH109545
MH119422

Title: Sex differences in rat insular cortex CRF and endocannabinoid systems provide a sex-specific mechanism for social affective behavior.

Authors: *A. J. NG, N. RIEGER, J. CHRISTIANSON;
Boston Col., Chestnut Hill, MA

Abstract: Stress is an important determinant of social behavior, and corticotropin releasing factor (CRF) initiates the central response to stressors, including responses to observing stress in others. CRF fibers and receptors are found within the neural circuits underlying social decision-making and, as such, provide a link between stress and social systems. We investigated CRF in the insular cortex (IC), a region involved in approach/avoidance decisions with stressed others. In acute IC slices from adult rats, application of CRF (50 to 300nM) increases field excitatory postsynaptic potentials (fEPSP) in males, but not females. The CRF enhancement of excitatory transmission depends on CRFR1 and $GABA_A$ which led us to hypothesize that CRF may modulate presynaptic inhibition via endocannabinoid signalling. Pretreatment of slices with CB1 receptor antagonist (AM251, 2μM) prevented the CRF-induced increased fEPSP in males. Behaviorally, insular CRF increased social interaction in male rats but not when co-administered with AM251. Similarly, AM251 prevented approach or avoidance, respectively, of stressed adult (PN50) and juvenile (PN30) conspecifics, suggesting that the insular cortex is a site of CRF modulation of neural activity via CB1. Because these synaptic and behavioral effects were only observed in males, we investiaged sex-differences in insular CRFR1 and CB1 receptor expression with RNAScope *in situ* hybridization. Males expressed significantly more CRFR1 and CB1 mRNA and more co-expression in insular glutamateric (vglut1) cells compared to females, providing a basis for the physiological and behavioral sex differences at the level of gene expression. These results identify novel roles for CRF and endocannabinoids in regulating cortical circuit function and further delineate the neurobiology of social decision-making.

Disclosures: A.J. Ng: None. **N. Rieger:** None. **J. Christianson:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.08

Topic: G.03. Motivation

Support: MH119422

Title: Semantic segmentation for analysis of social preference in rats.

Authors: *N. B. WORLEY[1], A. J. NG[2], N. RIEGER[1], J. P. CHRISTIANSON[2];
[2]Psychology, [1]Boston Col., Chestnut Hill, MA

Abstract: Automated analysis of animal behavior using machine learning holds the promise of reducing experimenter effort and bias while improving temporal precision. Markerless pose estimation uses a model trained through supervised machine learning to identify key points on an animal (such as body parts or joints) frame by frame in a video. A ball and stick model created from these points is then used to perform behavioral analysis. This method alone may be insufficient when investigating social behavior where multiple moving conspecifics must be identified. To resolve this issue, we combined pose estimation with another supervised machine learning based tool known as semantic segmentation, which can identify the moving targets location at the individual pixel level. Example targets are labeled to form binary masks of the target, in this case a rat in a social interaction test, and the background. Using videos of a rat social preference test, we applied pose estimation to identify the location of the focal test rat's head and its orientation relative to targets isolated in plastic chambers at either end of the arena. To determine the location of the targets, we trained a semantic segmentation model, and quantified model performance (intersection over union) under several conditions. We systematically varied the model architecture (e.g. pretrained backbone, size and number of transposed convolutional filters) and training specifications (e.g. size of test set, batch size, data augmentation). The resulting masks were used to identify the minimum distance between the test and target rats. This machine learning method successfully reproduced hand-scored results. Additionally, this method rivals hand-scoring in terms of experimenter effort, reduces experimenter bias, and increases temporal accuracy which will facilitate time-series analysis with tools such as fiber photometry and electrophysiology.

Disclosures: N.B. Worley: None. **A.J. Ng:** None. **N. Rieger:** None. **J.P. Christianson:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.09

Topic: G.03. Motivation

Support: NIDA IRP

Title: Waving through the window: an operant model of social reward in female mice

Authors: M. F. GARCIA, B. T. HOPE, Y. SHAHAM, M. VENNIRO, *L. A. RAMSEY;
NIDA IRP, Baltimore, MD

Abstract: Background: Maladaptive social behavior is a cardinal feature of neuropsychiatric disorders. Rodent models of social behavior classically rely on unconditioned measures of social preference such as time spent in contact with a social partner. We recently developed an operant self-administration and choice model in which rats lever-press for social interaction (Venniro et

al. Nat Neurosci 2018). Here we introduce a mouse version of this model.
Methods: We compared adult female C57/Bl6J to adolescent and adult female CD1 mice using custom-made social self-administration chambers. We first trained the mice to self-administer palatable food pellets (8 days – 1-h/d). Then, we trained them to lever-press for access to a female social partner (23 days – 1 h/d – fixed ratio 1 to 6 reinforcement schedule). We then tested their motivation to seek social interaction using a progressive ratio schedule (3 d). Finally, we investigated preference for food versus social interaction using a discrete trial choice procedure.
Results: The female mice of both strains showed reliable food self-administration. In contrast, only CD1 mice learned to lever-press for access to a social partner, whereas C57 mice did not. The CD1 mice continued to lever-press when the response requirements were increased under both fixed-ratio and progressive-ratio schedules. They also preferred social interaction over palatable food. We observed no differences between adult and adolescent CD1 mice.
Conclusions: Our new mouse social administration and choice model opens up new avenues for research on neurobiological substrates of social reward learning using multiple genetic tools that are only available in mice.

Disclosures: M.F. Garcia: None. **B.T. Hope:** None. **Y. Shaham:** None. **M. Venniro:** None. **L.A. Ramsey:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.10

Topic: G.03. Motivation

Support: UNL CEHS/EDPS Start-Up Funds

Title: Relationship between hostile sexism and gender-biased bullying mediated by nucleus accumbens volume

Authors: E. HAMEL[1], *H. SUZUKI[2];
[1]Dept. of Child, Youth and Family Studies, [2]Dept. of Educational Psychology, Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: Bullying is widespread in American schools. Social-group dimensions are often factors involved in bullying, with sexism showing the strongest association with bullying. Yet, no study has comprehensively investigated whether sexism is associated with altered brain structure and escalated gender-biased bullying. This study examined the relationships among sexism and bullying behavior with individual brain structure accounting for variation. We focused on the volume of the nucleus accumbens (NAc) as a mediator due to its role in social aspects of reward processing and involvement in aggression. We hypothesized that the relationship between sexism and gender-biased bullying existed indirectly, mediated by NAc

volume. Young adult participants (N=47) underwent T1-weighted Magnetic Resonance Imaging (MRI) scan (MP-RAGE, voxel volume = 1.0 x 1.0 x 1.0 mm^3) on a 3T Siemens Skyra scanner, with 32ch brain array coil. Then, participants were asked to report their history of bullying girls and boys respectively during schools. Gender-biased bullying was computed by dividing the number of girls who participants bullied by that of boys who participants bullied. Participants also completed the Ambivalent Sexism Inventory (ASI) to assess hostile and benevolent sexism. FreeSurfer was applied to MP-RAGE to measure the estimated intracranial volume (eTIV) and the bilateral NAc volumes. Then, we first tested correlations among hostile/benevolent sexism, NAc volumes, and gender-biased bullying in those with/without a history of bullying. Based on our correlations, we further tested the model where NAc volume mediated in the relationship between sexism and gender-biased bullying, with the bootstrapping method.Results revealed that hostile sexism, right NAc volume, and gender-biased bullying were associated with each other in those with a history of bullying (all p's<0.05). Furthermore, increased hostile sexism was associated with reduced right NAc volume (t=-2.50, p<0.05), which in turn increased gender-biased bullying (t=-2.46, p<0.05); when right NAc volume was controlled for, the relationship between hostile sexism and gender-biased bullying (t=2.79, p<0.05) turned to nonsignificant (t=1.47, p=0.16). In contrast, benevolent sexism or left NAc volume was not related to gender-biased bullying. These findings suggest that bullies with strong hostile sexism tend to have smaller right NAc volume. When their right NAc volume is reduced, they subsequently target female victims more than male victims. The role of the NAc in the context of sexism and bully needs to be further investigated.

Disclosures: E. Hamel: None. **H. Suzuki:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.11

Topic: G.03. Motivation

Support: NIH Grant MH119422

Title: Social investigation of stressed juveniles is mediated by basolateral amygdala projections to the insular cortex

Authors: *A. DJERDJAJ, N. B. WORLEY, A. J. NG, N. S. RIEGER, J. P. CHRISTIANSON; Psychology, Boston Col., Chestnut Hill, MA

Abstract: Social vocalizations, chemosignals, and behavioral expressions of one's internal affective state shape the selection of social behavior responses made by others who detect these cues. A network of interconnected brain structures called the social decision making network (SDMN) integrates these stimuli and network output yields social behaviors that are appropriate for a given interaction. Deficits in SDMN connectivity underlie several psychiatric disorders

such as schizophrenia and autism so further understanding the function of the SDMN may grant us new insight into aberrant social behaviors. The basolateral amygdala (BLA) and the insular cortex (IC) are reciprocally connected and each contribute to social and emotional behaviors. We explored the involvement of BLA projections to the IC in a social affective preference (SAP) test in which a test rat is presented with 2 juvenile conspecifics (PN30), 1 naïve to treatment and 1 stressed via 2 footshocks. Test rats prefer interaction with the stressed juvenile in this paradigm. Chemogenetic inactivation of the BLA via bilateral transduction of AAV-hSyn-hM4D(Gi)-mCherry followed by systemic administration of clozapine-N-oxide (3mg/kg) prior to SAP tests prevented test rat preference for the stressed juvenile. Further, inhibition of IC-projecting BLA neurons via CNO injection (1μM) directly into the IC also prevented preference for the stressed juvenile. Additionally, DeepLabCut, a machine learning algorithm that can be implemented for markerless pose estimation, was used in conjunction with semantic segmentation to obtain unbiased behavioral data detailing the amount of time each rat spent investigating each conspecific. Ongoing investigations include transsynaptic tracing to determine BLA-IC projection patterns and in vivo Ca2+ imaging of BLA neuronal activity during encounters with stressed conspecifics to observe neural signatures of this social behavior. The current results identify a new tract by which social emotional information is processed within the SDMN.

Disclosures: **A. Djerdjaj:** None. **N.B. Worley:** None. **N.S. Rieger:** None. **J.P. Christianson:** None. **A.J. Ng:** None.

Digital Abstract Session

P273. Motivation: Social Communication, Behavior, and Sex Differences in Animals and Humans

Program #/Poster #: P273.12

Topic: G.03. Motivation

Support: Intramural Research Program National Institutes of Health

Title: Mapping the neural correlates of approach and avoidance in adolescent social anxiety: An fMRI analysis of brain response during peer observation

Authors: *J. BEZEK, A. SMITH, Q. DO, M. YETTER, E. CARDINALE, D. PINE;
Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Introduction: Adolescence is a sensitive developmental period defined by ongoing prefrontal maturation and an increased focus on peer interactions. However, this emphasis is often marked by enhanced fear of negative evaluation and greater risk for developing social anxiety. The conflict between social motivation to engage and fear of judgement reveals a complex interaction between approach and avoidance behaviors. However, most previous work in pediatric anxiety has focused on the avoidance system. The present study expanded this work by investigating the neural mechanisms underlying both approach and avoidance motivations in an evaluative social context.

Methods: Adolescents aged 8-17 (N=52; Age: M=14.26, SD=2.46) with a range of anxiety symptoms completed the BIS/BAS questionnaire to assess avoidance and approach tendencies, respectively (Carver & White, 1994). Errors during a cognitive control task (Eriksen Flanker), defined as indicating the incorrect direction of the center arrow, were measured during functional magnetic resonance imaging. The task was adapted such that participants believed they were being observed by an age- and sex-matched peer for half of the task and alone for the other half. Whole-brain analyses used BIS and BAS scores as continuous, between-subjects variables, while social context (peer, alone) and trial type (error, correct) were within-subject variables. All analyses controlled for age.
Results: Preliminary analyses revealed that high approach and avoidance scores were associated with distinct prefrontal activation patterns. Adolescents with higher approach scores showed decreased ventromedial prefrontal cortex (vmPFC) activity during peer observation relative to when they were alone ($r=-.41$, $p<.005$, $k=63$). Higher avoidance scores were associated with decreased medial frontal gyrus (mPFC) activation when youths committed an error in front of a peer relative to alone ($r=-.44$, $p<.005$, $k=80$).
Discussion: The current analysis revealed that youths with higher approach motivations demonstrated less prefrontal activation in regions typically associated with self-referential thought (i.e., vmPFC) when observed by peers. Alternatively, youths with stronger avoidance tendencies showed less mPFC recruitment, a region essential to engaging cognitive control, during peer-observed errors. This work suggests there are unique prefrontal profiles underlying distinct social motivations during this sensitive neurodevelopmental period. Dissecting these neural mechanisms is an important step toward informing interventions against maladaptive approach and/or avoidance behaviors in adolescence.

Disclosures: J. Bezek: None. **A. Smith:** None. **Q. Do:** None. **E. Cardinale:** None. **D. Pine:** None. **M. Yetter:** None.

Digital Abstract Session

P274. Emotional-Cognitive Representations in The Human Brain

Program #/Poster #: P274.01

Topic: G.04. Emotion

Support: NIH Grant MH112558

Title: Mapping the time course of brain activation in affective picture processing

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Abstract: Viewing emotionally engaging scenes activates a large-scale brain network consisting of both cortical and subcortical regions. Mapping the time course of activation of these regions is a key step toward developing a theoretical account of affective scene processing. For aversive scene content, past work has suggested rapid processing along the subcortical pathway, superior

colliculus->pulvinar->amygdala, the function of which is to provide the context for the more deliberate processing along cortical visual pathway. Less is known about the neural processing of pictures displaying neutral and pleasant content. We recorded simultaneous EEG and fMRI data from 20 participants viewing pleasant (20), unpleasant (20) and neutral (20) pictures from the International Affective Picture System (IAPS). Following data preprocessing, representational dissimilarity matrices (RDMs) were obtained from fMRI in pulvinar, amygdala, early visual cortex, ventral visual cortex, and dorsal visual cortex for each of the three picture categories, as well as from EEG at each time point. RDMs from EEG at each time point were correlated with that from fMRI in each ROI. From the correlation functions peak latencies were estimated, the peak latency data suggested the following sequence of activation: (1) for unpleasant pictures: pulvinar->amygdala->early visual cortex->ventral/dorsal visual cortex, (2) for neutral pictures: pulvinar->amygdala/early visual cortex->ventral/dorsal visual cortex, and (3) for pleasant pictures: amygdala->pulvinar->early/ventral visual cortex->dorsal visual cortex. These results shed new light on affective scene processing and demonstrate that combining simultaneous EEG-fMRI with multivariate methods can yield spatial-temporal dynamics not possible with each method alone.
Keywords: emotion; representational similarity analysis; fMRI; EEG; multivariate pattern analysis.

Disclosures: **L. Cui:** None. **K. Bo:** None. **A. Keil:** None. **M. Ding:** None.

Digital Abstract Session

P274. Emotional-Cognitive Representations in The Human Brain

Program #/Poster #: P274.02

Topic: G.04. Emotion

Support: NIH Grant R01 MH112558.

Title: Decoding Neural representation of Affective scenes in Retinotopic Visual Cortex

Authors: ***K. BO**[1], S. YIN[1], Y. LIU[2], Z. HU[1], S. MEYYAPAN[1], S. KIM[1], A. KEIL[3], M. DING[3];
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Abstract: The perception of opportunities and threats in complex scenes represents one of the main functions of the human visual system. The underlying neurophysiological basis is often studied by having observers view pictures varying in affective content. It is shown that viewing emotionally engaging, compared to neutral, pictures (1) heightens blood flow in limbic, frontoparietal, and anterior visual structures, and (2) enhances late positive event-related potential (LPP). The role of retinotopic visual cortex in this process has, however, been contentious, with competing theories predicting the presence versus absence of emotion-specific signals in retinotopic visual areas. Recording simultaneous EEG-fMRI while observers viewed

pleasant, unpleasant, and neutral affective pictures, and applying multivariate pattern analysis, we found: (1) unpleasant-versus-neutral and pleasant-versus-neutral decoding accuracy, were well above chance level in all retinotopic visual areas, (2) decoding accuracy in ventral visual cortex (VVC), but not in early or dorsal visual cortex, was correlated with LPP, and (3) effective connectivity from amygdala to VVC predicted unpleasant-versus-neutral decoding accuracy, while that from ventral frontal cortex to VVC predicted pleasant-versus-neutral decoding accuracy. These results suggest that affective scenes evoked valence-specific neural representations in retinotopic visual cortex and that these representations were influenced by reentry signals from anterior brain regions.

Disclosures: **K. Bo:** None. **S. Yin:** None. **Y. Liu:** None. **Z. Hu:** None. **S. Meyyapan:** None. **S. Kim:** None. **A. Keil:** None. **M. Ding:** None.

Digital Abstract Session

P274. Emotional-Cognitive Representations in The Human Brain

Program #/Poster #: P274.03

Topic: G.04. Emotion

Title: Distracted by affective pictures: Neural mechanisms revealed by multivariate pattern analysis

Authors: K. BO[1], ***C. XIONG**[1], N. PETRO[1], A. KEIL[2], M. DING[2];
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Abstract: Distracted by affective pictures: Neural mechanisms revealed by multivariate pattern analysis
Ke Bo[1], Changhao Xiong[1], Nathan M. Petro [2], Andreas Keil[3], Mingzhou Ding[11]J Crayton Pruitt Family Department of Biomedical Engineering, University of Florida[2]Department of Psychology, University of Nebraska at Lincoln[3]Department of Psychology and the NIMH Center for Emotion and Attention, University of Florida
Affective pictures are highly potent distractors. In this study we examined the impact of picture valence on task-relevant visual processing and the underlying neural mechanisms. Simultaneous EEG-fMRI were recorded while participants detected instances of coherent motion in a random dot kinematogram (RDK) overlayed on IAPS pictures (pleasant=erotic couples, neutral=workplace people, and unpleasant=bodily mutilations). Applying support vector machines to BOLD responses in ventral visual cortex (VVC) and MT cortex we found the following results. First, decoding accuracy of both pleasant-vs-neutral and unpleasant-vs-neutral distractors was above chance level in both VVC and MT cortex, at 62.6% and 59.4% for VVC , 71.2% and 64.5% for MT, respectively. Second, in early period of VVC (3-5 TR after stimulus onset) across subjects, decoding accuracy of unpleasant-vs-neutral distractors was negatively correlated with the correctly identified instances of coherent motion ($p=0.01$), namely, the higher the decoding accuracy the lower behavior performance; decoding accuracy of pleasant-vs-neutral distractors, however, was not associated with behavioral performance. Third, in MT we found

similar effect (p =0.019) on unpleasant-vs-neutral distractors with VVC in late period (6-8 TR after stimulus onset) yet pleasant-vs-neutral distractors did not show such effect. In summary, these results demonstrated that (1) although pleasant distractors were better represented than unpleasant distractors in these two ROIs, it was the unpleasant distractors that had a stronger adverse influence on behavior and (2) Unpleasant distractor represents on VVC and MT to adversely impacted behavior of ongoing task in sequence.
Keywords: Emotional distractor; positive and negative emotion; simultaneously EEG-fMRI; multivariate pattern analysis; visual cortex

Disclosures: **C. Xiong:** None. **K. Bo:** None. **N. Petro:** None. **A. Keil:** None. **M. Ding:** None.

Digital Abstract Session

P274. Emotional-Cognitive Representations in The Human Brain

Program #/Poster #: P274.04

Topic: G.04. Emotion

Support: Hyundai NGV grant

Title: Deep Brain Stimulation of the Anterior Nucleus of Thalamus Neutralizes the Effects of Emotions on Attention.

Authors: ***H. N. SONG**[1], Y. RAH[2], S. A. LEE[2];
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Abstract: Deep brain stimulation (DBS) is an established treatment method for medication resistant epilepsy patients. The anterior nucleus of thalamus (ANT), a component of the Papez circuit, is one of main target regions for DBS treatment of epilepsy. However, there are reports that one of the adverse side effects of such treatment is abnormal emotional response. According to previous studies, the ANT-DBS may affect the interaction between attention and emotion. Taken together, this study focused on how the ANT-DBS changes the effects of emotions on attention. Five refractory epilepsy patients participated in this study, and one of them was rejected due to impairment of cognitive ability. For experimental conditions, three types of emotional pictures (neutral, positive, and negative) were selected from International Affective Picture System (IAPS). There were three DBS conditions (no-stim, left, and right), and the intensity of stimulation was 1.5V with 90usec of pulse width and 130Hz of frequency. In every trial, the picture was briefly presented (75ms) and masked by another neutral picture non-overlapping (225ms). Then, a cued flanker task was immediately presented in which patients had to press a button indicating the correct direction of a target arrow. Subjects were instructed to focus on the flanker task and to respond as quickly as possible. We found that, without DBS, patients tended to react more slowly when they were primed by negative emotion, replicating findings from previous studies that showed that emotional stimuli, in particular negative emotions, interfere with attention. With DBS, however, this effect was neutralized in that

reaction times following emotional pictures were significantly faster than that without DBS and identical to the neutral pictures condition. In contrast, DBS did not affect performance in the neural condition. ANT-DBS may have interrupted the processing of emotion and, consequently, its interference on attention. This effect of DBS was observed in every subject.

Disclosures: H.N. Song: None. **S.A. Lee:** None. **Y. Rah:** None.

Digital Abstract Session

P274. Emotional-Cognitive Representations in The Human Brain

Program #/Poster #: P274.05

Topic: G.04. Emotion

Title: The representations of realistic events are grounded in semantic and emotional brain regions.

Authors: *M. RIBERTO[1,2], R. PAZ[1], G. POBRIC[2], D. TALMI[3];
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Abstract: Background. Human beings render events meaningful by categorising and attributing an affective value to them. Investigating the neural correlates of high-level representations of realistic events is of paramount importance for the research on semantic and social cognition, as well as mood and anxiety disorders. Different questions are still unsolved in this field. Which regions are involved in the representation of realistic events? Does this depends on the aspects (visual, semantic, and emotional) of the stimuli participants focus on during experimental paradigms? We know that different regions encode and integrate the relevant information about each stimulus, but it is not known whether its emotional value is encoded separately.**Methods.** We selected 72 complex pictures from freely-available sources, which depicted 4 separate categories of realistic events. Two were neutral (people doing laundry, people on the phone) and 2 negative emotional (poverty scenes, car accidents). The categories differed in valence and arousal, and were balanced for visual properties. During a functional Magnetic Resonance Imaging (fMRI) scan, participants (n=29) performed a visual complexity rating of the pictures. After the scan, they arranged these pictures in a bidimensional space according to the similarity among them (multi-arrangements, MA, task). We used searchlight Representation Similarity Analysis (RSA) to unveil which brain region represented the participants' similarity space. The brain activation-patterns matrix was obtained by moving a 3x3x3 spherical cluster throughout the brain and at each location a correlational distance (1-correlation) among t values is assigned to the centre voxel of the sphere. This was then correlated with the behavioural similarity matrix. Inference was performed at each voxel by performing a signed rank test across subjects (p_{FDR} <0.05). **Results.** In the behavioural experiment, we found higher dissimilarity between categories, than within category (p_{FWE}<0.001), and lower dissimilarity within the 'car accidents' category compared to the other within category conditions (p_{FWE}<0.05). The behavioural data significantly correlated with the neural patterns in the Inferior Temporal Cortex (ITC) (right:

x=48, y=-58, z=-10; Z=5.28; left: x=-48, y=-46, z=-22; Z=5.32) and in the Precuneus (right: x=9, y=-58, z=-10; Z=4.86; left: x=-6, y=-58, z=29; Z=4.86).**Conclusions.** The ITC is involved in the semantic representations of realistic events and the Precuneus in the representation of their affective dimension. This is separately encoded, despite of the aspects participants focused on during the task.

Disclosures: **M. Riberto:** None. **R. Paz:** None. **G. Pobric:** None. **D. Talmi:** None.

Digital Abstract Session

P274. Emotional-Cognitive Representations in The Human Brain

Program #/Poster #: P274.06

Topic: G.04. Emotion

Support: Brain Research Program (NRF-2017M3C7A1031333) through the National Research Foundation (NRF)
Basic Science Research Program (NRF-2020R1A2C2007770) through the National Research Foundation (NRF)
Technology Innovation Program (20012464) funded by the Ministry of Trade, Industry & Energy (MOTIE) of Korea

Title: Individual negativity bias is associated with biased neural processing weighing negative information in the salience network

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Abstract: The recognition of emotions through facial expressions is essential for our interpersonal communication. Negativity bias in facial emotion recognition, a tendency to interpret ambiguous facial expressions more negatively than they actually are, can therefore lead to difficulties in our social interactions. The negativity bias is thought to be caused by over-weighted negative information over positive or neutral information during the process of information integration. However, this possibility has not yet been clearly determined. Given that the salience network (SN) is considered to play a key role in the multiple information integration to evaluate subjective saliency, here we investigated whether individual negativity bias in face emotional recognition is mediated by the SN information processing. Using functional magnetic resonance imaging and neural pattern analyses for facial emotions, we found that the degree of individual negativity bias depends on how much weighted negative information is integrated into the neural representation of the SN. This tendency was not observed for positivity bias, and was not detected in other networks such as the higher visual network or default mode network. These findings suggest that individual negativity bias is critically associated with the biased information integration weighing negative information in the SN.

Disclosures: **G. Kim:** None. **T. Kim:** None. **S. Lee:** None.

Digital Abstract Session

P274. Emotional-Cognitive Representations in The Human Brain

Program #/Poster #: P274.07

Topic: G.04. Emotion

Title: Emotion regulation training induces wide-spread changes in functional activation and connectivity

Authors: *O. L. COOK, J. KANE, K. HUNT, B. DEPUE;
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Abstract: Emotion Regulation Training Induces Wide-Spread Changes in Functional Activation and Connectivity Cook, O., Kane, J., Hunt, K., Naaz, F. & Depue, B.University of Louisville
"Brain Training" programs are becoming more ubiquitous in mainstream society. However, there are relatively few studies exploring whether specific task related training leads to functional plasticity in the brain. Furthermore, no studies exist, to our knowledge, that explore training an individual's ability to down-regulate emotion reactivity. Importantly, understanding which neural regions show changes across training highlights important brain mechanisms involved in emotion regulation processes; processes which are critical to efficient daily functioning. We explored this question using fMRI before and after emotion regulation training. Eighteen undergraduate students were recruited in a 5-day training study. Participants were scanned on day 1 and day 5. The emotion regulation task and training consisted of a standard emotion regulation paradigm, in which participants were asked to down-regulate their physiological response to negatively valanced IAPS pictures. A subjective emotion ratings task was performed before (baseline) and approximately 30 minutes later (ER-rating) required participants to interrogate their subjective emotional feeling toward IAPS pictures. Behaviorally, a significant decrease in subjective negative emotion ratings occurred on day 5 compared to day 1 ($p<.03$; M=2.1/2.6). Functional analyses revealed decreased activation in the dorsal medial prefrontal cortex (dmPFC), right inferior and middle frontal gyri (rIFG/rMFG), and both ventral and dorsal amygdalae on day 5 compared to day 1. Conversely, increased activation was seen in the orbitofrontal cortex (OFC) on day 5 compared to day 1. Using the above regions in functional connectivity analyses, revealed that while functional activation decreased, increased functional connectivity was observed in the rMFG-OFC and OFC-amygdalae.

Disclosures: O.L. Cook: None. **J. Kane:** None. **K. Hunt:** None. **B. Depue:** None.

Digital Abstract Session

P274. Emotional-Cognitive Representations in The Human Brain

Program #/Poster #: P274.08

Topic: G.04. Emotion

Support: NIH Grant DA023248
NIH Grant DA045189
NIH Grant DA044749

Title: Gray matter volumetric correlates of impulsivity in children: an adolescent brain cognition development project

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Abstract: Previous research has investigated the cerebral volumetric correlates of impulsivity, largely in moderate-sized samples, and the findings varied extensively across studies. Here, we followed published routines and performed voxel-based morphometry analysis of a data set (n=11,542; 5,491 girls, 9-10 years) curated from the Adolescent Brain Cognition Development project. Of the sample, 648 and 2,697 were identified as monozygotic twins and dizygotic twins/siblings, respectively. In linear regression with each of the subscores of the UPPS-P, we aimed to identify volumetric correlates of negative urgency, positive urgency, sensation seeking, lack of planning and lack of perseverance in girls and boys combined as well as separately. The results showed both sex- shared and specific correlates, involving a wide array of cortical and subcortical regions, for negative urgency, positive urgency, sensation seeking, and lack of planning. In contrast, lack of perseverance was distinctly associated with lower volumes of bilateral putamen across all subjects, with a steeper loss of volumes in boys than in girls as confirmed by a slope test. Further, genetic modeling showed that whereas negative urgency, positive urgency, sensation seeking, and lack of planning subscores were more amenable to unique environmental influences (h^2 ~0.25, e^2~0.74), lack of perseveration showed a strong heritability (h^2 ~0.79, e^2~0.47) in both girls and boys. The volumetric correlates showed strong heritability (h^2 ~0.70) for all five subscores. These findings highlight the neural markers of a critical personality trait and suggest a unique role of putamen volume in determining lack of perseverance. The findings may inform future studies in investigating the inter-relationship between genetics, cortical and subcortical volumes, and clinical manifestations, including those that specifically implicate putamen GMV deficits.

Disclosures: C. Li: None. **S. Zhornitsky:** None. **J.S. Ide:** None. **C.R. Li:** None.

Digital Abstract Session

P274. Emotional-Cognitive Representations in The Human Brain

Program #/Poster #: P274.09

Topic: G.04. Emotion

Support: uni:docs scholarship, University of Vienna
FWF Grant W1262-B29
WWTF Grant VRG13-007
Doctoral program Cognition and Communication, University of Vienna

Title: Is your pain my pain? Effects of localized placebo analgesia on empathy for everyday painful situations

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Abstract: Empathy for pain involves the affective-motivational and the sensory-discriminative pain processing network. The shared representations account of empathy postulates that sharing another person's pain recruits similar underlying brain functions that are also engaged during first-hand pain processing. Previous research underlines this, reporting reduced empathy for pain when reducing first-hand pain by means of placebo analgesia. Critically, causal experimental evidence for shared representations has so far only been shown in brain areas related to affective pain processing, while the specific contribution of one's own somatosensory system to empathic responding remains controversial. The experimental paradigms used in previous studies did not direct attention towards a specific body part in pain and relied on inferring the other's pain via abstract cues, which could explain the absence of effects. In this study, we thus aimed to test more specifically whether a localized causal manipulation of first-hand pain affects somatosensory processing during empathy for everyday painful situations in a body part-specific manner. This study was preregistered prior to data collection (https://osf.io/uwzb5) and the main analyses are thus confirmatory, but we also include exploratory analyses. Forty-five participants (right-handed, 23 females, age: M/SD = 23.8/2.7 years) underwent a localized placebo analgesia induction targeted at the right hand (with the left hand acting as a control) and completed an empathy for pain task in the MRI scanner where they observed pictures of either right or left hands in pain and rated the pain intensity of the person in the picture as well as their own unpleasantness. Contrary to our predictions, we did not find evidence for a location-specific modulation of empathy for pain as a result of the placebo induction, in neither behavioral nor neural measures. However, exploratory data analysis revealed a general downregulatory effect of the placebo on empathy for pain, and increased brain activity in bilateral anterior insula when viewing other's hands in pain that corresponded one's placebo hand. Hence, somatosensory sharing during empathy does not appear to be modulated by placebo analgesia in a location-specific manner. These results refine our knowledge regarding the mechanisms underlying empathy for pain.

Disclosures: H. Hartmann: None. **F. Riva:** None. **M. Rütgen:** None. **C. Lamm:** None.

Digital Abstract Session

P275. Emotions: Preclinical Models, Human Studies, and Therapeutic Approaches

Program #/Poster #: P275.01

Topic: G.08. Other Psychiatric Disorders

Support: emerging Research Innovators in Mental Health from the Royal Ottawa Institute of Mental Health Research

Frederick Banting and Charles Best Canada Graduate Scholarship from the Canadian Institute of Health Research

Title: Neural mechanisms and predictors of response to electroconvulsive therapy: a transdiagnostic approach

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Abstract: Background. Electroconvulsive therapy (ECT) is an effective treatment for many mental health disorders. Unfortunately, there are often cognitive side effects associated with ECT and not all patients will show clinical response. Developing a neurobiological predictor of outcome to identify which patients will benefit from ECT is essential to avoid unnecessary impairments for those who do not respond. In addition, the neural mechanisms of ECT are not well understood and research into the cognitive symptoms is inconsistent. **Objectives.** Using an open-label naturalistic approach, this exploratory study aims to identify neural mechanisms and neurobiological predictors of cognitive and clinical outcome, as well as monitor the effects of ECT on cognition. **Methods.** We will recruit 60 males and females, 18 to 65 years old, over two years with diverse mental health disorders (i.e. depression, psychosis) receiving ECT treatment. Using a Magpro X100 device (Magventure, Denmark), transcranial magnetic stimulation (TMS) will be applied to the left primary motor cortex to non-invasively explore how excitatory and inhibitory brain mechanisms are linked to clinical and cognitive outcome. We will use a standard TMS procedure: 1) single pulse TMS, 2) short interval intracortical inhibition, 3) long interval intracortical inhibition and 4) intracortical facilitation. For each of the four measures, 20 pulses will be sent every 5-7 seconds while electromyography signals are recorded on the left first dorsal interosseous muscle, reflecting corticospinal excitability. TMS recordings will be conducted pre- and post-ECT which will be correlated with clinical and cognitive outcome. We will use a novel cognitive screening tool, i.e. the ElectroConvulsive Therapy Cognitive Assessment (ECCA), which is a quick assessment of cognitive domains (orientation, attention, verbal delayed recall, factual knowledge, and autobiographical memory) commonly affected by ECT. ECCA will be administered before ECT and at two time points after. Repeated measures analysis of variance, correlational analyses and logistic regression models will be conducted for each subgroup diagnosis, ECT parameters, and biological sex. Due to COVID-19, our study is currently only collecting clinical and cognitive data remotely, and we do not have enough data at this time to report results. **Conclusion.** We are hopeful when research restrictions are lifted, this study will determine neural mechanisms of ECT, neurobiological predictors of clinical and cognitive outcome, and further validate the efficacy of the ECCA. This research is crucial to help guide clinicians and patients in their decision to receive ECT.

Disclosures: **M. Watson:** None. **S. Tremblay:** None. **L. McMurray:** None. **C. Tizzard:** None. **A. Gebara:** None. **S. Guimond:** None.

Digital Abstract Session

P275. Emotions: Preclinical Models, Human Studies, and Therapeutic Approaches

Program #/Poster #: P275.02

Topic: G.08. Other Psychiatric Disorders

Support: Max E. Binz Fellowship
RQHSA Student Award

Title: Profiling extracellular vesicles in the anterior cingulate cortex of individuals with Major Depressive Disorder

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Abstract: Major Depressive Disorder (MDD) is one of the leading causes of disability worldwide, affecting 20% of the population. MDD also confers a 20-fold higher risk of suicide. Environmental factors are thought to play a role in disease development, resulting in biological changes mediated by epigenetic mechanisms. MicroRNA's (miRNA) are well known epigenetic regulators that are disrupted in the depressed brain, and they are packaged into extracellular vesicles (EVs). EVs have emerged as means of intercellular communication, a process that is also disrupted in MDD. They are thought to transfer miRNA, as well as other bioactive molecules such as proteins, between cells. This can alter gene expression in recipient cells. Different cell types in the brain have been shown to release EVs, so it is possible that EVs might play a role in the pathogenesis of central nervous system disorders, including MDD. Therefore, we hypothesize that EV cargo from the anterior cingulate cortex, a brain region highly implicated in MDD, will have a disease specific profile that could mediate disease development in MDD subjects compared to healthy controls (HC). We aim to isolate EVs from human post-mortem anterior cingulate cortex, profile the miRNA and protein cargo, and compare it between MDD subjects and HC. EVs were isolated from post-mortem human brain tissue from the anterior cingulate cortex of 43 MDD subjects and 43 HC using size exclusion chromatography. The quality was assessed by western blots and transmission electron microscopy (TEM). RNA was extracted and a small-RNA library was constructed and sequenced using the Illumina Platform. Proteins were also extracted and profiled using LC-MS/MS. Differential expression analysis was then performed. Western blots showed little to no contamination with cellular debris, along with enrichment of the exosomal marker CD9. TEM images showed the typical cup-shaped morphology with sizes mostly between 30 and 200 nm. Preliminary differential analyses revealed that both the miRNA and proteomic profiles of the EVs are dysregulated in MDD. In conclusion, high quality EV extractions can be obtained from post-mortem brain tissue using our method. This will be the first study to profile brain-derived EV miRNA and protein in the context of depression. Future studies will be needed to determine the effect of the

dysregulated EV cargo in MDD. This could provide novel mechanistic insights into the pathophysiology of MDD and will serve as a starting point to examine the potential role of EVs in MDD pathology, which could be a starting point for the development of targeted therapeutic strategies as well as prevention measures.

Disclosures: P. Ibrahim: None. **C. Nagy:** None. **J. Théroux:** None. **Z. Aouabed:** None. **G. Turecki:** None.

Digital Abstract Session

P275. Emotions: Preclinical Models, Human Studies, and Therapeutic Approaches

Program #/Poster #: P275.03

Topic: G.08. Other Psychiatric Disorders

Support: T32 MH-15330
Dalio Philanthropies

Title: Activity based anorexia results in oxidative stress in adolescent female rats: Systemic and central evidence.

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Abstract: Anorexia Nervosa (AN) is a devastating eating disorder characterized by excessive exercise, starvation and weight loss. Our lab utilizes the activity based anorexia (ABA) rodent paradigm to study the behavioral and neural consequences of AN. The ABA paradigm combines time limited feeding with unlimited running wheel access, which results in voluntary hyperactivity, voluntary starvation and ultimately rapid weight loss. Previous studies have found that patients with AN have significant deficits in glutathione, one of the body's primary antioxidants, and patients with the lowest BMI also had the lowest glutathione levels, suggesting there may be a link between oxidative state and severity of AN. To determine whether alterations in oxidative state also occur in response to ABA, we examined plasma glutathione and cysteine, a precursor for glutathione, levels in ABA adolescent female rats (n=6/timepoint) sacrificed at maximum weight loss or 10 days weight recovered. These values were compared to levels in age-matched sedentary (ad lib chow + locked wheel; n=6/timepoint) controls. We found that ABA animals at max weight loss, but not weight recovered, have significant deficits in both cysteine ($p=0.0467$; $t=2.269$; $df=10$) and glutathione ($p=0.0478$; $t=2.255$; $df=10$). We then probed the medial prefrontal cortex (mPFC) for mitochondrial fission and found that ABA animals sacrificed at maximum weight loss, but not when weight recovered, have increased mitochondrial fission compared to sedentary control ($p=0.0056$; $t=3.509$; $df=10$), again suggesting increased oxidative stress. We conducted a follow up study to assess the impact of the

ABA paradigm on mPFC parvalbumin interneuron density as oxidative stress could lead to the permanent impairment of these cells. In brief, a separate cohort of animals (8 sedentary; 10 ABA) were perfused for double immunohistochemistry analysis of mPFC parvalbumin (PV) and 8-Oxo-2'-deoxyguanosine (8-oxo-dG), a single cell marker for DNA-oxidation. Raters counted the total number of PV positive cells in a 10x image and then quantified corrected total cell florescence of 8-oxo-dG in 20 of the PV positive cells. We found that ABA animals have significantly fewer PV cells ($p=0.0013$; $t=3.889$; $df=16$) than sedentary controls and, on average, the PV cells in the ABA animals had significantly greater 8-oxo-dG compared to levels in PV cells from sedentary controls ($p=0.0018$; $t=3.732$; $df=16$). Taken together, these data demonstrate that ABA produces significant peripheral and central signs of oxidative stress resulting in PFC neuronal loss.

Disclosures: **M.M. Hurley:** None. **K. Murlanova:** None. **S.C. O'Brien:** None. **M.V. Pletnikov:** None. **L.K. Macias:** None. **M. Tarekegne:** None. **A.N. Nawari:** None. **H. Bhasin:** None. **A.I. Sabir:** None. **K.L.K. Tamashiro:** None. **T.H. Moran:** None.

Digital Abstract Session

P275. Emotions: Preclinical Models, Human Studies, and Therapeutic Approaches

Program #/Poster #: P275.04

Topic: G.08. Other Psychiatric Disorders

Support: NIH NIA F30AG066329

Title: Characterizing gene-environment interactions in a preclinical model of psychiatric disease

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Abstract: Psychiatric disease remains one of the leading causes of disability worldwide, yet it is still unclear how these diseases develop. Both genetics and environmental factors are thought to play a significant role in the development of psychiatric illness and understanding how these factors might interact can help elucidate the underlying mechanisms of disease. In order to examine the gene-environment interaction that might occur in psychiatric disease, we paired the *Disc1 svΔ2* pre-clinical rat model of psychiatric disease with exposure to chronic early-life stress (CES), in the form of a limited bedding paradigm, and evaluated their individual and combinatory effects on behavior and brain microstructure. *Disc1 svΔ2* animals and wild-type controls were maintained in either cages with ample bedding or cages with limited bedding during postnatal days 2-9. A behavioral battery, including the open-field test, Y-maze, and elevated-plus maze, was conducted between P180-P190, and brains were imaged *ex-vivo* for subsequent DTI and multi-compartment diffusion weighted imaging studies. Both the *Disc1* mutation and CES individually resulted in hypoactivity and heightened anxiety in both male and female animals. However, *Disc1 svΔ2* animals also demonstrated global, sex-specific changes in white and gray matter microstructure, whereas CES preferentially affected female brains. *Disc1*

svΔ2 and CES combined was associated with hyperactivity and more severe anxiety in comparison to animals that received either genetic or environmental insult alone, and female *Disc1 svΔ2* brains were also more strongly impacted by CES. Our results suggest a significant interaction between the *Disc1 svΔ2* mutation and chronic early-life stress, and the ability of advanced multi-compartment diffusion weighted neuroimaging methodologies to dissect sex-specific gene-environment interactions in psychiatric illness.

Disclosures: S.Y. Yi: None. **B.R. Barnett:** None. **J.J. Yu:** None.

Digital Abstract Session

P275. Emotions: Preclinical Models, Human Studies, and Therapeutic Approaches

Program #/Poster #: P275.05

Topic: G.08. Other Psychiatric Disorders

Title: Anxiogenic effects of progesterone withdrawal and GABA/benzodiazepine receptor binding: Wistar Kyoto rats as a suitable experimental subject to study Premenstrual Dysphoric Disorder.

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Abstract: Anxiety is one of the most common emotional symptoms of Premenstrual Dysphoric Disorder (PMDD). This might be related to an abrupt drop of progesterone towards the late luteal phase of the menstrual cycle and to abnormalities in the hypothalamus-pituitary-adrenal (HPA) axis, which may lead to impairments in $GABA_A$/benzodiazepine receptors. PMDD is considered a chronic stress-related disorder, hence stress susceptibility could play an important role in developing symptomatology. Thus, it is crucial to have an animal model that represents the stress vulnerability observed in PMDD women. Therefore, we evaluated the response of a stress-vulnerable strain, the Wistar-Kyoto rats (WKY), to the Progesterone withdrawal (PW) challenge in some core aspects of the PMDD: anxiety symptoms, HPA axis activity and $GABA_A$ receptor sensitivity. All experiments were performed using Wistar rats (W) as a control strain. Thus, female WKY and W rats were ovariectomized and administered with 1.5 mg/kg of progesterone for five days, and on day sixth, were tested in the anxiety-like burying behavior test (BBT). After BBT completion, animals were euthanized and trunk blood was collected to measure corticosterone levels. Brains were extracted to measure $GABA_A$/Benzodiazepine binding. An independent group was administered with different doses of Diazepam to explore the $GABA_A$/Benzodiazepine sensitivity. Results showed that PW induced anxiety-like behaviors in both strains and is exacerbated in WKY rats along with corticosterone concentrations. Differential strain-dependent [3H]-Flunitrazepam binding pattern was observed, as PW increased the [3H]-Flunitrazepam binding in the DG and central amygdala only in WKY rats, suggesting a

greater sensitivity to PW in the stress-vulnerable strain. WKY rats showed higher sensitivity to Diazepam but induced an anxiogenic-like response only in PW rats, which may parallel the observed in PMDD women. Our findings suggest that WKY rats could be a suitable experimental subject to explore the neurobiology of PMDD.

Disclosures: D. Islas-Preciado: None. **C. Lopez-Rubalcava:** None. **I. Sollozo-Dupont:** None. **G. Ugalde-Fuentes:** None. **E. González-Trujano:** None. **E.M. Estrada-Camarena:** None.

Digital Abstract Session

P275. Emotions: Preclinical Models, Human Studies, and Therapeutic Approaches

Program #/Poster #: P275.06

Topic: G.08. Other Psychiatric Disorders

Support: the Tallinn University ASTRA project TU TEE financed by the European Union European Regional Development Fund (2014-2020.4.01.16-0033)
Horizon 2020 Programme Project CoCA Eat2beNICE (n° 728018)

Title: Associations between impulsivity and nutrient intake in a longitudinal birth cohort study

Authors: *D. MATROV[1], T. KURRIKOFF[2], I. VILLA[3], K. SAKALA[3], A. PULVER[1], T. VEIDEBAUM[4], R. SHIMMO[1], J. HARRO[2];
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Abstract: Introduction: Impulsivity has been associated with obesity, unhealthy food choices, and snacking. The neurobiological substrates that cause various types of impulsive behavior are dependent on metabolic pathways in CNS, therefore a more in depth study of gut-brain-behavior axis is warranted.
Methods: Two cohorts of schoolchildren from Estonian Children Personality Behavior and Health Study (ECPBHS) were followed into adulthood. In the current study, the results from the first cohort measured in 3 waves at ages of 15, 18, and 25 years were included. The second cohort was measured at ages of 18, 25, and 33. The available sample from the first cohort consisted of 550 subjects and 1377 observations. The second cohort included 613 subjects and 1509 observations. The impulsivity was measured with Adaptive and Maladaptive Impulsivity Scale (AMIS). AMIS is a Likert-type self-assessment questionnaire that measures functional (adaptive) and dysfunctional (maladaptive) categories of impulsivity. Additionally, averaged daily dietary intake was derived from 24 h to 72 h (the reported period varied at different measurement waves) self-reported eating diaries. The dietary intake was categorized into various macro and micronutrients using Micro-Nutrica and NutriData software. Several additional covariates such as aerobic fitness and BMI were included in the dataset. Because some observations within waves were missing (the missingness varied between 0.58% and 33%), a

multiple imputation of 100 plausible values was performed. Adaptive and maladaptive impulsivity scores were predicted from dietary data and covariates using linear mixed models. The 100 multiply imputed datasets were used for modeling and the final model coefficients were aggregated using Rubin's rules.
Results: Maladaptive impulsivity score was associated with a larger number of predictors. Negative associations included aerobic fitness, fish and vegetable consumption, while alcohol was positively associated. Among micronutrients, zinc was negatively associated in both sexes and sodium showed positive association only among females. Adaptive impulsivity score was positively associated with zinc and vitamin B6 intakes, while cereal products showed negative associations. Both types of impulsivity score declined with age and differed between sexes.
Conclusion: Maladaptive impulsivity was associated with unhealthy eating habits, while adaptive impulsivity was only associated with the reduced intake of cereal products. Several micronutrients that have been previously associated with ADHD symptomatology are also involved in the expression of impulsive behavior among young adults.

Disclosures: D. Matrov: None. **R. Shimmo:** None. **A. Pulver:** None. **J. Harro:** None. **T. Kurrikoff:** None. **I. Villa:** None. **K. Sakala:** None. **T. Veidebaum:** None.

Digital Abstract Session

P275. Emotions: Preclinical Models, Human Studies, and Therapeutic Approaches

Program #/Poster #: P275.07

Topic: G.08. Other Psychiatric Disorders

Support: CIHR FDN-147473

Title: Sex differences on the effects of consuming a high-fat diet in binge and/or ad libitum schedules on anxiety-like behaviour and the locomotor effects of acute and repeated cocaine exposure in adult male and female mice

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Abstract: Binge eating disorder (BED) can be characterized by a loss of control in food intake leading to rapid consumption of large quantities of highly palatable food. While binge eating disorder is the most common type of eating disorder the neurobiology is poorly understood. Furthermore, even though BED prevalence is higher in women than men, pre-clinical studies of BED has overwhelmingly been focused on male subjects. The current study aimed to evaluate sex differences in the effects of binge consumption of high-fat diet (HFD) on anxiety-like traits in the open field and elevated plus-maze. Because the mesolimbic dopaminergic system is critically involved in both BED and substance use disorder, we also examined the effect of binge eating (BE) on cocaine-induced locomotion over 5 days. Adult male and female mice were given ad libitum access to chow, HFD, or chow plus three times per week for 4 weeks, mice had 1-

hour access to unlimited HFD (BE). Daily food and body weight were recorded before and after BE protocol. Limited access to a high-fat diet induces BE in both male and female mice, although only the HFD group gained weight. BE female mice showed increased anxiogenic behaviour on the elevated plus-maze compared to males. Furthermore, female BE and HFD mice had greater cocaine-induced locomotor activity than male BE or HFD mice. These results indicate that there are sex differences in anxiety-like behaviour and responses to cocaine in BE mice and imply that treatment of comorbidities associated with BE should have a sex-specific focus.

Disclosures: **T. Kenney:** None. **L. Seabrook:** None. **M. Sobey:** None. **S. Borgland:** None.

Digital Abstract Session

P275. Emotions: Preclinical Models, Human Studies, and Therapeutic Approaches

Program #/Poster #: P275.08

Topic: G.08. Other Psychiatric Disorders

Support: NIH Grant R21MH122862

Title: Regional differences in various risk factors for postpartum depression: Applying mixed models to the PRAMS dataset

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Abstract: 10-15% of new mothers will experience depression symptoms of varying severity in the weeks following childbirth, known as postpartum depression. Susceptibility for postpartum depression has been linked to a variety of variables directly associated with socioeconomic, physical, psychological, and environmental factors. The purpose of this study was to assess the various risk factors for postpartum depression severity using a large dataset that included variables associated with previous mental health status, social factors, societal factors, health care access, as well as other state-wide or region-specific variables. We obtained the most recent (2016-2017) dataset from the Pregnancy Risk Assessment Monitoring System (PRAMS), which is a dataset compiled by the Centers for Disease Control (CDC) that collects state-specific, population-based data on maternal attitudes and experiences before, during, and shortly after pregnancy. A hierarchical linear model was used to analyze the data across various levels, with a symptom severity scale (0-8) as the dependent variable. Of the 21 predictor variables included in the final model, 9 were statistically significant predictors of symptom severity. Statistically significant predictors of postpartum depression symptom severity included previous depression diagnosis and depression symptoms during term, baby residing with mother, unintentional pregnancy, education, Women Infants Children (WIC) enrollment, and marital status. In contrast to these other factors, a postpartum follow up appointment significantly decreased symptom severity. Age revealed an inverted curve related to postpartum symptom severity. There was minimal discrepancy of symptom severity scores among the 39 participating states. Postpartum

depression symptom severity was associated most notably with previous depression diagnosis and previous symptom severity, but also important social and education factors that contribute to the support and well-being of the mother and child. Postpartum depression is more common than society is aware and these findings may aid in future studies that continue to investigate the causal relationship between specified variables and symptom severity.

Disclosures: J. Pluchino: None. **R. Della Valle:** None. **J. Schwarz:** None.

Digital Abstract Session

P276. Cortical and Subcortical Neurocircuitry of Emotion and Anxiety

Program #/Poster #: P276.01

Topic: G.04. Emotion

Support: NIH Grant R01MH117785
NIH Grant R01MH057414
NIH Grant R01NS024760

Title: Specialized projections of the primate subgenual cingulate area 25 to the amygdala

Authors: *M. P. JOYCE, H. BARBAS;
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Abstract: Communication between the primate prefrontal cortex and the amygdala is essential for the balance between cognition and emotion and the emergence of flexible behavior. Previous studies have shown that projections from medial prefrontal area 32 most densely innervate the basolateral nucleus of the amygdala. The posterior orbitofrontal cortex (pOFC, which includes area 13, the proisocortex, and the periallocortex) most densely innervates the inhibitory intercalated masses of the amygdala. The subgenual cingulate area 25 (A25) has unique contributions to mood and emotion states among prefrontal areas. A25 is positioned at the intersection of the medial prefrontal and the orbitofrontal cortex, and a comprehensive understanding of its innervation patterns in the amygdala remains unclear. Here we studied axonal termination patterns from five A25 sites in the amygdala, ranging from anterior to posterior, medial and orbital sectors of A25. Our results reveal that medial A25 innervates the basomedial and basolateral nuclei, while more orbital A25 shifts toward heavier innervation of the intercalated masses. These data suggest that in the context of amygdalar projections, A25 is a transitional area between the medial prefrontal and orbitofrontal systems. Our data also suggest that posterior medial A25 has a unique relationship with the amygdala among prefrontal areas, with denser projections extending more ventrally in the amygdala than projections from other sectors of A25. We also examined the postsynaptic inhibitory neurons targeted by medial and orbital A25 terminations across the basomedial, basolateral, and lateral nuclei of the amygdala. Preliminary data obtained using multiple labeling techniques for neuronal tracers and calcium binding proteins parvalbumin, calbindin, and calretinin suggest that A25 has a bias toward interacting with calretinin neurons in all basal nuclei of the amygdala. Terminations on calretinin

neurons, which are thought to be disinhibitory of nearby pyramidal neurons, may allow for the amplification of excitatory inputs.

Disclosures: **M.P. Joyce:** None. **H. Barbas:** None.

Digital Abstract Session

P276. Cortical and Subcortical Neurocircuitry of Emotion and Anxiety

Program #/Poster #: P276.02

Topic: G.04. Emotion

Support: MH096093 (ELB)
DA018184 (REJ)
Harvey Family Endowment (ELB)

Title: Medial prefrontal cortical projections are altered by a single acute fear experience: Evidence for biological mechanisms of anxiety and substance abuse disorders

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Abstract: Pyramidal neurons from medial prefrontal cortex (mPFC) project into striatum, thalamus, hypothalamus, and went as far as dorsal raphe nucleus (midbrain) and locus coeruleus (pons). Neural activity in mPFC is reciprocally modulated by monoamine systems arising in these distant brain regions: noradrenergic, dopaminergic and serotonergic. By deregulating these systems genetically, we previously reported that functional projections from mPFC are similarly regulated by these monoaminergic systems using brain-wide tracing based on manganese-enhanced magentic resonance imaging (MEMRI) followed by statistical parametric mapping (SPM). Here we report that a single experience of acute threat alters mPFC projections in wild type mice, and this change correlates with multiple subtle behavioral effects. Mice with or without acute threat (30m exposure to predator odor (2,3,5-Trimethyl-3-thiazoline, TMT) were group housed in environmentally controlled facility for 3 weeks and then underwent MEMRI tract-tracing. Behavior was video recorded before odor, during odor and before tract tracing. $MnCl_2$ (3-5nL of 0.6M in buffered saline) prepared with 0.5 mg/ml 3k rhodamine dextran-amine was injected into right mPFC and location confirmed by post-injection MRI: x = 0.59 ± 0.13 (R of midline), y = +0.72 ± 0.49mm (bregma), z = -1.04±0.36 mm (deep) (n= 24). Longitudinal images were collected at 6h and 24h after injection. Between imaging sessions animals were returned to their home cages. At conclusion of imaging, animals were sacrificed and perfusion-fixed brains processed for microscopy. Voxel-wise SPM comparisons between time points revealed progression of M(II) accumulation distally. Application of our new in vivo atlas allowed automated digital measurements of Mn(II) signal, revealing dramatic anatomical differences in projections between animals experiencing acute threat and those without which corresponded to subtle behavioral effects. Computational analysis of threat-exposed projections

with non-threat exposed mice carrying genetic disruptions of monoaminergic transporters suggested that fear-induced mPFC projections were most similar to altered projections induced by deletion of norepinephrine transporter (NET), which increases the amount of noradrenaline in the synaptic cleft. Acute threat further impacted mPFC projections in mice lacking the serotonergic transporter (SERT). Thus acute threat produces long-term changes in the reward circuitry of the limbic system, which may explain how life-threatening experiences pose a risk for long-term anxiety and substance use disorders. Supported by NIH RO1 MH096093 (ELB) and DA018184 (REJ).

Disclosures: E.L. Bearer: None. **T.W. Uselman:** None. **C.S. Medina:** None. **R.E. Jacobs:** None.

Digital Abstract Session

P276. Cortical and Subcortical Neurocircuitry of Emotion and Anxiety

Program #/Poster #: P276.03

Topic: G.04. Emotion

Support: NARSAD Young Investigator Grant 27202
Biogen 979338
NSF NRT UtB: Neurophotonics National Research Fellowship DGE-1633516

Title: Distinct interneuron ensembles in the cingulate cortex encode interactions with anxiogenic and social stimuli

Authors: *L. KRETSGE[1], C. JOHNSON[1], W. YEN[1], R. LIU[2], A. O'CONNOR[2], T. NGUYEN[2], E. CHO[2], T. JINADASA[2], E. FUCHS[2], E. SPEVACK[2], B. ESCUDE[2], B. SRIRAM[2], A. CRUZ-MARTIN[2];
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Abstract: Studies in humans and rodents indicate that the anterior cingulate cortex (ACC) is involved in both social behavior and anxiety, but it remains unclear how ACC neural activity encodes information across these diverse functions. Local ACC microcircuits are composed of excitatory and inhibitory neurons. One subclass of inhibitory interneuron, which is positive for vasoactive intestinal peptide (VIP), can modulate local activity by inhibiting other inhibitory cells, thereby driving excitatory cell activity. Previous work shows that cortical VIP cells exhibit diverse molecular, morphological, and electrophysiological properties, but little is known about their functional heterogeneity. Recent studies provided some insight into ACC function by monitoring the activity of excitatory neurons or of this region as a whole. These studies were limited by techniques without cellular resolution, so they could not address whether ACC cells all activate similarly or if distinct subgroups activate to different kinds of stimuli. To our knowledge, no existing studies have monitored VIP interneurons in the ACC (VIPACC) in vivo with cellular resolution. To determine whether VIPACC are functionally heterogeneous, we monitored their activity during behavioral tasks. We injected AAV9-flex-GCaMP6f into the

ACC of VIP-IRES-Cre mice to express this fluorescent calcium indicator in VIPACC. We then implanted gradient-index lenses and miniaturized microendoscopes into the ACC to image VIPACC calcium dynamics. Animals performed anxiety-related and social tasks while neural activity was recorded. Our data demonstrate that overall VIPACC activity did not change as animals navigated different stimuli in any of the tasks. Despite the lack of population level changes, we identified distinct subgroups of VIPACC that preferentially activated to anxiogenic or anxiolytic zones or to social or non-social stimuli. In each task, selective cells could predict the animal's behavior. When neurons were monitored across anxiety-related and social tasks, we determined that most VIPACC were neutral or selective for only one of these stimuli. In summary, our data show that VIPACC are functionally heterogeneous and non-overlapping subgroups of VIPACC activate preferentially to different types of stimuli.

Disclosures: **L. Kretsge:** None. **C. Johnson:** None. **W. Yen:** None. **R. Liu:** None. **A. O'Connor:** None. **T. Nguyen:** None. **E. Cho:** None. **T. Jinadasa:** None. **E. Fuchs:** None. **E. Spevack:** None. **B. Escude:** None. **B. Sriram:** None. **A. Cruz-Martin:** None.

Digital Abstract Session

P276. Cortical and Subcortical Neurocircuitry of Emotion and Anxiety

Program #/Poster #: P276.04

Topic: G.04. Emotion

Support: NARSAD Young Investigator Award to DF
Brain Research Foundation BRFSG-2017-02 to DF
NIH Grant R21MH114178 to DF
NSF Grant NSF1754831 to DF
NARSAD 2018 Young Investigator Award to EA
KV supported by NIMH T32MH112507 training grant

Title: Novel cerebello-amygdala connections provide missing link between cerebellum and limbic system

Authors: S. JUNG[1], ***K. VLASOV**[1], A. PARIGI[1], M. FERNANDEZ-FRENTZEL[1], M. ANGUIANO[1], E. G. ANTZOULATOS[1,2], D. FIORAVANTE[1,2];
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Abstract: The role of the cerebellum (CB) in executive functions, including emotional processing, is well established but lacks mechanistic understanding (Schmahmann et al., 2019). The major limitation has been lack of knowledge of the anatomical and functional connectivity that supports CB regulation of emotion. Early electrophysiological experiments in animals have hinted to the amygdala, a central structure of the limbic system, as a recipient of CB signals (Snider & Maiti, 1976), which raises the interesting but untested possibility of a CB-amygdala circuit as a mediator of CB limbic function (Otsuka et al., 2016; Strata, 2015). To explore this

hypothesis, we investigated the anatomical and functional characteristics of connections between the CB and amygdala in the mouse brain. We first used anterograde and retrograde viral tracing in CB and basolateral amygdala (BLA), respectively, and found significant overlap between these labeled populations in two major nodes: the intralaminar thalamus (Th) (part of the higher order thalamic nuclei) and the ventral tegmental area (VTA). Using confocal imaging, we discovered putative synaptic contacts between CB fibers and BLA-projecting neurons in both nodes. We provided support for the synaptic nature of these connections using anterograde transsynaptic viral techniques, and we examined monosynaptic connections between CB and the thalamic node using slice electrophysiology combined with optogenetics and pharmacology. These experiments revealed functional connections between CB and neurons in the intralaminar and medial dorsal thalamus, which were monosynaptic and primarily excitatory—indicating fast, robust transmission of information from the CB through this node. Using immunohistochemistry and high resolution imaging, we found that the axons of CB-Th projectors in the BLA colocalized with pre- and postsynaptic markers, indicating putative glutamatergic synaptic contacts. Additionally, we found CB-Th projections in the nucleus accumbens (Nac) as well as cingulate and prelimbic cortices. We are currently performing similar experiments for the VTA. We have also launched functional manipulations of CB-Th and CB-VTA projections to investigate the conditions under which these two different pathways might be recruited, and the kind of information they might convey to influence affective processing and behavior. These studies will advance our understanding for how the CB can modulate complex non-motor functions and play a major role in neuropsychiatric and neurodevelopmental disorders (Fatemi et al., 2012; Parker et al., 2014).

Disclosures: S. Jung: None. **K. Vlasov:** None. **A. Parigi:** None. **M. Fernandez-Frentzel:** None. **M. Anguiano:** None. **E.G. Antzoulatos:** None. **D. Fioravante:** None.

Digital Abstract Session

P277. Emotional and Social Behavioral Classification

Program #/Poster #: P277.01

Topic: G.04. Emotion

Support: NSERC Discovery Grant (06248,AA)

Title: Automated Emotion Classification of Free-Moving Mice from Video

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Abstract: Studies involving emotion often use animal models and currently rely on manual labelling by researchers. This human-driven labelling approach leads to a number of challenges such as: long analysis times, imprecise results, observer drift, and varying correlation between observers. These problems impact reproducibility, and have contributed to our lack of understanding of fundamental mechanical questions such as how emotions arise from neuronal

circuits. Recent success of machine learning models across similar problems show that it can help to mitigate these challenges while meeting or exceeding human accuracy.
We developed a classifier pipeline that takes in videos and produces an emotion label. The pipeline extracts body part positions from each frame using a pose estimator and feeds them into an Artificial Neural Network (ANN) classifier built using stacked Long Short Term Memory (LSTM) layers. The data was collected by treating nine rats with Lypopolysaccharide (LPS) injections (10mg/kg). First, rats were recorded for 10 minutes under control conditions with no manipulation and no observed symptoms of stress or malaise. A week later, rats were injected with LPS and filmed for 10 minutes two hours post injection.
The method developed correctly labelled 7 out of 8 test videos. The test videos had varying environments and used rats that were different from the training videos, providing evidence of a degree of robustness in the model. Future work will involve growing the training and test sets in order to improve performance and evaluation, as well as exploring new features and model architectures.

Disclosures: **A. Telfer:** None. **F. Sherratt:** None. **O. van Kaick:** None. **A. Abizaid:** None.

Digital Abstract Session

P277. Emotional and Social Behavioral Classification

Program #/Poster #: P277.02

Topic: G.04. Emotion

Title: Individual differences in social play behaviour in rats: behavioural microstructure and communication

Authors: C. J. BURKE[1], S. M. PELLIS[1], L. J. VANDERSCHUREN[2], *M. ACHTERBERG[2]; [1]Dept. of Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada; [2]Dept. Population Hlth. Sci., Utrecht University, Fac. of Vet. Med., Utrecht, Netherlands

Abstract: Social play behaviour is rewarding and important for the development of brain and behaviour. Disruptions of social play behaviour in rats have been associated with social and cognitive deficits. How natural variation in social play contributes to development of brain and behaviour remains unclear. It is common knowledge that there are differences in the amount of play behaviour between strain and sexes. Furthermore, even within a strain and within a sex, individuals differ in how much they play. Findings that show that individual differences in play lead to a different response to neural manipulations suggests that individual differences in playfulness may also be a valuable avenue for studying the neuro-behavioural mechanisms that regulate play. To this end, male Lister hooded rats were characterized as high, medium and low players (HP, MP and LP) based on the sum rank score of the amount of play they initiated and received across three days with an unfamiliar partner. Once animals were categorized in player-types, microstructural analyses of play were conducted to assess differences in the way animals play. To investigate differences in communication during play, ultrasonic vocalizations (USVs) were recorded. Our data shows that the player-types differ significantly in the amount of play

they initiate but less in the amount they receive. Furthermore, the microstructure of play differs between HP and LP as well as the communication during play. These results give insight into how individual animals behave and communicate during social play. Future research may indicate whether individual differences in play also lead to differences in the brain.

Disclosures: M. Achterberg: None. **C.J. Burke:** None. **S.M. Pellis:** None. **L.J. Vanderschuren:** None.

Digital Abstract Session

P278. Human Imaging, Animal Models and Neurological Disorders

Program #/Poster #: P278.01

Topic: G.05. Mood Disorders

Support: NIMH R21MH091553
NIH T35AG044303
NIH UL1TR000040

Title: Data-driven analysis of PET images reveals a pattern of in vivo kappa opioid receptor availability related to symptom severity in patients with Major Depressive Disorder

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Abstract: Preclinical studies have identified a central role of kappa opioid receptors (KORs) in stress response and depression-related behaviors. However, evidence in humans is limited. This work aimed to explore cross-regional patterns of variation in *in vivo* KOR availability in patients with major depressive disorder (MDD) in order to identify opioidergic networks that may be relevant to depression pathophysiology and treatment. A secondary analysis was performed of data acquired using positron emission tomography (PET) with the KOR radiotracer [^{11}C]GR103545 in 11 unmedicated, currently depressed individuals with MDD (32.6 ± 6.5 years, 5 women) and 13 matched healthy volunteers (34.8 ± 10 years, 6 women)[1]. Independent component analysis was performed at the region of interest (ROI) level to identify spatial patterns of coherent variance in KOR availability (tracer volume of distribution, V_T) across all subjects. Analyses were then repeated at the voxel level using whole brain images to better characterize spatial patterns. Expression of each component was compared between groups and relationships to symptoms were explored using the 17-item Hamilton Depression Rating Scale (HAMD). Three components of variation in KOR availability across ROIs were identified, spatially characterized by [^{11}C]GR103545 V_T contributions in (1) bilateral frontal lobe; (2) occipital and parietal cortices, right hippocampus, and putamen; and (3) right anterior cingulate, right superior frontal gyrus and insula. In MDD patients, component 3 was negatively associated

with symptom severity on the HAMD (r=-0.85, p=0.0021 uncorrected). Results were consistent in voxel-wise component analyses, with an ACC/insula component negatively correlated with HAMD score (r=-0.59, p=0.070). There were no group-wise differences in expression of any component between patients and controls. In MDD patients and controls, data-driven analyses identified independent patterns of variation in KOR availability across brain regions which could represent distinct signaling networks. KOR signaling in cortical regions relevant to depression, particularly the right anterior cingulate, may contribute to MDD pathophysiology.
1.Miller JM et al. (2018). Synapse 72(9):e22042.

Disclosures: K. Smart: None. **M.A. Oquendo:** None. **J.J. Mann:** None. **A.T. Hillmer:** None. **R.E. Carson:** None. **J.M. Miller:** None.

Digital Abstract Session

P278. Human Imaging, Animal Models and Neurological Disorders

Program #/Poster #: P278.02

Topic: G.05. Mood Disorders

Support: Intramural Research Program at the National Institute of Mental Health, National Institutes of Health

Title: MEG gamma-band dysregulation and clinical outcomes demonstrate reduced emotional reactivity during mood induction in a depressed sample

Authors: *G. E. ANDERSON, C. R. BURTON, C. FARMER, J. R. GILBERT, E. D. BALLARD, C. A. ZARATE, Jr.;
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Abstract: Individuals with depression may have cognitive biases that are activated by negative mood. This cognitive and emotional reactivity can be measured with mood induction paradigms, such as watching emotional movies. Here, we extend this research by collecting magnetoencephalography (MEG) during a mood induction paradigm in order to measure brain network-level differences in gamma power, a proxy measure of excitation-inhibition balance, between healthy control participants (HCs) and those with a mood disorder diagnosis (MDs). In this paradigm, a sample of MDs (n = 33) and HCs (n = 14) viewed emotional movie clips while undergoing MEG scanning. Two clips were used to induce positive mood, and two clips were used to induce negative mood (Negative 1 and 2; Negative 1 included a depiction of suicidal thoughts and behaviors). At baseline and after each clip, participants completed the Positive and Negative Affect Scale (PANAS) to rate mood and the Suicide Implicit Association Task (s-IAT) to asses *self-life/self-death* associations (after negative clips only). We hypothesized that HCs would experience a larger increase in positive affect after positive clips than MDs, while MDs would experience a larger increase in negative affect after negative clips than HCs coupled with stronger *self-death* associations. We also expected large-scale functional brain network gamma

power dysregulation in MDs, as evidenced by group-by-clip valence interactions. We used mixed effects models to compare group-level changes in mood ratings and *self-life/self-death* associations at baseline and following each clip and to test for gamma power group-by-valence interactions. Compared with baseline, HCs reported decreases in positive affect score following both positive clips and increases in negative affect following both negative clips. In contrast, MDs only reported a decrease in negative affect following Negative 2 and an increase in negative affect following Negative 1, along with no changes following both positive clips. On the s-IAT, both groups moved away from *self-life* associations following negative clips. Dysregulated gamma power was found in regions of the central executive network including dorsolateral prefrontal cortex, the salience network including insular cortex and amygdala, and the default mode network including inferior temporal cortex. Taken together, these preliminary findings suggest there is less emotional reactivity in MDs following emotional movie clips than HCs coupled with dysregulated gamma power in large-scale functional brain networks. Future work will examine the relationship between clinical presentation and brain network dysfunction.

Disclosures: G.E. Anderson: None. **C.R. Burton:** None. **C. Farmer:** None. **J.R. Gilbert:** None. **E.D. Ballard:** None. **C.A. Zarate:** None.

Digital Abstract Session

P278. Human Imaging, Animal Models and Neurological Disorders

Program #/Poster #: P278.03

Topic: G.05. Mood Disorders

Support: NIH NINDS BRAIN Initiative Grant 1UH3NS103549
McNair Foundation
NSF Graduate Research Program

Title: Modulation of cognitive control network in treatment-resistant depression with deep brain stimulation

Authors: *A. ALLAWALA[1], J. ADKINSON[3], K. BIJANKI[3], S. VARTANY[2], S. MATHEW[4], W. GOODMAN[4], N. POURATIAN[5], S. SHETH[3], D. BORTON[1];
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Abstract: Major Depressive Disorder is a common psychiatric illness affecting at least 4.4% of the global population, where a third of this population is treatment-resistant. Deficits in cognitive control, i.e., focal attention to relevant stimuli as well as inhibition of irrelevant material are thought to explain the inflexible behavior in depression. However, the underlying neurophysiological network state for control in depression and how this is modulated by DBS is unknown. The goal of this study is two-fold. First, to identify neural biomarkers involved in modulating control in depression. Second, to identify changes in neural biomarkers and cognitive control behavior driven by application of DBS.

Here, we report the outcome from a conflict task performed in our first human subject with Treatment-Resistant Depression (TRD) in an in-patient hospital setting with continuous psychiatric and intracranial monitoring, as part of a larger clinical trial study. The subject was implanted bilaterally with DBS electrodes in the ventral capsule/ventral striatum and subcallosal cingulate, as well as depth electrodes (stereo-EEG) spanning the prefrontal cortex (PFC), anterior cingulate cortex (ACC), orbitofrontal cortex and the amygdala for electrophysiology recordings collected across multiple days. The task was performed pre-, post-, and during DBS. In the task, a cloud of colored moving dots moving leftward or rightward is presented on a computer screen. The subject was asked to indicate the color of the dots (relevant feature) by pressing a button with their left or right index finger while ignoring the motion direction (irrelevant feature). Motion direction could either be congruent or incongruent with the required response side. Accuracy on the task improved from an average of 47% before DBS to 89% with DBS on and this effect was sustained after DBS was turned off the next day with 90% accuracy. Cognitive control is thought to be characterized by activity in theta band (4-7 Hz). Pre-DBS we found theta power to be greatest during high conflict and post-DBS there was an overall increase in theta power across all conflict conditions, especially in dorsolateral PFC and dorsal ACC compared to pre-DBS task states, suggesting that theta power may be a possible neural mechanism underlying cognitive control in depression. We will also present results from a second participant in the study, in addition to functional connectivity analysis to better understand the mechanistic account of control deficits, and how they are improved via DBS. Understanding these mechanisms would allow for optimized DBS treatment centered around electrophysiology for future closed-loop applications.

Disclosures: **A. Allawala:** None. **J. Adkinson:** None. **D. Borton:** None. **K. Bijanki:** None. **S. Vartany:** None. **S. Mathew:** None. **W. Goodman:** None. **N. Pouratian:** None. **S. Sheth:** None.

Digital Abstract Session

P278. Human Imaging, Animal Models and Neurological Disorders

Program #/Poster #: P278.04

Topic: G.05. Mood Disorders

Support: NCT02460666

Title: Regional grey matter volume predicts clinical improvements in a clinical trial of Tai Chi in geriatric depression

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Abstract: Background: Geriatric depression (GD) is associated with significant medical comorbidity, cognitive impairment, and suboptimal treatment response compared to depression in younger adults. More efficacious treatment to improve mood, cognition and quality of life in

GD are urgently needed. Mind-body interventions like Tai Chi are a promising adjunct treatment to antidepressants, as they may improve stress and body awareness in GD. We tested the effect of twelve weeks of weekly Tai Chi lessons on clinical symptoms, resilience and neuroplasticity.
Methods: Forty-nine older adults over 60 (38 women, 11 men) diagnosed with major depressive disorder undergoing concurrent antidepressant treatment were randomized to either Tai Chi training (N=26) or health education control (N=23). We administered the Hamilton Depression Rating Scale (HAMD), Montgomery-Asberg Depression Rating Scale (MADRS), Hamilton Anxiety Scale (HAMA), Apathy Evaluation Scale (AES) and the Connor-Davidson Resilience Scales (CDRISC). General linear models were used to test whether the groups differed in demographics and symptoms at baseline and repeated-measures analyses of covariance tested the interactions between time point and group for symptom scores including age and sex as covariates. T1-weighted imaging was performed using a 3T Siemens Prisma scanner. Processing, reconstruction and fully corrected voxel-wise whole-brain general linear models on grey matter volume (GMV) were performed using Freesurfer version 6.0.
Results: The groups did not differ in demographics or clinical scores at baseline (*ps*>.05) and there were no group differences in symptom change from baseline to follow-up (*ps*>.11). Further, there were no differences in GMV change over the twelve weeks between groups. Across both groups, higher baseline GMV was associated with lower anxiety and apathy, but higher resilience in widespread regions. Furthermore, separate clusters demonstrated that while higher baseline GMV in the Tai Chi group was associated with less improvement in anxiety, the control group showed the opposite effect. This pattern was similar for depressive symptoms (MADRS) in a cluster in the right precuneus.
Discussion: While we did not observe neuroplastic changes in the cortex of patients with GD undergoing Tai Chi training vs. health education control, we found correlations between clinical variables and GMV at baseline, as well as indications that higher GMV in some regions may yield unfavorable outcomes for those participating in Tai Chi compared to control interventions.

Disclosures: **M.M. Milillo:** None. **B. Krause-Sorio:** None. **L. Kilpatrick:** None. **P. Siddarth:** None. **L. Ercoli:** None. **K. Narr:** None. **H. Lavretsky:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; In the last 12 months, Dr. Lavretsky has received research grants from NIMH, NCCIH, PCORI and the Alzheimer's Research & Prevention Foundation. The other authors report no conflicts of interest..

Digital Abstract Session

P278. Human Imaging, Animal Models and Neurological Disorders

Program #/Poster #: P278.05

Topic: G.05. Mood Disorders

Title: A Comparative Analysis of Evoked Potential Referencing Methods in Schizophrenia and Bipolar I Disorder

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Abstract: The choice of reference location is a critical issue in recording evoked potentials (EPs) that has been debated often. EPs are brain responses to specific stimuli derived from the EEG that can be influenced by reference choice. In this study, we compared the effects of two different reference locations, linked mastoids (LM) and common average reference (CAR) on P50 sensory gating. Sensory gating is a process by which the brain filters out irrelevant or redundant stimuli in order to focus on the relevant information, and can be studied by comparing amplitudes of the positive EP around 50 ms post-stimulus to the first and second clicks using paired-click paradigm. LM is a unipolar reference obtained by averaging the left and right mastoids, while CAR is the average of the activity of all electrodes on the scalp. The subject groups included patients with bipolar I disorder with or without psychosis (n=45), schizoaffective disorder (n=39), paranoid schizophrenia (n=29), and controls (n=39), also evaluated by gender. A 64-channel EEG cap recorded EEGs while participants listened passively to 90 paired clicks (S1 and S2) 500 ms apart with pairs separated by a 10-second interval. A specialized software program (Cortech Solutions) was used to correct eye blinks and motor movements. The brain responses for each participant were averaged separately for S1 and S2 P50. These averages were measured from the pre-stimulus baseline to the maximum amplitude peak within the P50 window (40-80ms) using an automated peak-picking software feature. Missed peaks were measured by hand. The sensory gating ratio (S2/S1) and difference (S1-S2) were also calculated. Analysis of variance was used to compare these measures for each group with reference (CAR or LM) as a repeated factor in the design. The findings demonstrated that different locations influenced results for both click and gender. A gender by reference interaction was found showing that males and females produced different results at LM with males having a larger S1-S2 difference than females. Significant gender by reference and click by reference interactions also were found. Females had a larger P50 amplitude for LM than CAR and amplitude at LM for males was smaller than for females. Amplitude for CAR was smaller than LM for S1 but not for S2. For S1-S2 CAR a significant main effect of reference showed that S1-S2 for CAR was significantly smaller than for LM for all groups. Based on these findings, it was concluded that choice of reference location can affect interpretation of the results of a study, and comparison with previous studies may either require replication using the same reference or re-referencing to compare both reference locations.

Disclosures: M. Alhamody: None. **S. Arora:** None. **W.E. Bunney:** None. **J.V. Patterson:** None.

Digital Abstract Session

P278. Human Imaging, Animal Models and Neurological Disorders

Program #/Poster #: P278.06

Topic: G.05. Mood Disorders

Support: R25GM121231
NIH Grant R01MH10011

Title: Predicting Dimensional Symptoms of Psychopathology from Task-Based fMRI using Support Vector Regression

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Abstract: Extensive research in psychiatry has highlighted the potential utility of machine learning (ML) automation for disease diagnosis. For many years, studies aimed at uncovering biomarkers for traditional DSM diagnostic categories of mood disorders, such as Major Depressive Disorder, have presented challenges for many researchers. Machine learning models have been successfully applied in the context of disease diagnosis and prediction for treatment prognosis, using both structural and functional neuroimaging data. Due to its computational complexity, which does not depend on input space dimensionality, Support Vector Regression (SVR) provides a supplementary approach for studying whole-brain data, providing a data-driven perspective with sensitivity to relatively small effect sizes and increased reliability offered by multivariate analytic techniques. However, few studies have examined the relationship between brain activation features from task-based functional Magnetic Resonance Imaging (fMRI) data and symptom dimensions of anxiety and depression. In this study, we develop a novel application of feature-based type of supervised machine learning, SVR, approach to detect potential biomarkers in fMRI data and dimensional symptoms of anxiety and depression on 209 subjects enrolled in the study for risk for anxiety and depression. We trained the SVR classifier on fMRI data collected during the well-established Monetary Incentive Delay (MID) task, which is a widely used task to measure neural activity in response to reward and loss in healthy and clinical populations. The MID datasets were preprocessed into multi-dimensional feature space using a 264 regions of interest (ROI) mask, and the model was fit using ten fold cross validation on all 264 dimensions in order to identify patterns of activation associated with dimensional symptoms of depression and anxiety. We demonstrate MID task-fMRI data is not an accurate predictor of dimensional clinical symptoms. The results predicted dimensional symptoms with R2 values ranging from -0.269 to -0.658 indicating poor model fit compared to a horizontal line fit denoting a null effect. The poor model fit of linear models reported in this study suggests that a non-reliable linear relationship between reward-related neural activation and dimensional clinical symptoms related to the experience of depression and anxiety. Future analysis will explore additional non-linear modeling methods and clustering to further explore the relationship between reward-related brain activation and dimensional symptoms.

Disclosures: K. Garcia: None. **Z. Anderson:** None. **I.K. Chat:** None. **K.S.F. Damme:** None. **K. Young:** None. **S.Y. Bookheimer:** None. **R. Zinbarg:** None. **M. Craske:** None. **R. Nusslock:** None.

Digital Abstract Session

P279. Depression: Treatment and Discovery Preclinical To Clinical

Program #/Poster #: P279.01

Topic: G.05. Mood Disorders

Support: NIMH Grant P50MH096890
NIMH Grant R01MH051399
Hope for Depression Research Foundation

Title: Transcriptional Profiles of Treatment Resistant Depression in Mouse Models

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Abstract: Major Depressive Disorder (MDD) is the most prevalent psychiatric disorder worldwide, representing a high level of global economic burden. Fluoxetine (FLX) has been widely used to treat MDD, nonetheless, a majority of patients do not achieve full remission. Further, a subset of those afflicted is considered non-responsive to any orally-available treatments, which is termed treatment-resistant depression (TRD). More recently, Ketamine (KET) has been shown to induce a rapid antidepressant response in ~50% of TRD patients, providing a novel therapeutic approach. However, the molecular mechanisms underlying TRD and subsequent response vs. non-response to KET are poorly understood. This study was aimed at characterizing the transcriptional profile of successful vs. unsuccessful response to KET in mice that failed to respond to an initial course of FLX as a model of treatment resistance. We exposed adult male mice to chronic social defeat stress (CSDS), a validated mouse model of depression that differentiates between resilient and susceptible populations based on the social interaction test (SIT). Mice classified as susceptible underwent antidepressant treatment with FLX in their drinking water for 28 days or received water during the same period (water-treated). After FLX treatment, we identified a subset of mice (~35%) that continued to show reduced social interaction despite treatment (non-responders). FLX non-responders and water-treated mice were subsequently given a single injection of KET and assessed in the SIT 24h later. Transcriptome-wide changes in the prefrontal cortex (PFC) and nucleus accumbens (NAc) 48h after KET administration were profiled by RNA-sequencing. We found that ~50% of FLX-non-responder mice exhibited an antidepressant response to a single KET injection, a significantly greater response than that seen in susceptible mice treated with water (0%). We further identified a subset of treatment resistant mice who failed to respond to consecutive FLX and KET treatment. Pattern analysis of the differentially expressed genes in the PFC and NAc revealed transcriptional profiles associated with the antidepressant-like actions of FLX and of KET as well as a series of genes that were unique to treatment resistance to both drugs. We developed a novel paradigm of treatment resistance in mice that allows to identify potential mechanisms underlying TRD. The KET response rate in FLX-non-responders is similar to that seen in TRD

patients, lending further validity to our model. Moreover, our findings suggest that prior unsuccessful antidepressant treatment induces a "priming effect" that increases the likelihood of successful response to KET.

Disclosures: A. Torres Berrio: None. **E.M. Parise:** None. **M. Estill:** None. **T.M. Gyles:** None. **F.J. Martínez-Rivera:** None. **C.J. Browne:** None. **A. Godino:** None. **L. Shen:** None. **E.J. Nestler:** None.

Digital Abstract Session

P279. Depression: Treatment and Discovery Preclinical To Clinical

Program #/Poster #: P279.02

Topic: G.05. Mood Disorders

Title: Binding and electophysiological profiling of LCGA-17 - a novel peptide with stress-protective properties

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Abstract: Background. Major depressive disorder (MDD) remains a substantial clinical burden worldwide with an unmet need for treatment**.** A dysregulation of excitatory/inhibitory (E/I) balance within CNS might have an implication in the pathogenesis of MDD. LCGA-17 is a novel peptide with prominent stress-protective effects in zebrafish and rodent models. This peptide was designed using a proprietary software suite "Peptimize". The aim of the study was to validate specific targets of LCGA-17. **Methods.** Competitive radioligand binding assays in rat cerebral cortex preparations were carried out using tritium labeled [^{3}H]-LCGA-17 peptide. The half-maximal inhibitory concentration (IC_{50}) for the binding of [^{3}H]-LCGA-17 was determined by adding test compounds (ligands of different sites of GABA and VGCC as well as dopamine (DA), serotonin (5-HT), acetylcholine (Ach), glutamate (Glu), glycine (GlyRA1), cannabinoid (CB1), and TRPM3 receptors) to the incubation medium at final concentrations of 10^{-10}-10^{-4} . Electrophysiological profiling of LCGA-17 was carried out using automated patch-clamp recordings (SyncroPatch 384i platform, "SB drug discovery"). Six concentrations (0.04, 0.4, 4, 25, 40, 400 μM) of the peptide were applied against the seven GABAA cell lines (α(1-6)β2γ2 and α4β3δ) in NAM and PAM modulation modes. The peptide effects on evoked currents of VGCC ($Ca_{V1.2}$) were tested at the same dose range.**Results.** Specific binding sites of the [^{3}H]-LCGA-17 in rat cortex membranes were found with IC_{50}=2*10^{-6} M. Gabapentin (inhibitor of α2δ subunit-containing VGCCs), and Pregnenolone sulfate (GABAAR NAM-site ligand) displaced [^{3}H]-LCGA-17 with IC_{50} ~3.8*10^{-6} M and 36*10^{-6} M respectively. We also found that Diazepam (GABAAR BZD-site ligand), Baclofen, and Phenibut (both are GABABR and α2δ subunit

VGCC ligands) demonstrated a competition with [^{3}H]-LCGA-17. There was no cross-reactivity found with DA, 5-HT, Ach, Glu, GlyRA1, CB1, and TRPM3 receptors. The electrophysiological assessment showed that LCGA-17 produced a concentration-dependent inhibition of the $Ca_{V1.2}$ channels in a similar manner to Gabapentin. Also, LCGA-17 showed an inhibitory effect on α(2,3)β2γ2, α4β3δ GABAARs. **Conclusion.** The results propose LCGA-17 as a functionally active ligand of the GABAAR NAM site and the α2δ VGCC subunit. Both targets are in tight inter-regulation and are involved in maintaining E/I balance within CNS. GABAAR and VGCC are clinically relevant in neuropsychiatric disorders, and their engagement by LCGA-17 may underlie its anxiolytic and antidepressant effects in vivo.

Disclosures: A.V. Malyshev: None. **E.V. Vasileva:** None. **I.A. Sukhanova:** None. **I.I. Doronin:** None. **V.V. Pavshintcev:** None. **N.A. Mitkin:** None. **V.R. Gedzun:** None. **A.R. Kuchumov:** None. **G.I. Kovalev:** None. **Y.A. Zolotarev:** None. **G.A. Babkin:** None.

Digital Abstract Session

P279. Depression: Treatment and Discovery Preclinical To Clinical

Program #/Poster #: P279.03

Topic: G.05. Mood Disorders

Support: NSERC CGS-M
NSERC Discovery Grant

Title: Ketamine and reelin differentially impact peripheral clustering on lymphocytes after corticosterone administration.

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Abstract: Introduction: Depression is the leading cause of disability worldwide, with no reliable biomarkers for diagnosis or treatment responsiveness. With novel therapeutics such as ketamine, recent research has shifted away from the traditional monoaminergic hypothesis to focus on glutamatergic signaling and the mammalian target of rapamycin, shown to be essential for fast-acting antidepressant effects in the central nervous system. Reelin, an extracellular matrix glycoprotein downregulated in depression appears to activate similar molecular pathways at the synapse after exogenous administration. While there are parallels between ketamine and reelin in the brain, peripheral mechanisms play a large role in depressive pathophysiology and are often overlooked. Inflammatory events after chronic stress cause an upregulation of proinflammatory cytokines and alterations in peripheral cells such as lymphocytes. Our lab has shown that serotonin transporter (SERT) clustering on lymphocytes react to chronic stress, depression, and traditional antidepressants in both humans and animal models, providing a potential biomarker of treatment responsiveness. However, there is no data for the impact of

novel fast-acting therapeutics on these important peripheral measures.
Methods: Lymphocytes from male Long Evans rats were extracted through a Percoll gradient and incubated with 1mM of corticosterone (CORT) or a vehicle, which parallels the changes we observe in patients with depression. After the initial treatment, cells were incubated with varying concentrations of reelin (0.5nM - 5nM) and ketamine (10nM - 250nM), then rinsed and fixed. The lymphocytes were then stained with an anti-SERT antibody to determine changes in the size and number of protein clusters.
Results: CORT increased the size of protein clusters in comparison to vehicle ($p<0.05$). All concentrations of reelin decreased the size of the SERT clusters, but this was only significant at the highest concentration (5nM, $p<0.05$). Ketamine had no effect on the size of SERT clustering. No changes in number were observed across any of the groups.
Conclusions: The impact of reelin on SERT clustering that was not mimicked by ketamine indicates that they may have divergent mechanisms in the periphery, The effect of reelin paralleled what we have seen previously in patients who have responded to varying antidepressants, and supports reelin as a potential therapeutic. In a translational sense, these results could be greatly applicable to the clinic. Analyzing blood samples in this method is simple and inexpensive and could allow for the individualization of treatment as a putative biomarker of therapeutic efficacy.

Disclosures: J. Johnston: None. **R. Romay-Tallon:** None. **L.E. Kalynchuk:** None. **H.J. Caruncho:** None.

Digital Abstract Session

P279. Depression: Treatment and Discovery Preclinical To Clinical

Program #/Poster #: P279.04

Topic: G.05. Mood Disorders

Support: Brain and Behavior Research Foundation

Title: Neuron-glia interactions in the regulation of stress and depressive disorders

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Abstract: An estimated 264 million people globally suffer from psychiatric disorders and are treated by antidepressant drugs that were developed three decades ago. Selective serotonin reuptake inhibitor (SSRI) drugs are still the most widely prescribed antidepressants in the world, thought to influence mood by increasing levels of the neurotransmitter serotonin in the brain's synapses. They are effective in only two-thirds of patients and require weeks to alleviate symptoms. Mood dysregulation is a hallmark of stress- and depressive-disorders caused by

structural and functional impairments within critical neuronal networks. The lack of understanding of the precise biological mechanisms of remodeling and regeneration of these networks, as well as the molecular basis for weeks-long lag in response times, are major obstacles in antidepressant research. To gain insight into the mode of action of SSRIs, we analyzed the dynamics of fluoxetine (Prozac) in the prefrontal cortex, an important region in the cortico-limbic mood circuit. We identified the activation of sequential-signaling pathways that included growth-factor signaling and the stimulation of AP-1 transcription complex, which in turn modulated plasticity-inducing effector molecules that lead to improved mood. Blocking AP-1 formation in the first week of treatment attenuated the behavioral response, demonstrating time- and sequence-dependent activation of signaling pathways. Profiling AP-1 target genes revealed neuronal remodeling and plasticity-inducing effector molecules, many with known links to depression and antidepressant responses. To determine the identity of the cell types contributing to the antidepressant response and how signaling pathways integrated within these cell types, we performed single-cell RNA-sequencing in the mouse cortex subjected to chronic social-isolation stress and fluoxetine treatment. Remarkably, we found that the onset of the fluoxetine response required neuron-glia interactions and indicated that the glia shapes the neuronal properties that progressively drive recovery processes to improve mood. In addition, I developed a cellular model system and recapitulated the precise timeline of the antidepressant response, providing a powerful tool to study the delayed onset, with strong translational potential for rodent and human studies. These studies provide strong evidence for the crucial role of neuron-glia interactions within the cortico-limbic mood-circuit during mood dysregulation and recovery.

Disclosures: R.U. Chottekalapanda: None. **V. Yao:** None. **A. Aly:** None. **S. Kalik:** None. **J. Gresack:** None. **W. Wang:** None. **A. Schaefer:** None. **O. Troyanskaya:** None. **P. Greengard:** None.

Digital Abstract Session

P279. Depression: Treatment and Discovery Preclinical To Clinical

Program #/Poster #: P279.05

Topic: G.05. Mood Disorders

Support: PSYBIO Therapeutics Research Grant

Title: Effects of E. Coli Derived Psilocybin in Rats are enhanced by Norbaeocystin

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Abstract: Preliminary clinical research has shown that Psilocybin has potential therapeutic utility for the treatment of depression, anxiety, PTSD, and substance use disorders. However, our basic understanding of the drug and its possible clinical mechanisms are lacking. Additionally, it

is unclear if related tryptamines, such as baeocystin and norbaeocystin, could have similar or better efficacy, but with different side-effect profiles. Given their similar chemical structure and possible pharmacological targets, these substances may also interact with psilocybin to enhance or alter its efficacy (e.g. entourage effect). To address this topic, our lab examined the behavioral effects of psilocybin and norbaeocystin, individually and in combination. Psilocybin and norbaeocystin were synthesized using genetically modified E. coli, and verified for purity using mass spectroscopy. Varying doses of filtered E. coli broth containing each compound were then gavaged directly into male rat subjects, and head twitch responses and locomotion assessed. Head twitch responses were exhibited following the administration of both substances individually and were found to be dose-dependent. Additionally, the number of head twitches produced by low-dose psilocybin was enhanced when combined with low dose norbaeocystin, beyond simple additive levels, suggesting a pharmacological interaction may be occurring. Locomotor results showed similar trends but also exhibited a marked reduction of locomotion following high dose psilocybin. Combined, our data demonstrate the pharmacological efficacy of E. coli-derived psilocybin, and suggest that other tryptamines may supplement/augment its effectiveness, and should be considered for therapeutic testing alone and in combination with psilocybin. Additionally, our results demonstrate the relative safety of directly gavaging filtered E. coli broth containing these compounds. Future studies will assess the therapeutic potential of both compounds, as well as determine their mechanism of interaction.

Disclosures: **N. Anas:** None. **J. Hinegardner-Hendricks:** None. **P. Hamilton:** None. **K. McCloy:** None. **J.A. Jones:** None. **M.S. McMurray:** None.

Digital Abstract Session

P279. Depression: Treatment and Discovery Preclinical To Clinical

Program #/Poster #: P279.06

Topic: G.05. Mood Disorders

Support: NIMH Intramural Research Program

Title: Behavioral and activity changes in response to ketamine in depressed subjects

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Abstract: Standard measures of antidepressant response, e.g., rating scales, which play an invaluable role in assessing treatment efficacy, may benefit from supplementation by real-time behavioral measurement using techniques such as actigraphy. Wrist activity monitors can be used to inexpensively and noninvasively assess 24-hour patterns of motor activity. Prior work has indicated that ketamine produces rapid antidepressant effects in individuals with depression and that actigraphy data may be of use in assessing this response. In order to better understand the relationship between treatment, symptomatology, and biological rhythms, we examined

associations between one behavioral measure, the Montgomery-Åsberg Depression Rating Scale (MADRS), and several nonparametric actigraphic variables within a randomized crossover study of ketamine.Thirty depressed subjects (18 female, mean age:36.5 years+-10.7) wore an Actiwatch (AW64; Philips, Amsterdam, the Netherlands) for one day before and two days after two intravenous infusions of either 0.5mg/kg ketamine or placebo over a 40-minute period. Infusions took place two weeks apart. Subject ratings on the MADRS at baseline and following infusions were used to quantify antidepressant response to ketamine. Five nonparametric variables were calculated by day using pyActigraphy, an open-source Python actigraphy analysis package: the most ten-hour active period (M10), the least five-hour active period (L5), the relative amplitude (RA), the intradaily variability (IV), and the interdaily stability (IS).We ran five linear mixed effects models examining the relationship between actigraphy metrics and fixed effects of age, gender, and the interaction between day (in relation to infusion), drug (placebo or ketamine), and MADRS score. We found an effect of drug by day ($t(183)=2.80$,$p=0.006$) and MADRS by drug by day ($t(183)=-2.74$,$p=0.007$) on the IS. No other variables appeared to be associated with day, drug condition, or treatment response.Preliminary work suggests that ketamine may impact motor activity patterns of depressed inpatients. However, there is a need to identify the most suitable metrics to best characterize these patterns in a short-term, inpatient setting. Factors such as restricted activity patterns may inadvertently obscure findings. Therefore, while these results suggest associations between ketamine administration and the IS, future work will need to replicate these findings, as well as explore alternative calculation techniques and other metrics of interest.

Disclosures: C. Punturieri: None. **W.C. Duncan:** None. **D. Greenstein:** None. **J.W. Evans:** None. **C.A. Zarate:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on patent for use of ketamine for MDD/SI, Co-inventor on patent for use of (2R,6R)- hydroxynorketamine in depression/neuropathic pain, Co-inventor on patent for use of (S)-dehydronorketamine in depression/neuropathic pain, Co-inventor on patent of use of other stereoisomeric dehydro and hydroxylated metabolites of (R,S)-ketamine metabolites in the treatment of depression and neuropathic pain, Co-inventor on a patent application for the use of (2R,6R)-hydroxynorketamine and (2S,6S)-hydroxynorketamine in the treatment of depression, anxiety, anhedonia, suicidal ideation, and PTSD, All patent rights are assigned to the U.S. government, but Dr. Zarate will share a percentage of any royalties that may be received by the government.

Digital Abstract Session

P280. Therapeutics For Mood Disorders: Animal Models and Neural Mechanisms

Program #/Poster #: P280.01

Topic: G.05. Mood Disorders

Support: CoMRAD

Title: Assessment of orotic acid as a superior carrier for lithium in the management of mania

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Abstract: Lithium carbonate (Li_2CO_3) is a mainstay treatment option for the control of mania. However, Li_2CO_3 is not without its faults, specifically a narrow therapeutic index and common thyroid and renal complications. Lithium orotate (LiOr) is an alternative compound that is suggested to have uptake properties that would allow for markedly reduced dosages in treatment regimens, which would theoretically mitigate toxicity concerns and improve patient compliance. As a first step in exploring LiOr as a replacement for traditional lithium compounds in therapy, we established dose response curves for a range of LiOr and lithium chloride (LiCl) concentrations (0, 1.5, 2.5, 5, 10 and 15 mg/kg, normalized to elemental lithium) following a single injection (single injection protocol; SI) in male wild-type mice using an amphetamine-induced hyperlocomotion (AIH) model (3 mg/kg *d*-amphetamine). AIH is mania-mimetic and is sensitive to dose-dependent blockade by lithium. Lithium was injected intraperitoneally (IP) 30 minutes prior to IP administration of *d*-amphetamine. Animals were recorded for 120 minutes in an open field and scored for total locomotion. Next, to assess whether daily administration would further reduce the dose necessary for attenuation of AIH, mice were injected once daily over a span of 7 days (repeated injection protocol; RI) with LiOr at concentrations of 0, 1.5, 2 and 2.5 mg/kg, and with LiCl at concentrations of 5 and 10 mg/kg. The AIH protocol was performed on the 7^{th} day. The maximal effects of *d*-amphetamine on locomotor activity for SI were observed within the 5-35- and 70-120-minute time bins post-injection. LiOr, but not LiCl, elicited a complete blockade of AIH between minutes 5-35 at concentrations of 10 mg/kg and 15 mg/kg. For minutes 70-120, a partial attenuation of AIH was maintained by LiCl doses of 10 mg/kg or greater, while a complete blockade was induced by LiOr at concentrations of 5 mg/kg and beyond. Following RI, LiOr elicited a complete block at all concentrations of 1.5 mg/kg or greater within the 75-120-minute time bin, while LiCl remained effective only at doses of 10 mg/kg or above, with the strength of blockade unchanged (~40%). Neither compound attenuated AIH during the first window of peak hyperlocomotion. In conclusion, LiOr demonstrates superior efficacy to LiCl in the attenuation of mania-mimetic AIH, as evidenced by reduced dosage requirements and an enhanced strength of blockade at equivalent concentrations. Further, the reduced dosage requirements following RI for LiOr, but not LiCl, may suggest that the orotic acid carrier facilitates enhanced accumulation of lithium within the CNS over time relative to LiCl.

Disclosures: A.G. Pacholko: None. **L.K. Bekar:** None.

Digital Abstract Session

P280. Therapeutics For Mood Disorders: Animal Models and Neural Mechanisms

Program #/Poster #: P280.02

Topic: G.05. Mood Disorders

Support: NIH DP5 OD01790
T32 HD007430-19

Coulter Biomedical Accelerator (BioMedX)
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I. I. Rabi Scholars Program

Title: (*R,S)*-ketamine and (2*R,*6*R*)-hydroxynorketamine differentially alter hippocampal HCN1 expression

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Abstract: Major depressive disorder (MDD) is the leading cause of global disability, yet current pharmacological approaches to treating MDD are both slow and not universally effective. In recent years, the drug (R,S)-ketamine has been promoted as a fast-acting, efficacious antidepressant. (R,S)-ketamine acts within an hour of administration and has single-dose effects that can last for weeks, but also has psychotropic and other unwanted side effects. Therefore, there is a need for alternative drugs that retain (R,S)-ketamine's potent antidepressant effect without its psychotropic side effects. (R,S)-ketamine is also active when administered in a prophylactic manner, prior to stress: both (R,S)-ketamine and its active metabolite (2R,6R)-hydroxynorketamine ((2R,6R)-HNK) can prevent the onset of depressive-like symptoms following exposure to stress. Despite its efficacy, (R,S)-ketamine's mechanism of action is still not clear, as multiple receptors, brain regions, and pathways have been indicated. As such, there is a need for more research into how (R,S)-ketamine exerts both its antidepressant and prophylactic effects. Recently, the hyperpolarization-activated cyclic-nucleotide gated channel 1 (HCN1) has been shown to be necessary for (R,S)-ketamine's antidepressant effect, and downregulation of HCN1 in the hippocampus (HPC) has been shown to be sufficient to induce an antidepressant effect. Thus, we were interested in how levels of HCN1 might mediate both the antidepressant and prophylactic effects of (R,S)-ketamine. We found that an antidepressant administration of (R,S)-ketamine, but not (2R,6R)-HNK, prevented a stress-induced increase in dorsal and ventral HPC HCN1 expression following behavioral stress in male, but not female, mice (7-8 week old 129S6/SvEvTac mice: Taconic, Hudson NY). Conversely, prophylactic administration of (R,S)-ketamine decreased dorsal HPC HCN1 expression prior to stress in male mice, while in female mice, prophylactic administration of both (R,S)-ketamine and (2R,6R)-HNK prevented a stress-induced decrease in dHPC HCN1 expression following behavioral stress. These results suggest that (R,S)-ketamine and (2R,6R)-HNK have sex- and time-specific effects on the expression of HCN1, and that (R,S)-ketamine may exert its prophylactic effect through downregulation of HCN1 prior to stress exposure. Further investigation will be necessary to establish a causal relation between (R,S)-ketamine, HCN1 expression, and antidepressant and prophylactic effects.

Disclosures: **A. Shah:** None. **B.K. Chen:** None. **X. Xu:** None. **S. Deng:** None. **D.W. Landry:** None. **J.V. Kupferman:** None. **C.A. Denny:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent for prophylactic use of ketamine and related compounds against stress-related psychiatric disorders.

Digital Abstract Session

P280. Therapeutics For Mood Disorders: Animal Models and Neural Mechanisms

Program #/Poster #: P280.03

Topic: G.05. Mood Disorders

Title: The putative antidepressant pyro-glutamyl-glutamyl-prolineamide (EEP), an analog of Thyrotropin Releasing Hormone, decreased Bdnf mRNA in animals showing a positive response in the forced swim test.

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Abstract: Thyrotropin releasing hormone (TRH) has been demonstrated to demonstrates rapid efficacy in treating major depression. However, this treatment must be given intrathecally, and the duration of effect is foreshortened due to? degradation byalpha aminopeptidase degradation. Thus, the mode of administration and duration of action makes TRH untenable as a therapeutic agent. Pyroglutamyl-glutamyl-proleneamide (EEP), a naturally occurring analogue, has been demonstrated to have a positive effect in the forced swim test (FST) with peritoneal, subcutaneous, and nasal administration. Interestingly, electroconvulsive seizures have been found to increase both TRH and EEP in brain tissue. Furthermore, both escitalopram and ketamine increase TRH and TRH-like peptides in limbic tissue, including EEP itself. Peripherally, both valproate and corticosterone increase TRH. In addition to its putative antidepressant effects, EEP has been found to have anxiolytic effects, as assessed by the elevated plus maze test. Virtually all antidepressant treatments (reuptake blockers, ECT, ketamine) enhance BDNF (Brain-derived neurotrophic factor) protein. Serum BDNF protein? has been reported to be elevated in patients undergoing psychotherapy, but only in those demonstrating a positive response. BDNF has been found to stimulates dendritic arborization, synaptogenesis, neuronal neogenesis in the hippocampus of adult rats, and enhanced enhances both the survival of new neurons, and activation of “silent neurons”. In turn, the hippocampus shows atrophy in those with major depression, which reverses upon treatment. We assessed the effect of EEP up\\on BDNF expression by measuring the mRNA amount abundance of several *BDNF Bdnf* isoforms mRNA in the hippocampus of EEP treated Wistar ratsanimals (3 injections of 0.5mg/kg EEP vs. saline) compared to vehicle treated controls (all animals ~42 days old upon arrival). While semi-chronic treatment with EEP enhanced swim time in the FST ($p=0.03$, 1-tail), there was a significant **decrease** in total *BDNF Bdnf* mRNA in treated animals ($p=0.017$, 2-tail), with the percent of variance accounted for being 28%. The expression levels of individual *Bdnf*

mRNA isoforms were not altered by EEP treatment. The outcome of this experiment was highly unexpected. The dose and rout of administration has given us a much stronger response in the FST in the past, and we anticipated that this behavioral effect would be associated with an increase in messenger RNA coding for BDNF.Add a summary/implications type sentence?

Disclosures: T.W. Bastian: None. **R.L. Lloyd:** None. **D. Leff:** None.

Digital Abstract Session

P280. Therapeutics For Mood Disorders: Animal Models and Neural Mechanisms

Program #/Poster #: P280.04

Topic: G.05. Mood Disorders

Support: NIMH R01 086828

Title: An α5-containing benzodiazepine site on the $GABA_AR$ is required for the fast antidepressant-like actions of MRK-016 on stress-induced anhedonia and weakened synaptic function

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Abstract: Major depressive disorder (MDD) is a common psychological disorder characterized by chronic low mood, anhedonia and is comorbid with suicidal ideation. Traditional serotonergic antidepressants have a delayed onset of action, requiring 6-8 weeks of chronic dosing and up to half of MDD patients will remain refractory to treatment. Ketamine has shown promise as a rapid acting antidepressant by promoting surges in glutamatergic activity, but its broad antagonism of NMDARs throughout the brain causes side effects that limit its clinical use. We showed previously that negative allosteric modulators of α5-containing $GABA_A$ receptors (GABA-NAMs) have a rapid and persistent anti-anhedonic action in chronically stressed mice and selectively promote excitatory activity within reward regions of the brain with high endogenous $GABA_A$ α5 expression. Chronic multimodal stress was used to induce anhedonia in 8-week old male C57BL/6J mice as assessed through sucrose and female urine preference tests. Treatment with 3mg/kg of the GABA-NAM MRK-016 rapidly reversed stress-induced deficits in hedonic TA-CA1 synaptic strength as assessed via AMPA:NMDA ratios. Pretreatment with 20mg/kg of the benzodiazepine antagonist flumazenil was sufficient to prevent both behavioral and synaptic effects of MRK-016, suggesting that MRK-016's antidepressant-like effects are mediated through the benzodiazepine site of the $GABA_AR$. *In vitro*, MRK-016 has similar affinities for α1, α2, α3 and α5-containing $GABA_ARs$, though it produces the greatest inhibition of GABA responses at α5-containing receptors. We predicted that global genetic deletion of the α5 subunit *in vivo* would be sufficient to block the rapid antidepressant-like effects of MRK-016.

Cohorts of 8-week old, male C57BL/6J α5 KO mice and WT littermate controls were chronically stressed to induce an anhedonic phenotype. Treatment with 3mg/kg MRK-016 was sufficient to restore deficits in hedonic behavior and TA-CA1 synaptic strength in wildtype, but not α5 KO mice. The long-term behavioral and synaptic effects of ketamine and other rapid antidepressant interventions are believed to follow a transient period of high-frequency network activity to strengthen synapses in a use-dependent manner. We demonstrate that WT, but not α5 KO animals generate increases in high-frequency qEEG gamma power in response to 3mg/kg MRK-016. Both strains of animals, however, demonstrated increased gamma-power following 10mg/kg ketamine. We conclude the rapid-acting antidepressant-like activity of MRK-016 is mediated at an α5-containing benzodiazepine site of the $GABA_AR$ and that drugs of this class have potential in treating human depression.

Disclosures: T. Troppoli: None. **P. Zanos:** None. **P. Georgiou:** None. **U. Rudolph:** None. **T.D. Gould:** None. **S.M. Thompson:** None.

Digital Abstract Session

P280. Therapeutics For Mood Disorders: Animal Models and Neural Mechanisms

Program #/Poster #: P280.05

Topic: G.05. Mood Disorders

Title: Effect of agomelatine on the CPF 5-HT2C receptor in a rat model of major depression

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Abstract: Major depressive disorder (MDD) is a disease that affects people of all ages, showing a higher incidence in the female sex. The monoamine theory proposes that a decrease in the concentration of serotonin is the primary cause of this disorder, also affecting the levels of dopamine and norepinephrine. Among the different antidepressant drugs, selective serotonin reuptake inhibitors (SSRIs) stand out, including fluoxetine, which increases serotonin levels in the inter synaptic space, however, they have been described adverse effects with this drug, therefore, the investigation of new drugs for the treatment of this disorder is required, one of them is agomelatine, which is an antagonist of $5HT_{2C}$ receptors and an agonist of melatoninergic receptors, has shown the restoration of dopamine and norepinephrine levels within a few hours after their administration but not of serotonin, so further research is required on this drug. The aim of this work is to evaluate the effect of agomelatine on the $5\text{-}HT_{2C}$ receptor of prefrontal cortex (CPF) in a model of major depression in rats. 120 male rats of the Wistar strain weighing between 250-300g, 6 animals were randomly assigned to the following 5 groups: Sham (Sham surgery), OBX (bulbectomy) OPBX-V (Bulbectomy and vehicle), OBX-F (bulbectomy and fluoxetine) and OBX-A (bulbectomy and agomelatine). The depression model included bilateral

rat bulbectomy surgery (OBX) which was characterized by the olfactory discrimination test, the open field test and the Light/dark test, and the forced swim test. The administration of drugs was carried out daily for periods of 0, 7, 14, 21 days. The agomelatine dose was 40 mg/Kg and 10 mg/Kg for fluoxetine, both had 1% carboxymethylcellulose as vehicle during the aforementioned periods. Once the treatment was finished, the samples were kept at -80ºC. Subsequently, the quantification of serotonin in the supernatant was performed using the Serotonin ELISA kit ADI-900-175. The results of the behavioral tests showed an improvement in the motor behavior of the animals after the treatment. In addition, an increase in the concentration of serotonin in the prefrontal cortex occurs after prolonged treatment with agomelatine. In conclusion, both antidepressants reduced the hyperactivity of the animals with OBX after the different periods, as well as the regulation of neurotransmitters, which together suggest agomelatine as a good drug for the treatment of MDD.

Disclosures: D. Juárez Serrano: None. **I. Cesar Arteaga:** None. **C. Garcia-Olvera:** None. **P. Aguilar-Alonso:** None.

Digital Abstract Session

P280. Therapeutics For Mood Disorders: Animal Models and Neural Mechanisms

Program #/Poster #: P280.06

Topic: G.05. Mood Disorders

Support: Indian Council of Medical Research, Government of India

Title: Brain stimulation rewarding experience ameliorates depression-associated cognitive deficits by modulation of astroglial plasticity

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Abstract: Major depressive disorder (MDD) is a multifactorial disease and often co-exist with profound cognitive impairment and anxiety. One of the key features of MDD is anhedonia, which indicates altered reward circuitry. Recent studies have demonstrated that impaired reward processing or negative bias could precipitate anxiety and cognitive decline. Interestingly, reward-related behaviors and depression-induced dysfunctions are crucially regulated by astroglial cells. Moreover, brain stimulation of rewarding brain areas also modulates the astroglial expression and functions in the prefrontal cortex (PFC), which is a pivotal region for modulation of reward, memory and emotions. However, it is unknown whether brain stimulation rewarding experience could ameliorate depression-associated anxiety, cognitive deficits and accompanying aberrant astroglial plasticity in the PFC. Accordingly, we induced depressive-like behaviors in adult male Wistar rats by neonatal administration of clomipramine from postnatal days 8-21. Then, rats were subjected to intracranial self-stimulation (ICSS) of the medial forebrain bundle (MFB) at the level of the lateral hypothalamus (LH) for 14 days. Rats from control and ICSS groups were

subjected to assessment of depressive-, anxiety-like behaviors, and rewarded alternation task in T-maze. Following behavioral assessments, rats were sacrificed, and $GFAP^+$ astroglial cells were localized using immunohistochemistry. Astroglial expression was quantified using an optical fractionator, and their processes were reconstructed using Neurolucida in the prelimbic and anterior cingulate subregions of PFC. We demonstrated that neonatal clomipramine administration causes depressive-, anxiety-like behaviors, and precipitated cognitive deficits in adulthood. Depression-induced anxiety and cognitive deficits were associated with astroglial cell loss and arbors in the PFC. Interestingly, ICSS of LH-MFB restored affective symptoms and cognitive deficits in depressed rats. The beneficial effects of ICSS treatment were associated with improved astroglial plasticity in the PFC. Our results indicate that brain stimulation rewarding experience could restore astroglial dysfunctions in the PFC leading to amelioration of anxiety and cognitive deficits in depression. We speculate that brain stimulation rewarding experience might be a potential therapeutic tool for the management of depression-induced astroglial dysfunctions in the PFC.

Disclosures: S. Chakraborty: None. **S.J. Tripathi:** None. **B.S. Shankaranarayana Rao:** None.

Digital Abstract Session

P280. Therapeutics For Mood Disorders: Animal Models and Neural Mechanisms

Program #/Poster #: P280.07

Topic: G.05. Mood Disorders

Title: Eukaryotic initiation factor 4E-binding proteins mediate depressive behaviours in mice exposed to chronic variable stress.

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Abstract: Major depressive disorder (MDD) is one of the most common mental illnesses worldwide and chronic stress has been correlated with an elevated risk of developing depression. Ketamine, an N-methyl-D-aspartate receptor antagonist, has been demonstrated to rapidly reduce depressive symptoms in individuals with treatment-resistant MDD. Previously, we demonstrated that ketamine exerts antidepressant-like behavioural effects through regulation of negative regulators of protein synthesis termed eukaryotic initiation factor 4E binding proteins (4E-BPs) in naive mice. However, it is unclear whether this mechanism extends to depressogenic conditions, such as chronic variable stress (CVS). Therefore, we tested how male mice lacking 4E-BP1 and 4E-BP2 (Eif4ebp1/2 double knock out [DKO]) responded to CVS and ketamine. CVS consisted of 2 different stressors per day, for 5 weeks. Stressors included cage tilting, 15-minute restraint, wet bedding, no bedding, forced swimming, exposure to odour-induced variation, and altered light/dark cycle. Twenty-four hours after the last stressor, wildtype and Eif4ebp1/2 DKO mice received either a saline (IP, 10 ml/kg of body weight) or ketamine (IP, 10

mg/kg) injection, and were observed one hour later during a splash test. We found that in wildtype mice, CVS reduced grooming time in the splash test. In contrast, Eif4ebp1/2 DKO mice presented increased grooming in the splash test at baseline and this behaviour was not significantly changed by CVS or ketamine. Our results suggest that 4E-BPs are central in determining the response to ketamine and may be critical mediators of chronic stress.

Disclosures: E. Arsenault: None. **A. Daneshmend:** None. **M. Zhang:** None. **M. Parkhill:** None. **E. Matta-Camacho:** None. **S. Simard:** None. **N. Salmaso:** None. **A. Aguilar-Valles:** None.

Digital Abstract Session

P280. Therapeutics For Mood Disorders: Animal Models and Neural Mechanisms

Program #/Poster #: P280.08

Topic: G.05. Mood Disorders

Support: R01MH051399
P50MH096890

Title: FEDORA long noncoding RNA is a cell-type and sex-specific regulator of mood

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Abstract: Major depressive disorder, the leading cause of disability worldwide, strikes women twice as often as men, yet the molecular mechanisms contributing to this sex difference are poorly understood. There is an urgent need for novel sex-specific molecular targets, which can aid the development of new diagnostics and therapeutics for depression. We recently reported that lncRNAs, a class of regulatory transcripts, are robustly regulated in a sex-specific manner in postmortem brain tissue from depressed subjects compared to controls (Issler et al., *Neuron* 2020). Utilizing advanced bioinformatic tools, we identified sex-specific lncRNAs linked to depression risk. Here we highlight one such target, which is upregulated in multiple brain regions in depressed females only; therefore, we named it FEDORA (FEmale DepressiOn lncRnA). FEDORA is primarily expressed in oligodendrocytes as well as in neurons. Our analysis suggests that it has a potential role in regulating oligodendrocyte function, as its expression levels in female brains correlate with multiple genes that have been implicated in myelination. To test if FEDORA has a sex-specific causal role in depression, we utilized a cell type-specific viral approach to express it in the prefrontal cortex (PFC) of mice of both sexes. We found that

expressing FEDORA exclusively in neurons promoted anxiety- and depression-like behaviors in females only, which mirrored the human sex-specific phenotype. These behavioral changes were associated with transcriptional changes that resemble the sex-specific transcriptional signature of human depression and were associated with changes in neuronal electrophysiological properties. Expression of FEDORA in oligodendrocytes again had a female-specific effect on motivational behavior, which was associated with changes in myelin thickness and gene expression. Finally, we found that circulating FEDORA levels are elevated in depressed women only and that these levels are normalized following antidepressant treatment. Together, these findings support our hypothesis that lncRNAs play key roles in depression and contribute to the sex-differences in this disorder, and may lead to the identification of novel targets for treatment and diagnosis.

Disclosures: O. Issler: None. **Y.Y. Van Der Zee:** None. **C. Tan:** None. **Z.S. Lorsch:** None. **D.M. Walker:** None. **P.J. Hamilton:** None. **A. Ramakrishnan:** None. **M. Salery:** None. **C.J. Pena:** None. **C. Browne:** None. **R. Futamura:** None. **A. Torres-Berrio:** None. **B. Labonté:** None. **J.L. Dupree:** None. **C.A. Tamminga:** None. **Y. Dong:** None. **J. Murrough:** None. **L. Shen:** None. **E.J. Nestler:** None.

Digital Abstract Session

P281. Brain and Behavioral Alterations By Stress and Anxiety

Program #/Poster #: P281.01

Topic: G.04. Emotion

Support: 1R01MH102729-01A1
PSC CUNY Research Award 62664-0050

Title: Prefrontal-limbic circuitry in the children of Superstorm Sandy: a preliminary MRI study

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Abstract: Rising rates of childhood psychopathology mandate investigation into the antecedents of symptom onset. Growing evidence shows prenatal maternal stress experienced in utero is a strong contributor to offspring neurodevelopmental deficits, including emotion dysregulation, a core feature of many types of psychopathology. Evaluation of the prefrontal-limbic circuit, thought to underlie emotion regulation processes, has begun to illuminate aberrations linked to prenatal maternal stress and the offspring's emotion dysregulation. Functional differences in this circuit associated with prenatal maternal stress, particularly in early childhood, remain understudied. We present preliminary MRI data from a small pilot study of <20 5-7 year old children who were prenatally exposed to maternal stress related to a natural disaster, Superstorm Sandy, comparing with children who were not prenatally exposed to the storm. Gray matter volume was assessed via structural MRI scan and brain activity was assessed using an emotional face viewing task during functional MRI (fMRI) scan. Among children prenatally exposed to

maternal stress related to the storm, we found marginally or significantly increased gray matter volume in the bilateral hippocampi and amygdalae as well as decreased gray matter volume in the left lateral orbitofrontal cortex and right parahippocampal gyrus. Further, prenatal exposure was associated with significantly increased bilateral amygdalae activation while viewing fearful or angry facial expressions compared with children who were not prenatally exposed to the storm. Male and female children were included, and sex effects are explored. This represents the first attempt to document prefrontal-limbic differences in prepubertal children on the basis of prenatal natural disaster exposure in boys and girls. These findings suggest structural and functional differences in the prefrontal-limbic circuit on the basis of prenatal stress exposure, which may contribute to risk for psychiatric symptomatology. Our sample size was hindered by the COVID-19 pandemic and current findings need to be interpreted as preliminary. However, our future plan to resume the investigation will target confirming the implication of prenatal stress exposure on the prefrontal-limbic circuit. Gaining insight into the repercussions of the increasing frequency and intensity of natural disasters on the next generation of children will have important public health implications.

Disclosures: J. Buthmann: None. **T. Wu:** None. **A. Shereen:** None. **Y. Nomura:** None.

Digital Abstract Session

P281. Brain and Behavioral Alterations By Stress and Anxiety

Program #/Poster #: P281.02

Topic: G.04. Emotion

Support: NIMH Grant R15MH110951

Title: Gary matter volume correlates of attentional bias to threat in high anxious individuals

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Abstract: The preferential allocation of attentional resources to threatening stimuli is referred to as an attentional bias to threat. This attentional bias to threat is adaptive as it prepares individuals to face potential danger. Yet, exaggerated attentional bias to threat is associated with elevated levels of anxiety and can be maladaptive. The relationship between individual differences in brain structure and attentional bias to threat has been understudied. Limited evidence from a single study suggests that greater gray matter volume in the anterior cingulate cortex is linked to attentional bias to threat (Carlson et al., 2012). Yet, no studies have explored this relationship in high anxiety individuals. To address this, 111 right-handed high trait anxious adults (female = 75) between 18 and 38 (M = 21.90, SD = 4.71) years of age participated in the study. Participants performed a dot-probe task of attentional bias to threat using fearful and neutral faces. Gray matter volume was acquired from whole brain structural T_1-weighted MRI scans. A voxel based morphometry (VBM) regression analysis was used to assess the association between attentional bias to threat and regional gray matter volume. Small volume correction of an *a priori* region of

interest identified a significant positive correlation between gray matter volume and attentional bias in the anterior cingulate cortex (t = 4.02, k = 90, xyz = -9, 35, 20). This association was observed in a number of other regions including middle frontal gyrus, striatum, right posterior parietal cortex, cerebellum, and other distributed regions. No negative correlations between attentional bias to threat and gray matter volume were found. Lastly, exploratory analyses provide initial evidence that distinct sub-regions of the right posterior parietal cortex may contribute to attentional bias in a sex-specific manner: (1) greater gray matter volume in females compared to males: t = 4.53, k = 232, xyz = 44, -41, 42 and (2) greater gray matter volume in males compared to females: t = 3.57, k = 70, xyz = 51, -44, 30. In short, we extend previous findings in unselected samples that elevated attentional bias to threat is linked to greater gray matter volume in the anterior cingulate cortex. Our results illuminate how differences in gray matter volume morphology relate to attentional bias to threat in individuals with high trait anxiety. This knowledge could inform neurocognitive models of anxiety-related attentional bias to threat and targets of neuroplasticity in anxiety interventions such as attention bias modification.

Disclosures: J.M. Carlson: None. **L. Fang:** None.

Digital Abstract Session

P281. Brain and Behavioral Alterations By Stress and Anxiety

Program #/Poster #: P281.03

Topic: G.04. Emotion

Support: NARSAD Young Investigator Grant to CMR
F32MH110135 to CMR
R01DA038063 to PG
1R01DA043676 to PG

Title: Assessing COVID-related stress on mental health and decision-making

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Abstract: The COVID-19 pandemic is an unprecedented and pervasive stressor that poses a direct threat to individuals' health and survival and can also have downstream effects on mental health and decision-making. Understanding the effects of COVID-related health challenges and stress will be critical for informing interventions throughout the pandemic. To comprehensively assess these effects, we conducted a large-scale, mobile (smartphone) app-based, longitudinal study to track how COVID-related stressors affects psychological health (stress levels, depression) and economic decision-making (risk and ambiguity preferences, impulsivity). Each day, participants spent 5-10 minutes completing gamified decision-making tasks and survey instruments on their smartphones. To measure COVID-related stress, we developed the *COVID Stress Assessment* (CSA), a weekly self-report survey that identifies general and domain-specific

stressors as well as how unpredictable, uncontrollable and overwhelming each are perceived to be. Depression was measured using the *PHQ-8*. To measure risk and ambiguity aversion, participants made a series of binary choices between a certain gain and a lottery option with winning probabilities that were explicitly stated (risk) or partially hidden (ambiguity). We measured impulsivity using a delay discounting task. Initial trajectories of subjective stress responses and how they related to mental/physical health and decision-making were assessed in our current sample (n=171; 82% female; mean age = 42.77, SD=14.32). Overall, higher perceived controllability, predictability, and coping all predicted lower global stress levels as measured by the *CSA,* suggesting they serve as protective factors that buffer perceived stress. These protective factors were (on average) negatively correlated with depression levels (*PHQ-8*; Spearman's rho= -0.39, $p<.05$). In terms of physical health, these protective factors were negatively related to the stressfulness of COVID infections that participants experienced (subset reporting positive COVID infection: n=35; Spearman's rho = -0.68, p = 0.0034). While no effect of stress was observed on risk and ambiguity preferences, we found that our protective factors were related to lower impulsivity choice as measured through our delay discounting task (rho= -0.49, $p<.05$). Our results provide an initial assessment of subjective stress responses, as well as stress resilience factors, during the COVID-19 pandemic and set the stage for future analyses tracking how real-world stress predicts changes in physical/mental health and choice behavior.

Disclosures: C.M. Raio: None. **A. Mellis:** None. **A. Vina-Albarracin:** None. **P. Glimcher:** None.

Digital Abstract Session

P281. Brain and Behavioral Alterations By Stress and Anxiety

Program #/Poster #: P281.04

Topic: G.04. Emotion

Title: Empathy and its relationship with peer victimization in school

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Abstract: Introduction: Empathy has been a topic of special attention between all kinds of scientists, in special in these days when societies across the world strive to fight racism, homophobia, sexism and many other types of prejudice. Empathy is frequently defined as a phenomenon comprising cognitive, emotional and behavioral aspects. Its cognitive compound relates to the ability of inferring someone's feelings and emotional state while emotional empathy is described by feelings of compassion and sympathy that guide towards a genuine motivation to help someone in need. The behavioral aspects of empathy are those related to the way the feelings are expressed through verbal and non-verbal behavior. Evolutionarily speaking, empathy is a form of prosocial behavior, especially important for social animals, that helps their members to maintain a social cohesion and the survival of their kins. On the other hand, we have bullying behavior as an aggressive type of interaction that occurs mainly among kids and

teenagers. According to the United Nation Children's Fund (2007), around 30% of children are targets of peers' physical and emotional aggression and many studies suggest that peer victimization, as a form of early life stress, impact the onset of psychopathologies later on in life. But despite all the deleterious effects of peer victimization in a child's life, could such sort of early stress give rise to positive emotions in adults? **Objectives:** This is an exploratory, cross-sectional, descriptive study in which self-reported peer victimization during school life was correlated with general score of empathy in adulthood. **Methods:** 236 brazilian human subjects (M_{age} = 28.61; SD_{age} = 13.04; 78.8% women and 21.2% men; 69.1% single; 41.5% middle class; 47.9% incomplete higher education) answered the Olweus Victim Questionnaire (OVQ) and the Basic Empathy Scale in Adults (BES-A). The items of the OVQ were adapted to evaluate past experiences. All ethical precepts for research with humans based on the Declaration of Helsinki were respected, including the Informed Consent, and data was collected from an aggregated database without the possibility of individual identification. **Results:** A statistically significant association was found between the variables peer victimization and empathy (r = 0,477; p < 0,01). **Conclusions:** This research showed that peer victimization in school is positively related to more empathetic adults, reinforcing pre-existing literature that shows a link between early stressful events and development of more empathetic people. Therefore, it contributes to the existing literature and shines a light towards the dynamics of positive emotions like empathy.

Disclosures: R. Benà De Araujo: None. **R.P. Monteiro:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.01

Topic: G.06. Anxiety Disorders

Support: R01MH122712
R00MH106757
Brain and Behavior Research Foundation Young Investigator Award
Margaret Q. Landenberger Foundation

Title: The role of the kappa opioid system in stress-induced anxiety-like behaviors in females

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Abstract: Women are twice as likely as men to be diagnosed with a mood or anxiety disorder. Given that stress plays a significant role in the development of these disorders, sex differences in the response to stress are likely to be a critical factor in the enhanced vulnerability of females to these pathologies. Across both human populations and animal models, males and females exhibit divergent responses to stress at all levels, from molecular signatures to behavioral adaptations. Sub-chronic variable stress (SCVS) is a model of depression and anxiety in which female mice

develop anhedonia and anxiety-like behaviors but males do not.
Dysregulation of the mesolimbic reward circuitry is implicated in the pathophysiology of stress-related illnesses such as depression and anxiety. Dopaminergic (DA) neurons in the ventral tegmental area (VTA) are a major target of stress. Previous work has shown that acute stress leads to a days-long activation of kappa opioid receptors (KORs) in the VTA. We hypothesize that similar changes may underlie the female-specific behavioral deficits induced by SCVS. If this hypothesis is correct, pre-stress treatment with NorBNI (10 mg/kg, ip) would prevent the stress-induced behavioral deficits observed in females. To test this, we used both male and female C57Bl/6J mice at 8-weeks old in a SCVS protocol (n=9 per group). Using dark-light box to assess anxiety-like behavior, we first confirmed that SCVS causes an increased latency to enter the light compartment in female mice only. Interestingly, this deficit is prevented by a pre-treatment with NorBNI. Next, we will inject the NorBNI post-stress to see if it can reverse this anxiety-like behavior in females. Together, this data indicates the important role of the kappa opioid receptor in the development of maladaptive behavioral responses in female mice.

Disclosures: A. Khurana: None. **C. Bouarab:** None. **A.M. Polter:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.02

Topic: G.06. Anxiety Disorders

Support: MR/NO2530X/1
EP/R025398/1

Title: Structural and molecular signature in the Locus Coeruleus of the vulnerability to develop compulsive adjunctive drinking

Authors: *C. VELAZQUEZ-SANCHEZ[1], L. MARTI-PRATS[1], L. MURESAN[2], B. J. EVERITT[1], D. BELIN[1];
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Abstract: Although the neural mechanisms underlying the vulnerability to develop compulsivebehaviours remain to be established, increasing evidence points to a role ofnoradrenergic (NE) mechanisms in the transition from impulsivity to compulsivity.We showed that chronic treatment with the noradrenaline reuptake inhibitor,atomoxetine (ATO), decreased impulsivity in highly impulsive rats, and selectivelyprevented the associated development of compulsive drinking in a schedule-inducedpolydipsia procedure (SIP).The effects of ATO on impulse control are mediated by the nucleus accumbens shell,the medial portion of which receives NE afferents from the Locus Coeruleus (LC). Wetherefore investigated the structural and cellular mechanisms within the LC that areassociated with the individual vulnerability to develop compulsive drinking.96 male Sprague Dawley rats trained under SIP for 21 days were

screened as high-or low-compulsive drinkers (HD or LD) based on their water intake. We theninvestigated whether LC neuronal ensembles are involved in mediating the anxiogenicproperties of the SIP, or the anxiolysis brought about by the adjunctive response or itscompulsive nature. Rats were challenged in a last session during which the waterbottle was removed for half rats so they were unable to express adjunctive drinkingbehaviour. Brains were harvested 45- or 90 min after the test and processed forRNAscope or immunohistochemistry in order to measure structural features of, andthe expression level of cellular activation or plasticity markers in different cell types inthe LC.Immunohistochemical analysis revealed that HD rats had a larger LC and more cFos+cells in the LC than LD rats. Also, at the population level, dimensional analysesrevealed that the size of the LC was correlated with the compulsive nature of theadjunctive behaviour.
RNAscope data confirmed that cFos expression was higher in the LC of HD than inLD rats, and further showed that it was restricted to GABA neurons, since cFosmRNAs were increased in GAD2 but not in GFAP+ or, following immunohistochemicalanalysis, in TH+ cells. Moreover, the inability to cope resulted in increased cFosexpression in LC astrocytes specifically in HD rats.These results show that an individual's vulnerability to develop compulsive drinking isassociated with structural differences in the LC, and the functional recruitment ofGABAergic neurons, while the inability to cope with distress is associated withactivation of LC astrocytes, suggesting a differential involvement of these twomicrocircuits in the LC in the vulnerability to switch to compulsivity.

Disclosures: C. Velazquez-Sanchez: None. **L. Marti-Prats:** None. **L. Muresan:** None. **B.J. Everitt:** None. **D. Belin:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.03

Topic: G.06. Anxiety Disorders

Support: NIH MH080318
NIH MH112688

Title: Differential effects of diazepam and yohimbine on hippocampal local field potentials during avoid-approach conflict in rats

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Abstract: Anxiety is thought to depend on evaluations of potential future threats, but the neural mechanisms by which this may occur remain unclear. To examine how hippocampus (HC) may relate to anxiety and decision-making, we examined effects of Diazepam and Yohimbine on HC local field potentials (LFP) in rats on the robogator task. In this task, a rat runs back and forth on a linear track for food rewards with a robot lurking near one reward site, lunging forward and

attacking on 20% of the laps. This task has been shown to induce anxiety-like behaviors in which the rat hesitates at the nest doorway (stretch-attend posture, SAP) and quits laps more often, returning to the nest without reaching the far food site (mid-track aborts, MTA). We recorded LFPs from dorsal HC of brown norway rats (6M, 5F) with silicon probes. Rats received Diazepam (2 mg/kg), Yohimbine (2.5 mg/kg), or their respective vehicle controls before each session. Diazepam increased hesitation (SAP) behaviors, but also decreased quit (MTA) behaviors, replicating previous work. Hesitation at the nest doorway coincided with increased sharp wave ripples (SWRs) in HC. Diazepam also reduced MTA events. Animals remained in theta throughout the MTA events. Yohimbine reduced running speed while the rats were running towards the robot, but not on the return journey. Diazepam decreased the incidence of SWRs at the pyramidal layer, decreased low gamma (40-80 Hz), and increased high gamma (80-140 Hz). Diazepam reduced both the power and central frequency of theta. Yohimbine increased low SWR frequencies (150-200 Hz) but decreased high SWR frequencies (200-250 Hz) and broadly inhibited power in the theta, low gamma, and high gamma ranges. Low gamma reflects inputs from CA3 while high gamma reflects inputs from Entorhinal cortex (EC), which would suggest that Diazepam is reinforcing internal hippocampal processing over inputs from EC, which could create the observed reduction in SWRs and could affect hippocampal replay. HC SWRs and theta play important roles representing future outcomes. Given how hippocampal circuits are disrupted by Diazepam and Yohimbine on this task, we expect effects on hippocampal representations of future outcomes could partially explain their effects on anxiety-like behaviors.

Disclosures: M.T. Erickson: None. **A.D. Redish:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.04

Topic: G.06. Anxiety Disorders

Support: NIH-NIGMS Grant SC2GM109811

Title: Exposure to concomitant fluoxetine and aripiprazole leads to a depressive-like outcome in adult female c57bl/6 mice

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Abstract: Major depressive disorder (MDD) is a highly debilitating illness affecting millions across the globe. The first line of defense against this disorder is fluoxetine (FLX), a selective serotonin reuptake inhibitor. Unfortunately, FLX does not alleviate MDD symptoms in most patients - with close to 50% being resistant to this pharmacotherapeutic treatment. A current alternative approach for treatment-resistant MDD is the prescription of FLX in combination with the atypical antipsychotic aripiprazole (ARIP). This is surprising, given that the long-term consequences of this combination treatment (FLX+ARIP) have not been thoroughly assessed at

either the clinical or preclinical level. To make things worse, females are often excluded in studies that assess potential long-term consequences of drug exposure, despite making up more than half of the clinically depressed population. Thus, the purpose of this study is to examine if FLX+ARIP exposure influences reactivity to anxiety-inducing situations, as well as drug-seeking behavior, in later life. To achieve this, adult (postnatal day [PD] 70) female C57BL/6 mice were administered either vehicle (DMSO) or FLX (10 mg/kg) with aripiprazole (0.03 mg/kg) for 15 consecutive days (PD70-84). Twenty-one days later (PD105+), mice were tested on the open field, light/dark box, or cocaine (5 mg/kg) conditioned place preference (CPP) paradigm. The results showed that animals pretreated with FLX+ARIP displayed greater sensitivity to anxiety-inducing situations, and spent significantly less time in cocaine-paired environments, when compared to DMSO-treated controls. Likewise, mice with FLX+ARIP history displayed decreases in preference for cocaine-paired environments, when evaluated on the CPP test. Collectively, these data indicate that the combination treatment of FLX+ARIP induces an enduring anxiogenic and anhedonia-like behavioral profile - recapitulating some of the core symptoms of MDD. Of concern, this long-term enhanced sensitivity to anxiety-inducing environments and reduced preference to reward-related stimuli may reflect general dysfunction(s) of brain reward systems.

Disclosures: **S.A. Castillo:** None. **C. Amaya:** None. **D. Pardo:** None. **S.D. Iñiguez:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.05

Topic: G.06. Anxiety Disorders

Title: Coding of anxiety states by coordinated neural ensembles in the mPFC

Authors: ***G. SHAH**[1], J. POPOVITZ[2], B. HOLT[1], S. P. MYSORE[1], H. ADWANIKAR[1]; [1]Johns Hopkins Univ., Baltimore, MD; [2]Charles River Labs., San Francisco, CA

Abstract: Coding of anxiety states by coordinated neural ensembles in the mPFC

Authors
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Disclosures

G. Shah: None. **J. Popovitz:** None. **B. Holt:** None. **S.P. Mysore:** None. **H. Adwanikar:** None.

Abstract Distributed neural circuits in the prefrontal and limbic regions are known to be involved in the control of anxiety-like behaviors. Much less is known about the neural encoding of anxiety states, with the primary finding, thus far, being the presence of individual neurons that are selective for high or low anxiety states in the medial prefrontal cortex, the amygdala and the ventral hippocampus. The complexity of an affective state such as anxiety suggests that additional insights may be gained from the investigation of coding by ensemble activity patterns

- an approach that has proved effective in studies of other encoding problems in neuroscience.Inspired by these, here, we examine the joint neural ensemble activity patterns (or 'code') in mPFC using calcium imaging in mice engaged in a classic assay of anxiety, the elevated zero maze. Using a miniature fluorescence microscope, we longitudinally tracked the Ca^{2+} dynamics of mPFC neurons in freely moving mice as they traversed the exploration or avoidance zones of the elevated zero maze (EZM).Our data reproduce the classic results of subsets individual neurons being selective but go beyond to show that the joint activity patterns are distinct. These patterns occupy distinct subsets of n-d space, with the open arm representational cloud being denser (more stereotypical patterns), than that of the closed arm. An additional powerful finding is that the distinctive coordinated activity patterns between the two arms is not purely driven by the selective neurons. Surprisingly, sub-ensembles of neurons that are not individually selective also convey distinctive information about anxiety states. Results reveal a novel, distributed, coordinated neural code for anxiety states in the mPFC.

Disclosures: G. Shah: None. **H. Adwanikar:** None. **J. Popovitz:** None. **B. Holt:** None. **S.P. Mysore:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.06

Topic: G.06. Anxiety Disorders

Support: NIMH K99MH124434
BBRF NARSAD
NIH R37HD083217
NIAAA-R01AA023181
NIMH-F32MH112232

Title: Bidirectional control of infant social behavior by dopaminergic innervation of the basolateral amygdala

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Abstract: Social interaction deficits seen in psychiatric disorders emerge in early-life and are most closely linked to aberrant neural circuit organization and function. Due to technical

limitations, we have had little understanding of the typical ontogeny of social behavior neural circuits or how early caregiving adversity impacts infant neurobiology to perturb lifelong social interaction. Using a suite of invasive procedures in awake, behaving young infant rats, including optogenetics, microdialysis and microinfusions, we charted the gradual increase in social behavior deficits following adversity-rearing and dissected circuits controlling this process. Persistently elevated dopamine in basolateral amygdala (BLA) was necessary and sufficient in initiating social behavior pathology, as demonstrated by manipulation of amygdala dopamine during adversity and during expression of social behavior deficits with the mother and peers. Taken together, these data highlight mesolimbic dopamine circuit organization and function as a potential therapeutic target in understanding behavioral deficits associated with psychiatric disorders.

Disclosures: **M. Opendak:** None. **C. Raineki:** None. **R.E. Perry:** None. **E. Wood:** None. **K. Packard:** None. **J. Woo:** None. **R. Zanca:** None. **K. Martinez:** None. **K.Y. Vinod:** None. **R. Brown:** None. **G.A. Deehan:** None. **P.A. Serrano:** None. **D.A. Wilson:** None. **R.M. Sullivan:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.07

Topic: G.06. Anxiety Disorders

Support: NIH Grant RO1AA020444
NSF GRFP

Title: Marble burying behavior is influenced by sex-related chemosensory cues in adult sex-naïve male C57BL/6J mice

Authors: ***C. L. JIMENEZ CHAVEZ**, E. VAN DOREN, E. RIVERA, K. K. SZUMLINSKI; Psychological and Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: In rodents, exposure to chemosensory cues from the opposite sex have be implicated in reward and motivation. For example, interaction with vaginal secretions from sexually mature female rodents have indicated a reduction in anxiety-like behaviors as they are believed to be naturally rewarding stimuli to sex-naïve males. Conventional preclinical behavioral paradigms of anxiety, such as the marble-burying assay, are often employed to measure levels of anxiety-like behaviors but are vulnerable to environmental perturbations (e.g. chemosensory cues) known to alter levels of anxiety and consequently affect marble-burying behavior. To understand the role of chemosensory cues in the marble-burying test, adult male (n=12) and female (n=10) C57BL/6J mice were subjected to marble-burying under three conditions: clean bedding, male soiled bedding, and female soiled bedding. Animals were separated by sex and tested in counterbalanced order in regard to the bedding type. On each test day, animals in the clean bedding group were ran first to avoid any lingering chemosensory cues from the used bedding

from previous testing. After confirming the absence of any carryover effects with the consecutive days of marble-burying, our results identified an anxiolytic effect with the adult male mice in female soiled bedding reflected via a significant decrease in total time spent burying marbles compared to the clean and male soiled bedding. This decrease in anxiety-like behavior demonstrates the influence of chemosensory stimuli in the reduction of anxiety-like behavior and thus an overall behavioral change in the marble burying behavior. Ongoing studies from our group will provide a behavioral profile for both male and female adolescent mice. These future results will provide further insight into the role on how chemosensory social stimuli can alter neurodevelopment during adolescence and result in altered affective responses. The importance in validating these behavioral models is imperative in maintaining the integrity of classic behavioral paradigms particularly with the expansion of behavioral profiling both males and females in preclinical studies.

Disclosures: C.L. Jimenez Chavez: None. **E. Van Doren:** None. **E. Rivera:** None. **K.K. Szumlinski:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.08

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant R21 MH111104
NIH Grant R01 MH121603

Title: Deletion of vasopressin cells in the suprachiasmatic nucleus increases anxiety and sucrose consumption in male and female mice

Authors: *J. WHYLINGS[1], N. RIGNEY[1], G. J. DE VRIES[2,1], A. PETRULIS[1];
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Abstract: The suprachiasmatic nucleus (SCN) is critical for maintaining circadian rhythms, in part through central projections of arginine vasopressin (AVP). However, other functions of SCN AVP outside of rhythmic behavior have not been directly tested. Nuclei that receive AVP projections from SCN, such as the paraventricular nucleus of the hypothalamus, bed nucleus of the stria terminalis, and preoptic area, are important for typical expressions of anxiety-like behavior and social behaviors. Additionally, the SCN is involved in regulating immune responding, which also influences anxiety and social behaviors during sickness. However, whether SCN AVP is involved in sickness behavior has also not been directly tested. We examined the role of SCN AVP cells in the expression of anxiety, social, and sickness behaviors by selectively deleting SCN AVP cells using viral-based, Cre-dependent expression of a cell-death construct (activated caspase) in the SCN of adult male and female AVP-Cre expressing mice (or their Cre-negative littermates; C57/B6 background). We then characterized anxiety behavior, social behavior, and sickness behaviors (induced via i.p. injection of 0.5 mg/kg LPS) in

these animals. We found that SCN AVP cell ablation increased anxiety-like behavior in the elevated plus maze in both sexes, increased sucrose intake in both sexes, and increased urination in males. We found no effects of removing SCN AVP cells on social investigation or communication, sex behavior, aggression behavior, or sickness expression. These results support novel roles for AVP outputs from the SCN in regulating behavior.

Disclosures: J. Whylings: None. **N. Rigney:** None. **G.J. De Vries:** None. **A. Petrulis:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.09

Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC RGPIN-05570-2014

Title: Pubertal Novel Rouxiella badensis subsp. acadensis (Canan SV-53) Mitigates LPS-Induced Depression in Adulthood in a Sex Specific Manner

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[1]Univ. of Ottawa, Ottawa, ON, Canada; [2]Univ. of Cardiff, Cardiff, United Kingdom; [3]Univ. of Franch-Comté, France, France

Abstract: Puberty/adolescence is a critical period of development and a phase with high emergence of psychiatric disorders. During this period, the brain undergoes significant remodeling that affects behavior later in life. Exposure to stress during puberty triggers modifications in brain function resulting in the development of mental disorders in adulthood, such as depression. A single exposure to LPS during puberty resulted in enduring depression-like behaviour in female mice in adulthood. The objective of the current study is to examine the ability of a novel probiotic (*Rouxiella badensis* subsp. *acadiensis (Canan SV-53))*[1,2]treatment during puberty to reverse LPS-induced depression in adulthood. 5 weeks old CD1 mice (n=96: 48 males and 48 females) were daily provided the novel probiotic in 1%sucrose or 1%sucrose (control group) by oral gavage for 14 consecutive days. At week 6 old (puberty), mice received a single intraperitoneal injection of 1.5 mg/kg LPS or an equivalent volume of sterile saline. At 10 week old (adulthood), mice are exposed to forced swimming test and tail suspension test to assess depression-like behaviors. Both behavioral tests revealed the ability of *the* novel probiotic to reverse LPS induced immobility in females while no immobility changes were observed in males. Given the critical role 5-HT1A receptors in depression; immunohistochemistry was used to quantify these receptors in the hippocampus. In mice, a decrease in 5-HT1A receptors in projection areas is associated with depressive-like behaviors. Our results show that female adult mice with depressive-like behaviors presented a significant lower expression of 5-HT1A in CA1 while a decrease trend is observed in CA3 after LPS injection. The consumption of the novel probiotic *(Canan SV-53)* at puberty amplified 5-HT1A receptors expression in these specific

investigated areas while no changes were observed in males. On the other hand, pubertal LPS challenge correlated with an upsurge of the numbers of 5-HT1A receptor in the dorsal raphe nuclei in both males and females while the probiotic alleviated these changes and no difference was observed among probiotic-LPS and probiotic-saline groups in both sexes. Taken together, these findings indicate that exposure to this novel probiotic during puberty decreases LPS-induced vulnerabilities to depression-like behaviours later in life, in a sex-specific manner.
References:1- A **U.S. Provisional Application No. 62/916,921 entitled "Probiotics Composition and Methods"** has been filed related to the bacterium 2- the taxonomy of the bacterium has been submitted to the *International Journal of Systematic and Evolutionary Microbiology*.

Disclosures: N. Yahfoufi: None. **E. Ah-Yen:** None. **R. chandrasegaram:** None. **C. Matar:** None. **N. Ismail:** None. **S. Aly:** None. **C. Hay:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.10

Topic: F.02. Behavioral Neuroendocrinology

Support: NIDA, Grant# 1R15 DA044500-01A1
NIH-ENDURE R25-NS080268706

Title: The interplay between oxytocin and CB1 receptors in the endocrine, mesolimbic, and limbic systems of rats followed by the effects of chronic oxytocin intranasal administration in exercise and anxiety-like behaviors

Authors: P. A. MUÑOZ-RODRÍGUEZ, *A. P. RAMOS-ROLÓN, W. NORZE, L. G. RODRÍGUEZ-SANTOS, V. S. ENCARNACIÓN-CORTÉS, J. M. PADILLA-ESCALONA, L. L. MENDEZ-SANTACRUZ, C. S. MALDONADO-VLAAR;
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Abstract: Oxytocin (OT) is a neuropeptide primarily synthesized in the hypothalamus associated with social behaviors, stress responses, and drug-addiction. A previous study showed a cross-talk between the type 1 cannabinoid receptor (CB1R) of the endocannabinoid system (ECS) and oxytocin receptor (OTR) within the mesolimbic system to modulate anxiety-like behavior. Several studies support that intranasal oxytocin administration and voluntary wheel running are treatments endowed with anxiolytic properties. Additionally, other studies demonstrated that ECS is crucial for voluntary wheel running performance. For this reason and because the presence of OT and endocannabinoids in the limbic, mesolimbic, and endocrine systems has been associated with the anxiolytic response, the purpose of the present study was: (i) to examine whether OT intranasal pretreatment enhances voluntary wheel running behavior and potentiates the anxiolytic properties of exercise in adults male Sprague-Dawley rats and (ii) to characterize the OTR and CB1R expression within the endocrine, limbic, and regions. Rats were divided in

two groups, the first group received intranasal infusions of OT 1 µg /µl, 10 µl or vehicle in each nostril before being exposed to the running wheel two hours daily for 10 consecutive days. The second group received the same dose of OT or vehicle and was not exposed to the running wheel. Results showed that rats treated with OT, exercised more than those treated with vehicle. Using the Light and dark box as a behavioral paradigm, we found that OT potentiated the anxiolytic effect of exercise. Western blots showed that OT treatment significantly increased OT receptors within the pituitary gland of rats exposed to the running wheel. These findings suggest that when both treatments are provided, exercise upregulates OT receptor expression in specific regions related to OT regulation. Future biochemical studies are required to examine the potential cross-talk between OT and the ECS as mediators of the anxiolytic response and exercise performance. We are currently examining OTR's and CB1R's expression in other brain regions associated with anxiety-like behaviors to better understand the neurocircuitry underlying the anxiolytic response and the implications it can have as a treatment for drug addiction, sedentary lifestyle, and anxiety disorders.

Disclosures: P.A. Muñoz-Rodríguez: None. **A.P. Ramos-Rolón:** None. **W. Norze:** None. **L.G. Rodríguez-Santos:** None. **V.S. Encarnación-Cortés:** None. **J.M. Padilla-Escalona:** None. **L.L. Mendez-Santacruz:** None. **C.S. Maldonado-Vlaar:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.11

Topic: F.02. Behavioral Neuroendocrinology

Support: Depaul University URC, FSRG, URAP

Title: Early life exposure to environmental contaminant (PCBs) alters display of motivated and anxiety-like behavior in adolescent male and female rats.

Authors: K. M. FORTUNE[1], *Y. LOWE[2], S. T. RHODES[3], D. A. LIBERMAN[3], K. M. BELL[3], B. A. PLOURDÉ[2], A. E. DEVANEY[4], M. R. BELL[3,1,2];
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Abstract: Polychlorinated biphenyls (PCBs) are a ubiquitous class of persistent organic pollutants and endocrine disrupting compounds. Beyond being implicated in a range of reproductive disorders, PCBs have profound effects on the developing brain. Previous research suggests that early life exposure to PCBs alters dopaminergic and neuroimmune endpoints, often in a sex-specific manner. Both systems are important in motivated behavior and substance abuse, which are also sex-differentiated and begin during adolescence. This experiment seeks to determine the effects of perinatal PCB exposure on motivated behaviors and ethanol intake in both male and female adolescent rats. Pregnant Sprague-Dawley rats were orally exposed to an environmentally relevant mix of PCBs (Arocolor 1242, 1248, 1254, 1:1:1, at 20 ug/kg / day) or

oil vehicle daily during gestation and offspring were behaviorally characterized in adolescence. Animals underwent a light-dark test on postnatal day 35. Males exposed to PCBs showed greater number of entries into, distances traveled in, and time spent in the light chamber, while PCBs only caused minor effects in females. Rats were then singly housed in their home cages over 12 days and provided the choice to drink from bottles that contained 1) water or saccharin (0.25% or 0.75%), to determine sweet preference as a measure of anhedonia OR 2) water or sweetened ethanol (3% - 12%), to determine inclination towards alcohol intake. Rats exposed to PCBs showed significantly greater saccharine intake than Oil exposed animals, especially among females at higher concentrations. However, only minor effects of PCB exposure were found on ethanol intake. The day after the last ethanol/saccharine test, the rats were pair-housed and their social behavior was immediately recorded for 15 minutes in the dark. Across sexes, PCB treated rats performed more play pounces than oil controls; it appears that males treated with PCBs were also quicker to pounce than oil controls. Conversely, females treated with PCBs were slower to show nape-contact. Together, these results indicate that perinatal PCB exposure alters motivation for non-social (sweet) and social (play) reward, in male and female adolescent rats. Ongoing analysis will determine effects on forced-swim tests. Before euthanasia, animals were exposed to a bolus gavage of ethanol or water, so neuroimmune responses to this challenge will also be determined and correlated with behavioral outcomes. Results could inform mitigation and therapeutic strategies to limit effects of environmental contaminants onpresentation of sexually differentiated mood disorders and maladaptive motivated behaviors during adolescence.

Disclosures: K.M. Fortune: None. **Y. Lowe:** None. **S.T. Rhodes:** None. **D.A. Liberman:** None. **K.M. Bell:** None. **B.A. Plourdé:** None. **A.E. Devaney:** None. **M.R. Bell:** None.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.12

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: SUNY Old Westbury FDG
SUNY Old Westbury CSTEP/LSAMP

Title: Lead exposure disrupts rat's developmental milestones with lasting effects on anxiety-like behaviors: Implications for difficulties related to threat and safety learning in later-life

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Abstract: Lead is neurotoxicant, that causes early neurodevelopment pathologies that persist into adulthood and manifest as affective, cognitive, and neurobehavioral disorders. Underrepresented minority children are frequently exposed to lead poisoning, and face

significant health disparities and quality of life challenges across their development due to deficits in cognition and aberrant behaviors. In rat models of lead poisoning, a pronounced negative effect of neurodevelopmental lead exposure is anxiety-like behaviors with reduced inhibitory responses that would otherwise serve to regulate threat and safety context/experience-dependent learning. In order to better understand these later-life difficulties, a dose-dependent rat model was employed to evaluate early- and late-developmental behaviors induced by lead poisoning. The present study evaluated the effects of Perinatal (from 4-weeks prior to breeding through parturition) lead exposure (150 ppm and 1,000 ppm) on rat pup's developmental milestone (DMBs) behaviors (i.e., body weight, body and tail length, righting reflex, positive and negative geotaxis, eye opening, ear pinnae detachment, cliff aversion, vibrissa reflex, forepaw grasping, bar level grip, and auditory startle response) from postnatal day (PND) 2-14 in Long Evans Hooded rats when compared to Control rats (0 ppm). Male and female Control rats did not significantly differ across these DMBs. However, developmental lead exposure altered the DMB profiles in a sex-dependent, more so, than a dose-dependent manner. These rat pups were then followed into adulthood (i.e., PND 36-60 and tested for locomotor activity in the Open Field (OF), anxiety-like behaviors in the Elevated Plus Maze (EPM), and threat and safety experience-dependent learning behaviors in the Active Avoidance Test (AAT). The effects of developmental lead exposure persisted into the later-life rat behaviors thereby increasing locomotor hyperactivity in the OF, increasing hyperactivity and anxiety-like behaviors in the EPM, and decreasing learning performance in the AAT. Taken together, the effects of early neurodevelopmental lead exposure disrupt the normal trajectory of critical DMBs that serve to organize local as well as broad ranging sensori-motor, cognitive, and affective systems that are critical during development. This work serves to increase the developmental psychobiology field's understanding of how psychopharmacotherapies can be investigated in specific contexts such as threat and safety learning, to better serve children in underrepresented minority communities to improve their developmental trajectories and quality of life outcomes.

Disclosures: **J.N. Joseph:** None. **G.B. Cruz:** None. **E. Cabañas:** None. **M.A. Vasquez:** None. **Z. Ayaz:** None. **J.C. Skeen:** None. **E.B. Khairi:** None. **J.R. Bonitto:** None. **I. Ahmed:** None. **K.P. Lynch:** None. **V.D. Hackert:** None. **W. Zhu:** A. Employment/Salary (full or part-time):; part-time, SUNY Old Westbury. **P. Cadet:** A. Employment/Salary (full or part-time):; full-time, SUNY Old Westbury. **L.S. Neuwirth:** A. Employment/Salary (full or part-time):; full-time, SUNY Old Westbury.

Digital Abstract Session

P282. Preclinical Models for Anxiety and Depression

Program #/Poster #: P282.13

Topic: G.06. Anxiety Disorders

Support: PROMEP/103.5/12/4857
UCOL-CS 2015.2017

Title: Dose dependent anxiogenic effect of levetiracetam exposure during pregnancy and lactation in juvenile and adult offspring of Wistar rats with an epilepsy model

Authors: *M. F. PINTO-GONZÁLEZ, N. A. MOY-LÓPEZ, J. GUZMÁN-MUÑIZ, M. ORTIZ, R. PEDRAZA-MEDINA;
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Abstract: Maternal untreated epilepsy and classic anticonvulsive use during pregnancy are related with teratogenic effects over the central nervous system development. Levetiracetam (LEV), has shown less teratogenic effects on pregnancy outcomes, for which, it may be a safe option its use during pregnancy and lactation. However, LEV consumption in adult patients has reported increased anxiety as a side effect, bringing out the necessity to evaluate later in life behavioral changes related to perinatal development. Our aim was to analyze the effect of growing doses of LEV during pregnancy on an epilepsy model and its relationship with anxiety in juvenile and adult offspring. 12 nulliparous female Wistar rats at 90 days of age were divided in 6 groups with different conditions: 1) negative control, saline solution (SS); 2) Positive control LEV 25 mg/kg in SS (LEVSS); 3) LEV 25 mg/kg with previous exposition to Picrotoxin (PTX)-(LEV25); 4) 50 mg/kg LEV-PTX (LEV50); 5) 100 mg/kg LEV-PTX (LEV100); and 6) Exposure to PTX without LEV treatment (PTX). For the epilepsy model, 1.5 mg/kg of PTX or saline solution for 10 days were given to females before induction to pregnancy. Resulting female and male pups were considered as the study subjects (n=120, 20 per group). Anxiety evaluation was made on postnatal 42 (P42) and P90 using the elevated plus maze (EPM), results from EPM were analyzed by anxiety index. To see if there was an effect over mobility, open field test was also applied to identify changes in total distance moved and speed also at P42 and P90. According to data normality, One-way ANOVA was applied. At P42, results from the anxiety index showed no significant differences according to treatment ($F=0.672$, $P>0.05$); while at P90, there were differences between groups ($F=3.649$, sig=0.006), which post-hoc Tukey revealed corresponded to LEV100 group, who showed increased anxiety compared with LEVSS (P=0.002), LEV25 (P=0.029) and PTX (P=0.046) groups. By other hand, mobility also showed significant increase in distance traveled ($F=3.889$, sig=0.003), and speed ($F=3.892$, sig=0.003) when evaluated at P42, corresponding to groups LEVSS-LEV25 (P= 0.010) in distance traveled and LEV25-LEVSS (P=0.009) in speed. There were no differences at P90. When results were compared by sex using univariant analysis, there was no statistical significance neither in P42 or P90 ($F=1.609$, sig=0.163). Results suggest that LEV exposure during critical periods of brain development does not appear to have effects on anxiety during juvenile stages but on mobility, however, there are indicators of increased anxiety at high doses when evaluated during adulthood, indicating possible dose and age related changes.

Disclosures: M.F. Pinto-González: None. **N.A. Moy-López:** None. **J. Guzmán-Muñiz:** None. **M. Ortiz:** None. **R. Pedraza-Medina:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.01

Topic: G.07. Post-Traumatic Stress Disorder

Support: BoR (LEQSF (2018-21)-RD-A-17)
R01 (R01MH122561)

Title: Role of prefrontal cortex afferents to the amygdala in regulation of defensive behavior

Authors: *C. D. BORKAR, Q.-S. LE, R. VUTUKURI, C. VO, A. RESENDEZ, J. FADOK; Tulane Univ., New Orleans, LA

Abstract: Exaggerated responses to the perceived threat are associated with anxiety and post-traumatic stress disorders. Previous studies demonstrated that animals exhibit behavioral scaling to favor flight responses over freezing behavior in the face of high threat intensity. Recently, we developed a modified Pavlovian threat conditioning model to induce freezing-to-flight shifts within a subject and revealed a mutually inhibitory neuronal motif in the central amygdala (CEA) involved therein. However, whether an integrated upstream brain network provides top-down regulation of action selection processes in the CEA is not yet understood. Herein, injection of fluorescent retrobeads into the CEA of mice retrogradely labeled a large number of cells in the dorsal peduncular nucleus (DP), along with fewer cells in the infralimbic region. Conditioning in the modified threat conditioning paradigm induced abundant cFos expression in CEA-projecting DP cells, suggesting activation of the pathway. Double neuronal tracing experiments showed that these projections were distinct from hypothalamus-projecting neurons known to elicit sympathetic responses. Viral labeling revealed that DP afferents to the CEA are predominantly positive for VGLUT1. Furthermore, these afferents strongly innervate the anterior part of the CEA, which contains a large population of CRH neurons known to regulate flight responses. Using in vivo optogenetic and DREADD techniques, inhibition of DP-CEA projections during conditioned stimuli leads to higher freezing and reduced flight, suggesting an important role in regulating defensive behavioral. Similarly, inhibition of this pathway during extinction training significantly increased freezing behavior and decreased flight. Collectively, our results suggest that rapid and flexible action selection in the CEA is regulated by top-down cortical afferents.

Disclosures: C.D. Borkar: None. **Q. Le:** None. **R. Vutukuri:** None. **C. Vo:** None. **A. Resendez:** None. **J. Fadok:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.02

Topic: G.07. Post-Traumatic Stress Disorder

Support: ANR PTSDmemo (ANR-14-CE13-0026-01)
EquipEx Grant OptoPath (ANR-10-EQX-008-1)
Fondation pour la Recherche sur le Cerveau
Institut National de la Santé et de la Recherche Médicale
University of Bordeaux
Ministère de l'Enseignement supérieur et de la Recherche
Conseil Régional d'Aquitaine

Title: The hippocampus-dependent trauma contextualization can prevent and treat PTSD-like memory

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Abstract: Post-traumatic stress disorder (PTSD) is characterized by a paradoxical memory alteration with hypermnesia for salient traumatic cues and amnesia for the traumatic context. Clinical studies strongly suggest that a deficit in trauma contextualization would leave traumatic memory out of control and thus prone to be automatically reactivated upon trauma-related cues in any context through flashbacks. In this view, promoting the contextual memory of the trauma would prevent and/or treat PTSD-related hypermnesia. Yet, preclinical research focuses exclusively on the emotional hypermnesia, leaving unexplored the putative causal role of contextual amnesia and the underlying hippocampal alterations. Using the first animal model that precisely recapitulates PTSD-related hypermnesia and amnesia, we first show that compared to normal fear memory, PTSD-like fear memory is specifically associated with hippocampal hypoactivation and dendritic atrophy. Second, we show that optogenetic inhibition of the hippocampus (dCA1) during stress can produce PTSD-like memory in mice, whereas activating the dCA1 prevents its formation and promotes normal contextual fear memory. Third, the hippocampal dendritic atrophy is shown to be causally involved in PTSD-like memory formation, and blocking such neuronal atrophy prevents the formation of this abnormal fear memory. Finally, trauma re-contextualization, which is shown to be hippocampus-dependent, normalizes PTSD-like memory, promoting the expression of a long-lasting normal fear memory. These findings indicate that PTSD-like memory depends on contextual amnesia and that promoting the hippocampal function promotes a switch from PTSD-like to normal fear memory *via* trauma contextualization. Therefore, these data call for promoting therapeutic approaches of PTSD centered on trauma contextualization and its underlying hippocampal mechanisms.

Disclosures: A.S. Al Abed: None. **E.G. Ducourneau:** None. **C. Bouarab:** None. **A. Sellami:** None. **D.N. Abrous:** None. **E. Pacary:** None. **A. Marighetto:** None. **M. Koehl:** None. **A. Desmedt:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.03

Topic: G.07. Post-Traumatic Stress Disorder

Support: DOD W81XWH-16-1-0016

Title: Intranasal NPY at High Doses to Females Can Prevent Development of Depressive Symptoms with Single Prolonged Stress PTSD Model

Authors: R. J. NAHVI, A. TANELIAN, C. NWOKAFOR, L. PEACOCK, *E. L. SABBAN; New York Med. Col., Valhalla, NY

Abstract: Sex is implicated in susceptibility of stress-elicited disorders. Women are twice as likely as men to develop PTSD, depression, anxiety disorders, social anxiety, and eating disorders. However, most of the studies in animal models examining putative therapeutics, including single prolonged stress (SPS), were performed mostly with males. Previous studies in males demonstrated intranasal NPY provided therapeutic relief of stress-elicited symptoms of SPS, but was ineffective in females at this dose (Nahvi et al, 2019). The overwhelming majority of studies found that NPY levels in many brain areas are lower in females than in male rodents. Thus, they may need a higher dose of exogenous NPY to attain a therapeutically significant concentration or the NPY system may not be as important in females compared to males. Here, we examined SPS as an appropriate model to elicit PTSD-associated symptoms in females. We evaluated whether intranasal NPY at higher doses is able to alter the development of SPS triggered behavioral impairments. Sprague Dawley females rats (7 weeks old), were purchased from Charles River. After 2 week accommodation, they were exposed to SPS and then immediately infused intranasally with one of several doses of NPY, starting with 600 µg/rat - 4 times the dose effective in males. After at least 14 days they were tested on several behavioral tests. SPS elicited significant depressive/despair like behavior on Forced Swim Test (FST), anxiety behavior on the open field and elevated plus maze (EPM), as well as impaired social interaction. On the FST, there was a dose-response effect of intranasal NPY, with 1200µg, but not 600µg, effectively preventing development of the SPS-elicited increased immobility (depressive-like behavior) as compared to stressed, vehicle-treated females. In a separate cohort of animals, females were infused intranasally with 600µg NPY, a dipeptidyl peptidase IV (DPP4) inhibitor, or both immediately after the SPS stressors. This inhibitor is expected to prevent N-terminal cleavage of NPY and increase activity at Y1R. ANOVA revealed significant differences and Tukey comparison showed that DPP4 inhibitor and 600µg NPY combined treatment, but not alone, was sufficient at preventing depressive-like behavior on the FST. The results demonstrate that SPS elicits behavioral manifestations of PTSD, such as depressive-like behavior, anxiety and social impairment in females. This was prevented with early intervention with a high dose of NPY, indicating its therapeutic potential also for females, although a higher dose will likely be required. Furthermore, NPY degradation may play a role in the higher dose requirement for females.

Disclosures: **R.J. Nahvi:** None. **A. Tanelian:** None. **C. Nwokafor:** None. **L. Peacock:** None. **E.L. Sabban:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.04

Topic: G.07. Post-Traumatic Stress Disorder

Support: U.S. Department of Veterans Affairs Biomedical Laboratory Research and Development Program Merit Award 1I01BX003512
William and Ella Owens Medical Research Foundation

Title: Antidepressant Effects of Extinction Learning by Promoting Neuroplasticity in the vmPFC of Chronically Stressed Rats

Authors: *J. LIU, S. E. BULIN, L. F. FERREIRA, A. R. KNIPPENBERG, D. A. MORILAK; Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Posttraumatic stress disorder (PTSD) is a serious mental disorder, which affects 7-8% of the population in the USA. About half of patients with PTSD also have symptoms of major depression disorder (MDD). Treatment of co-occurrence of PTSD and MDD is complex. Our lab established a model of cognitive behavioral intervention in rats, namely extinction therapy, which resembles exposure-based cognitive behavioral therapy (CBT). We found previously that extinction learning ameliorated chronic unpredictable stress (CUS)-induced deficits in cognitive flexibility and active coping behavior in rats. Recently, exposure-based CBT has been demonstrated to be associated with significant reductions in depressive symptoms in PTSD or MDD patients. Thus, the potential antidepressant effects of extinction therapy are examined in the current studies. First, the antidepressant actions were evaluated in the forced swim test (FST), a widely used animal model to screen for potential antidepressant activity. The results showed that extinction learning reduced immobility and increased climbing in the FST 24 h following extinction, which was conducted 2 weeks after fear conditioning. We further found that extinction learning reversed CUS-induced anhedonia, a core symptom of MDD, in the sucrose preference test (SPT) in stressed male, but not stressed female rats. Then, we demonstrated that extinction learning significantly evoked c-fos expression in the ventral medial prefrontal cortex (vmPFC) of stressed rats. The antidepressant effects of extinction learning on CUS-induced anhedonia were blocked by inhibiting neuronal activity in the vmPFC during extinction learning. Furthermore, we found that extinction learning resulted in increased optogenetic potentiation (opto-LTP) of evoked responses in the mediodorsal thalamic - vmPFC pathway in stressed male, but not stressed female rats, which was consistent with the behavioral effects of extinction learning in the SPT. Taken together, these results demonstrate the potential antidepressant effects of extinction learning, which are associated with increased neuronal responsivity and neuroplasticity in the vmPFC.

Disclosures: **J. Liu:** None. **S.E. Bulin:** None. **L.F. Ferreira:** None. **A.R. Knippenberg:** None. **D.A. Morilak:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.05

Topic: G.07. Post-Traumatic Stress Disorder

Support: VA CDA-2 (5-IK2-RX002686-02)
CURES NIEHS P30 (ES020957)
VA Merit (5-I01-RX002252-02)
Shimadzu Grant
John D. Dingell VA Medical Center, Detroit, MI

Title: Combined traumatic stress and chronic alcohol exposure uniquely alters endogenous endocannabinoid system proteins in mice

Authors: *V. M. PIGGOTT[1,3], S. C. LLOYD[1,3], J. I. MATCHYNSKI[2,3,1], S. A. PERRINE[1,3,2], A. C. CONTI[1,3,2];
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Abstract: The cannabinoid (CB) system plays an important role in both stress and alcohol dependence as evidenced by enriched content of CB system components in brain regions that are involved in the reward pathway and limbic system. Pre-clinical studies report that anandamide (AEA) levels are decreased while 2-arachidonoylglycerol (2-AG) levels are increased in limbic-associated brain regions after acute and chronic stress. In alcohol-related animal studies, chronic alcohol exposure has been shown to increase AEA, but not 2-AG levels, in reward-related brain regions. While extensive research has shown that PTSD or alcohol dependence alone affects the CB system, the role of the CB system in the combined PTSD and alcohol dependence/withdrawal condition remains unclear. In this study, we used a mouse model of PTSD, single-prolonged stress (mSPS) combined with chronic intermittent ethanol (CIE) vapor exposure, to quantify AEA and 2-AG contents, and their hydrolase enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAG-L), respectively, in the prefrontal cortex, anterior striatum, nucleus accumbens, dorsal hippocampus, and amygdala. Adult male mice were exposed to mSPS/Control and CIE/Air, and AEA and 2-AG contents were analyzed by ultra-high-performance liquid chromatography-tandem mass spectrometry, while FAAH and MAG-L protein levels were analyzed by immunoblotting. Based on published reports mentioned above, we hypothesized higher AEA levels as a result of decreased FAAH protein levels with unchanged 2-AG and MAG-L levels in mSPS-CIE mice compared to the rest of the groups in reward-related brain regions. In limbic-associated regions, we hypothesized lower AEA and higher FAAH levels with higher 2-AG and lower MAG-L protein levels in mSPS-CIE mice

compared to the rest of the groups. Results showed that while AEA and 2-AG levels, as well as their hydrolase enzymes levels, remained the same among groups in the PFC, AEA and 2-AG levels increased in the anterior striatum in mSPS-CIE mice compared to mSPS-Air mice. In addition, 2-AG content decreased in the nucleus accumbens in mSPS-CIE mice compared to Control-Air and Control-CIE groups. In the dorsal hippocampus, Control-CIE mice had a higher AEA content compared to mSPS-CIE mice, and mSPS-Air mice had higher 2-AG content compared to the rest of the groups. Finally, Control-CIE mice had higher AEA content in the amygdala compared to the rest of the groups. Results in this study indicate that mSPS-CIE comorbidity impairs the CB system in the reward pathway, mainly driven by CIE exposure, and these results could help to examine a neurotherapeutic approach to reduce alcohol cravings in those with PTSD.

Disclosures: V.M. Piggott: None. **S.C. Lloyd:** None. **J.I. Matchynski:** None. **S.A. Perrine:** None. **A.C. Conti:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.06

Topic: G.07. Post-Traumatic Stress Disorder

Support: University of Houston Small Grants Program

Title: Comparative Analysis of Behavioral Responses to Early Life Stress in Male and Female Rats

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Abstract: *Background*: Negative consequences of early life trauma/stress are often expressed much later in life as anxiety disorders, depression and in complicated cases as post-traumatic stress disorder (PTSD). The relationship between early life stress and/or trauma exposure and development of later life psychiatric symptoms in males versus females is not fully understood. This knowledge is critical for early intervention. Animal studies can offer useful insights. *Objective:* The goal of this study was to determine the impact of early life single prolonged stress (EL-SPS) on later life behavioral functions including anxiety- and depression-like behaviors in female and male rats. *Methods:* We utilized the early-life single prolonged stress (EL-SPS), a rodent model of PTSD. Male and female Sprague-Dawley rats at on post-natal day (PND) 25 were exposed to SPS. Assessment of anxiety- and depression-like behavior were performed at early adolescence (PND32), late adolescence (PND60) and adult stage (PND90). *Results:* EL-SPS induced anxiety-like behavior in female rats (SPS-F) at early life (PND32) as indicated by elevated plus maze test (EPM), while male SPS rats (SPS-M) developed anxiety like behavior at adult life (PND90). Interestingly, anxiety-like behavior was concurrent with depression-like behavior in both male and female groups as SPS-F showed depression like behavior at early life

(PND32) as indicated by sucrose preference test, while SPS-M showed depression-like behavior at PND90 as indicated by splash test. *Conclusions:* Our findings suggest differential impact of EL-SPS in male versus female rats. EL-SPS induced behavioral deficits in SPS-F rats (anxiety- and depression-like behaviors) at early life, deficits which disappeared at adult life. Interestingly, EL-SPS induced delayed behavioral deficits in SPS-M rats as anxiety- and depression-like behaviors were expressed much later at adult life in this group.

Disclosures: G. Alrousan: None. **F. Atrooz:** None. **Z. Akbar:** None. **S. Salim:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.07

Topic: G.07. Post-Traumatic Stress Disorder

Title: A systematic review of the link between post-traumatic stress disorder and substance use disorders: insights from animal models

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Abstract: Post-traumatic stress disorder (PTSD) and substance use disorders (SUD) are chronic, debilitating human neuropsychiatric disorders that frequently co-occur. It is estimated that more than half of PTSD patients suffer from SUD, a rate 3-5 times higher than in the general population. Despite research advances over the past decades, neuropharmacological treatment of co-occurring PTSD and SUD remains poor, in part from ineffective preclinical animal models. Thus, the overarching goal of this review was to comprehensively synthesize the existing research and discuss the strengths and weaknesses of currently used animal models that aim to model comorbid PTSD and SUD. A systematic literature search on empirical findings over the last ten years was conducted on the PubMed electronic database. These results indicate that there are numerous gaps in the animal to human translatability of key mechanisms and pathogenesis of co-occurring PTSD and SUD in these models. This translatability is essential to identify novel therapeutic targets, and these findings demonstrate a critical need to stimulate this field of research with more translational preclinical models. This review points out the key research gaps that will need to be resolved in future studies in order to adequately address the link between PTSD and SUD.

Disclosures: G. Gonzalez-Cuevas: None. **F. Alen:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.08

Topic: G.07. Post-Traumatic Stress Disorder

Support: VA Merit Grant BX001075
F32MH117913

Title: A novel prefrontal neurocircuit regulating fear-associated threat responding relevant to PTSD

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Abstract: Post-traumatic stress disorder (PTSD) is a debilitating disorder associated with fear dysregulation. While prior research primarily focuses on fear responses to external threats, recent evidence suggests PTSD patients show heightened reactivity to homeostatic stressor CO_2 inhalation (non-hypoxic). CO_2 inhalation triggers acidosis, representing a homeostatic stressor which evokes fear-associated defensive behaviors. A prior study in veterans found that pre-deployment CO_2 sensitivity predicted trauma-induced PTSD symptoms suggesting CO_2-sensitivity may predict vulnerability to develop PTSD following later trauma. We recently developed a rodent paradigm to examine the effect of CO_2-inhalation on PTSD relevant behaviors where prior CO_2 exposure compromised fear extinction in a contextual fear conditioning paradigm one week later. Importantly, neuronal activation within infralimbic (IL) cortex was significantly reduced *both* immediately following CO_2 and after delayed fear extinction deficits and correlated with CO_2 freezing. Collectively, our data support convergence of homeostatic CO_2-sensing nodes and extinction regulatory circuits, however, underlying neurocircuits are unknown. IL cortex has been strongly associated with fear extinction learning. Previously, we reported the subfornical organ (SFO) as a key site regulating CO_2-evoked fear. In recent studies, using viral tract tracing we identified direct SFO projections to the IL, but not other regions within medial prefrontal cortex such as prelimbic (PL) cortex, suggesting SFO-IL circuits may regulate the long term fear extinction deficits evoked by homeostatic threat, CO_2. Here, we used an intersectional chemogenetic strategy to test the hypothesis that inhibiting SFO-IL projections during CO_2-inhalation would attenuate the CO_2-evoked fear extinction deficits. Consistent with our hypothesis, DREADD-Gi mediated inhibition of the SFO-IL circuit significantly reduced CO_2-evoked defensive behaviors and prevented contextual fear extinction deficits. These data elucidate a novel SFO to IL projection that promotes convergence of homeostatic threat sensing and long-term modulation of fear memory. Our findings highlight the SFO as a novel hub through which interoceptive triggers can regulate vulnerability to external threats and trauma of relevance to PTSD.

Disclosures: K.M. McMurray: None. **S. Shukla:** None. **R. Sah:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.09

Topic: G.07. Post-Traumatic Stress Disorder

Title: Identifying neuronal circuits mediating MDMA's prosocial effects by brain-wide activity mapping

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Abstract: Introduction: MDMA (a.k.a. ecstasy) shows promise in the treatment of PTSD, facilitating psychotherapy by producing strong feelings of social connection, empathy and trust. However, MDMA has abuse potential and long-term heavy use can precipitate neurological, psychiatric and cardiovascular complications. Improving on MDMA to develop a safe, scalable treatment requires understanding its therapeutic mechanism. We found that supraphysiological serotonin release in the nucleus accumbens accounts for MDMA's prosocial effect. Elucidating how this local pharmacological mechanism alters brain-wide activity could yield novel targets to mimic MDMA's therapeutic effects.
Methods: To image brain-wide neuronal activity, *TRAP2* mice (Targeted Recombination in Active Populations 2), in which the neuron firing-dependent *Fos* promoter drives CreER expression, were crossed to a reporter line (*Ai14*). In effect, 4-hydroxytamoxifen transiently enables CreER to trigger tdTomato expression. *TRAP2;Ai14* mice were singly housed and i.p. injected with saline or MDMA (7.5 mg/kg), along with 4-OHT (50 mg/kg). Mice were later regrouped and injected after 2 weeks with saline or MDMA. 90 minutes later mice were perfused with fixative, brains were bisected, made optically transparent (iDISCO+), immunolabeled, and imaged via light sheet microscopy. Using Ilastik and MIRAcL we detected active cells, registered the Allen brain atlas to images, and quantified regional cell counts.
Results: MDMA (333522 ± 17136 cells; N = 7) significantly increased the total number of tdTomato$^+$ (active) neurons compared to saline (160475 ± 36440 cells; N = 6). Counts from 491 grey matter regions were normalized to total counts. MDMA enhanced neuronal activity in several prefrontal cortical regions in both social and non-social contexts, including the prelimbic, agranular insular, infralimbic, anterior cingulate, and orbital areas. In the social context only, MDMA enhanced activity in several areas including the nucleus accumbens, lateral amygdalar nucleus, taenia tecta, rhomboid nucleus, and intermediodorsal nucleus of the thalamus. In the non-social context only, MDMA selectively, decreased activity in regions such as the pre/post subiculum, superior colliculus, red nucleus, and posterior pretectal nucleus.
Conclusions: Through unbiased whole-brain mapping of neurons differentially activated by saline vs MDMA in social and non-social contexts we identified a novel network of regions that may mediate some of MDMA's prosocial effects. Future work will test for causal links between these structures' activity and MDMA's effects on sociability and relief from traumatic experiences.

Disclosures: **D.R. Rijsketic:** None. **P. Llorach:** None. **S.R. Schlozman:** None. **Z. Rastegar:** None. **J. Salgado:** None. **D. Barbosa:** None. **D. Cardozo Pinto:** None. **P. Neuman:** None. **M. Hell:** None. **K. Beier:** None. **R.C. Malenka:** None. **B.D. Heifets:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.10

Topic: G.07. Post-Traumatic Stress Disorder

Support: NIH-NIMH R21MH117483
T32NS007453-19

Title: Understanding Asthma-PTSD risk and comorbidity: Models and Mechanisms

Authors: ***E. N. JOHNSON**[1], I. LEWKOWICH[2], R. AHLBRAND[1], J. MCALEES[2], R. SAH[1]; [1]Univ. of Cincinnati, Cincinnati, OH; [2]Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: The experience of trauma is unfortunately common, however only 10-20% of trauma-exposed individuals develop PTSD. A subset of research in PTSD is dedicated to predisposition factors, and our lab focuses on the role of inflammation, a rising topic of interest in the field. We have specific focus on allergic asthma for which there is evidence of a strong association. Our objective was to determine if a model of chronic asthma would create changes in fear behavior, as well as determine the influence of immune players in those behavioral changes.Allergic asthma has historically been known by a prominent T helper cell, Th2, however recently, severe asthma has shown a mixed phenotype with Th2/Th17. Higher levels of IL-17a, one of the prominent cytokines of Th17, have also been noted in the plasma and saliva of PTSD subjects. A previous publication in our lab using a mouse strain prone to the mixed Th2/Th17 asthma phenotype and increased airway hyperresponsiveness (AHR) following house dust mite (HDM) exposure were found to have compromised fear extinction as well as increased IL-17a in the brain. Thus, we sought to determine the role of Th17/IL-17a in this altered fear outcome.We conducted fear conditioning in a mouse strain, Balb/c, that typically only develops a Th2 phenotype following HDM. To shift the immune response to Th2/Th17, we blocked the complement C5R receptor (C5R antibody) in the lungs. This intervention was previously demonstrated to induce increased AHR as well as the Th2/Th17. Significantly higher freezing during extinction was selectively observed in HDM mice treated with blocked C5R (Th2/Th17) while HDM-IgG control group (Th2) showed freezing similar to PBS groups. Preliminary tissue analysis revealed significantly reduced delta-FoSB within the infralimbic (IL) but not prelimbic cortex of HDM-C5R mice. In a separate cohort, animals underwent the same allergen and antibody intervention, and tissue was collected 72 hours after the last treatment to assess lung and brain cytokine and T cell milieu. Ongoing regional tissue measurements will reveal contributory molecular targets.These results indicate that the mixed Th2/Th17 phenotype seen in HDM-C5R animals produces altered fear extinction compared to the animals with only the Th2 phenotype seen in HDM-IgG control animals. Overall, our work provides novel information on

regulation of PTSD-relevant fear-behavior by inflammatory mediators Th17/17A, central to severe asthma. In addition to mechanistic understanding of the asthma-PTSD association, our studies will provide important insight on how peripheral immune mechanisms can regulate brain function and behavior.

Disclosures: E.N. Johnson: None. **R. Ahlbrand:** None. **I. Lewkowich:** None. **J. McAlees:** None. **R. Sah:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.11

Topic: G.07. Post-Traumatic Stress Disorder

Support: NIH R15 MH114026

Title: Estrous cycle modulation of conditioned fear extinction and relapse

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Abstract: Estrous Cycle Modulation of Conditioned Fear Extinction and Relapse Alyssa A. Hohorst, Margaret K. Tanner, Aleezah Balolia, Troy J. Hubert, Benjamin N. Greenwood. Women are at higher risk than men to develop common stress-related psychiatric disorders such as post-traumatic stress disorder (PTSD). Therefore, identifying effective treatment strategies for females is especially important. Prior research has shown that female sex hormones can modulate fear extinction learning. For example, female rats that learn fear extinction during pro-estrus, when levels of estrogen are high, tend to have better fear extinction retrieval compared to males and females that learn extinction in other estrous phases. Conditioned fear responding, however, tends to relapse after successful fear extinction, even when manipulations that enhance fear extinction are employed. Because protection from fear relapse, rather than strength of the fear extinction memory, per se, is most important in determining the long-term efficacy of extinction-based exposure therapy, it is critical to determine whether estrous cycle phase during fear extinction modulates conditioned fear relapse. The goal of this experiment was to determine whether estrous phase during extinction modulates extinction memory relapse. Estrous cycle of female, Long-Evans rats was monitored daily. All subjects were exposed to auditory fear conditioning in Context A. The following day, subjects were exposed to fear extinction training in Context B. Twenty four hours later, rats were re-exposed to the conditioned stimulus in either the same context used from extinction (Context B), to test fear extinction retrieval, or a different, novel context (Context C) to assess fear renewal. A week later, rats were placed into Context B and tested for spontaneous recovery of fear. Females in pro-estrus during fear extinction training showed less fear renewal and spontaneous recovery compared to males and females exposed to fear extinction training in other estrous phases. Preliminary results suggest that males and females, regardless of estrous phase, had similar extinction recall when tested in Context B

twenty four hours following extinction training. These data suggest that phase of the estrous cycle during fear extinction learning modulates conditioned fear relapse, whereby learning fear extinction during pro-estrus seems to be protective against later relapse. These results could have important clinical implications for the use of exposure therapy in females.

Disclosures:

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.12

Topic: G.07. Post-Traumatic Stress Disorder

Support: Department of Veterans Affairs Merit Research Award I01BX002661
NIH Grant AA028175-01

Title: Antalarmin reduces anxiety and binge alcohol consumption in mice exposed to predator odor stress

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Abstract: Introduction: Anxiety and stress are intimately linked to alcohol abuse. People with anxiety and stress disorders display binge drinking, a common pattern of alcohol abuse. Evidence suggest that people drink alcohol in an attempt to alleviate anxiety. However, the exact relationship between anxiety and binge drinking is not clearly understood. We hypothesized that mice displaying anxiety-like behavior will show increased binge drinking. In addition, since stress promotes the expression and release of anxiogenic corticotropin releasing factor (CRF), blockade of CRF-1 receptor will attenuate anxiety and reduce binge drinking in mice exposed to predator odor. **Methods:** To test our hypothesis, anxiety-like behavior was induced by using predator odor. Drinking-in-the-dark (DID) paradigm was used to examine binge alcohol consumption. Two experiments were performed in C57BL/6J mice. Experiment 1 investigated the effects of anxiety-like behavior on binge drinking in mice. Experiment 2 examined the effect of systemic administration of CRF-1 receptor blockade on anxiety-like behavior and binge drinking. The selective CRF-1 receptor antagonist, antalarmin, was used. Blood alcohol concentration was measured to confirm binge drinking. Sucrose (10% w/v) consumption was also verified in separate groups of mice exposed to predator odor using the DID paradigm in both the experiments. **Results:** Mice displaying anxiety-like behavior following exposure to predator odor exhibit alcohol abuse behavior as evident by increased binge drinking without affecting sucrose consumption. Blockade of CRF-1 receptor by systemic administration of antalarmin attenuated anxiety and reduced binge drinking in mice exposed to predator odor.

Disclosures: V. Mishra: None. **R. Sharma:** None. **M. Parikh:** None. **P. Sahota:** None. **M. Thakkar:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.13

Topic: G.07. Post-Traumatic Stress Disorder

Support: VA Merit 1I01BX003890
VA Merit I01BX001978
American Legion

Title: Time-course of inflammatory markers in the RISP model (Revealing Individual Susceptibility to PTSD-like Phenotype)

Authors: K. ALEXANDER[1], R. NALLOOR[2], K. BUNTING[2], ***A. I. VAZDARJANOVA**[3]; [2]Pharm & Tox, [1]Augusta Univ., Augusta, GA; [3]Charlie Norwood VA Med. Ctr., Augusta, GA

Abstract: Post-Traumatic Stress Disorder (PTSD), which develops as a consequence of trauma in a subset of exposed people, has several hallmark components: intrusive memories, avoidance, hyperarousal, and altered mood and cognition; and is in part a memory disorder. As PTSD is difficult to treat, it is important to reveal susceptibility and increase resilience before trauma. To this end, we have developed the RISP (Revealing Individual Susceptibility to PTSD-like Phenotype) in male rats which allows us to classify animals as Susceptible or Resilient to developing PTSD-like behaviors prior to trauma. We have previously reported that Susceptible, compared to Resilient rats, have a delayed fear extinction and long-lasting trauma-related startle response, as well as altered expression of plasticity-related immediate-early genes in the hippocampus and prefrontal cortex. Inflammation can adversely affect learning and memory and pro-inflammatory factors are elevated in people with established PTSD. We hypothesized that elevated pro-inflammatory factors are also a susceptibility factor. To test this hypothesis, we evaluated levels of IFNy, IL-10, IL-1b, IL-6, KC/GRO, and TNFa in plasma samples from classified rats at different stages of the RISP model: prior to trauma, and 1, 3 or 60 day post trauma. We found that Susceptible, compared to Resilient, rats have higher expression of IL-1b at 1, 3, and 60 days post trauma when normalized to their pre-trauma values. Susceptible rats also had higher expression of TNFa at 1 and 60 days post trauma. The preliminary time-course of inflammatory markers suggests that 1) a susceptibility state is associated with increased pro-inflammatory markers after trauma; and 2) the mild stressor, which is necessary to reveal susceptibility in the RISP model, may be triggering an inflammatory response prior to trauma. While these results are consistent with our hypothesis, further work is needed to elucidate the effect of exposure to the mild stressor on early inflammatory markers in specific brain regions associated with PTSD: prefrontal cortex, hippocampus, and amygdala, as well as assess whether mitigating this inflammatory response will confer resilience.

Disclosures: A.I. Vazdarjanova: None. **K. Alexander:** None. **R. Nalloor:** None. **K. Bunting:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.14

Topic: G.07. Post-Traumatic Stress Disorder

Support: MAPS

Title: A Randomized, Double-Blind, Placebo Controlled Phase 3 Study Assessing Safety and Efficacy of MDMA-Assisted Psychotherapy for the Treatment of Severe PTSD

Authors: *J. MITCHELL[1], C. NICHOLAS[2], A. LILIENSTEIN[3], B. YAZAR-KLOSINSKI[3]; [1]UCSF: Dept. of Neurol., San Francisco, CA; [2]Univ. of Wisconsin, Madison, WI; [3]Multidisciplinary Assn. for Psychedelic Studies, Santa Cruz, CA

Abstract: Introduction
Posttraumatic stress disorder is a prevalent mental health condition with substantial impact on daily functioning that lacks sufficient treatment options. Previous research has led to the designation of 3,4-methylenedioxymethamphetamine (MDMA) as a Breakthrough Therapy for treatment of post-traumatic stress disorder (PTSD) when administered as an adjunct to psychotherapy.
Objectives
Here we report the findings of the first randomized, double-blind, Phase 3 trial assessing the efficacy and safety of 3 sessions with a flexible dose of MDMA or placebo administered under direct observation to participants with severe PTSD (n = 100) as an adjunct to inner-directed psychotherapy.
Methods
Change in PTSD symptoms (CAPS-5) and functional impairment (SDS) were assessed by a central, blinded Independent Rater Pool at baseline and following each treatment session. Adverse events (AEs), concomitant medications, suicidal ideation and behavior were tracked throughout the study. Vital signs were measured during experimental sessions. The primary endpoint was 18 weeks post-randomization.
Results
Change in CAPS-5 and SDS, placebo and therapy-subtracted Cohen's d effect size, and a responder analysis will be presented. There were three serious AEs of suicidal ideation or behavior reported. MDMA was well tolerated, with some treatment emergent AEs occurring at greater frequency for the MDMA group during and after experimental sessions.
Conclusions
If MDMA-assisted psychotherapy significantly attenuates PTSD symptomatology and associated functional impairment, these results will form the basis for marketing authorization applications worldwide, including among participants with dissociative subtype of PTSD, depression, history of alcohol and substance use disorders, and adverse childhood experiences.

Disclosures: **J. Mitchell:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; MAPS. **C. Nicholas:** None. **A. Lilienstein:** None. **B. Yazar-Klosinski:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.15

Topic: G.07. Post-Traumatic Stress Disorder

Support: NINR Intramural Research Program

Title: Changes in inflammatory markers and trophic factors following sleep-focused treatments in military service members with and without PTSD

Authors: ***J. LEETE**[1], A. JIANG[1], C. DEVOTO[1], J. M. GILL[1], H. L. RUSCH[1,2];
[1]Natl. Inst. of Nursing Res., NIH, Bethesda, MD; [2]Dept. of Clin. Neurosci., Div. of Psychology and Osher Ctr. for Integrative Medicine, Karolinska Inst., Stockholm, Sweden

Abstract: Sleep disturbance is one of the most common residual symptoms following treatment for posttraumatic stress disorder (PTSD). PTSD has been associated with inflammation and disruption of trophic factors. Previous research suggests that sleep-focused treatments may lead to decreases in inflammation and restoration of trophic factors, but more research is needed, especially in comorbid sleep disordered and PTSD populations. This study examined the effect of standardized sleep therapy on sleep quality, posttraumatic stress symptoms, plasma-derived inflammatory markers [C-reactive protein (CRP) and interleukin 6 (IL-6)], and trophic factors [brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1)] in sleep disturbed military service members with varying degrees of posttraumatic stress symptoms. Military service members (n=100, 98% male, mean age = 34.8 years) with insomnia and/or obstructive sleep apnea (OSA) were enrolled. Participants with insomnia received cognitive behavioral therapy for insomnia (CBT-I), participants with OSA received automatic positive airway pressure (APAP), and participants with comorbid OSA and insomnia received APAP and either CBT-I or sleep hygiene education. Baseline (week 1) and follow-up (week 12) visits included self-report questionnaires and blood draws, which were assessed with the SIMOA® HD-1 Analyzer. For analysis, participants were assigned to three groups according to baseline PTSD Checklist - Military Version scores: PTSD (n=29), Subclinical PTSD (n=39), and No PTSD (n=32). Pre-post analyses revealed improvements in sleep quality in the Subclinical PTSD (t_{31}=2.27, p=0.030) and No PTSD groups (t_{28}=2.83, p=0.008), but not in the PTSD group. In the PTSD group, levels of CRP decreased (t_{19}=2.70, p=0.014), while levels of BDNF (t_{15}=-2.37, p=0.032) and IGF-1 (t_{14}=-2.78, p=0.015) increased. In the Subclinical PTSD group, levels of IGF-1 (t_{17}=-2.71, p=0.015) increased, and in the No PTSD group levels of BDNF (t_{19}=-2.88, p=0.010) increased. In the PTSD group, posttraumatic arousal (t_{28}=3.01, p=0.005) decreased following treatment. Our findings suggest that standardized sleep treatments may reduce

posttraumatic arousal and alter peripheral inflammatory markers and trophic factor levels in military personnel with PTSD. While the participants with PTSD did not report significant improvements in self-reported sleep quality, we observed significant changes in biomarker concentrations. Measuring levels of inflammatory markers and trophic factors following sleep-focused treatments may provide added value for assessing treatment efficacy, and future research is warranted.

Disclosures: J. Leete: None. **A. Jiang:** None. **C. Devoto:** None. **J.M. Gill:** None. **H.L. Rusch:** None.

Digital Abstract Session

P283. Preclinical and Human Models of Stress and PTSD

Program #/Poster #: P283.16

Topic: G.07. Post-Traumatic Stress Disorder

Support: NIH grant R01-M1H106574
Medical College of Wisconsin CTSI Pilot Award
TL1TR001437 (Webb)

Title: Stability of hippocampal subfield volumes after trauma and relationship to development of PTSD symptoms

Authors: *K. WEBB[1], C. WEIS[1], A. HUGGINS[1], M. KALLENBACH[1], T. MISKOVICH[2], J. FITZGERALD[3], K. BENNETT[4], J. KRUKOWSKI[3], T. DEROON-CASSINI[5], C. LARSON[1]; [1]Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]VA Northern California Hlth., Martinez, CA; [3]Marquette Univ., Milwaukee, WI; [4]Montana VA Healthcare Syst., Fort Harrison, MT; [5]Med. Col. of Wisconsin, Milwaukee, WI

Abstract: The hippocampus plays a central role in fear learning and memory. The majority of neuroimaging research on post-traumatic stress disorder (PTSD) has studied the hippocampus in its entirety. Although the extensive literature demonstrates changes in hippocampal volume are associated with PTSD, fewer studies have probed the relationship between PTSD symptoms and the hippocampus' functionally and structurally distinct subfields. We utilized data from a longitudinal study examining post-trauma outcomes to determine whether subfield volumes change over time after a trauma and whether specific subfields are significantly associated with, or predictive of, PTSD. We leveraged our unique study design sample to also investigate reliability of hippocampal subfield volumes using pipelines available in *FreeSurfer*. Two-hundred and fifteen trauma-exposed individuals were recruited from an urban Emergency Department. Two-weeks post-traumatic injury, participants underwent two consecutive days of neuroimaging (T1 and T2) and completed self-report assessments. Six-months later (T3), participants underwent an additional scan and were administered a structured interview assessing PTSD symptoms. First, we compared we compared reliability of hippocampal measurements at T1 and T2 (automatically segmented with FreeSurfer v6.0). We then examined the prospective

(T1 subfields) and cross-sectional (T3 subfields) relationship between volumes and PTSD. Finally, we tested whether change in subfield volumes between T1 and T3 explained PTSD symptom variability. None of the subfield volumes (T1) significantly predicted T3 PTSD symptoms. Hippocampal tail volumes (T3) were associated cross-sectionally with PTSD symptoms (T3) after controlling for other relevant variables. Tl - T2 reliability of all hippocampal subfields ranged from good to excellent (intraclass correlation coefficient (ICC) values > 0.83), with poorer reliability in the hippocampal fissure. Our results provided a novel examination of the predictive relationship between hippocampal subfield volumes in relation to PTSD in a large trauma-exposed urban sample and demonstrated that hippocampal tail volumes are associated with PTSD symptoms. In addition, we demonstrated *FreeSurfer* hippocampal subfield segmentation is a reliable tool. Although hippocampal subfield volumes may be an important marker of individual variability in PTSD, findings are conditional on the timing of the measurements (e.g. acute or chronic post-trauma periods) and analysis strategy (e.g. cross-sectional or prospective).

Disclosures: K. Webb: None. **C. Weis:** None. **A. Huggins:** None. **M. Kallenbach:** None. **T. Miskovich:** None. **J. Fitzgerald:** None. **K. Bennett:** None. **J. Krukowski:** None. **C. Larson:** None. **T. deRoon-Cassini:** None.

Digital Abstract Session

P284. Mechanisms Contributing To Psychiatric Symptomatology: Insights From Animal Models

Program #/Poster #: P284.01

Topic: G.05. Mood Disorders

Title: Ventral hippocampal Egr1 expression is mechanistically implicated in mediating the role of ovarian hormone fluctuations on sex-specific psychiatric risk

Authors: *D. ROCKS[1], I. JARIC[1], E. F. GALLO[1], J. M. GREALLY[2], M. SUZUKI[2], M. KUNDAKOVIC[1];
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Abstract: Females exhibit a two-fold increased risk for anxiety disorders compared to males. Due to the exclusion of females in animal research of the brain and behavior, little is known about the mechanisms driving this sex-specific risk. We previously demonstrated a role for ovarian hormone fluctuations in mediating sex-specific vulnerability to anxiety-related behavior in female mice. We found that the physiological drop in estrogen during the estrous cycle is linked to increased indices of anxiety-related behavior. Further, we identified widespread changes in neuronal chromatin accessibility and gene expression in the ventral hippocampus, a brain region relevant to anxiety, underlying this behavioral phenotype. Subsequent bioinformatic analyses indicated Egr1, an estrogen-responsive transcription factor, as a potential upstream regulator of differential chromatin accessibility and gene expression in females across the estrous cycle. To understand the mechanistic role of ventral hippocampal Egr1 expression in estrous

cycle-dependent behavioral changes, we used stereotaxic surgery to deliver an adeno-associated viral vector carrying Egr1 and GFP (or a GFP-only control virus) under control of a neuronal-specific promoter into the ventral hippocampus of ovariectomized(OVX) female (n=12/group) and male (n=12/group) mice. We demonstrate that OVX females, at baseline, exhibit high anxiety-related behavior in the open field (OF) and elevated plus maze (EPM) tests (n=8-12/group), similar to low-estrogenic intact females. Remarkably, overexpression of Egr1 in ventral hippocampal neurons of OVX females mimics the high-estrogenic state, as Egr1-OVX females display reduced anxiety-related behavior in the OF and EPM tests compared to GFP-OVX females. We observed no behavioral changes in male mice overexpressing Egr1, indicating that the effect of neuronal Egr1 expression on behavior is sex-specific. We explore differential expression and chromatin accessibility of Egr1 target genes relevant to neuronal function and behavior as a plausible mechanism by which neuronal Egr1 expression in the ventral hippocampus drives anxiety-related behavior. Previous results from our lab indicate an important role for sex hormone fluctuations in female-specific susceptibility to anxiety disorders. Here we demonstrate a mechanistic role for Egr1 in driving the low-anxiety phenotype associated with the high-estrogenic state. Cyclical Egr1 expression in intact female mice presents a plausible mechanism through which fluctuating ovarian hormones can confer sex-specific risk for anxiety-related phenotypes.

Disclosures: **D. Rocks:** None. **M. Kundakovic:** None. **E.F. Gallo:** None. **I. Jaric:** None. **M. Suzuki:** None. **J.M. Greally:** None.

Digital Abstract Session

P284. Mechanisms Contributing To Psychiatric Symptomatology: Insights From Animal Models

Program #/Poster #: P284.02

Topic: G.05. Mood Disorders

Support: NIH Grant MH103848
NIH Grant NS073899

Title: Fkbp5 and early life stress impacts neuroendocrine response, depression and hippocampal cells in an age-dependent mechanism

Authors: M. CRIADO-MARRERO, *L. J. BLAIR;
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Abstract: Early life stress (ELS) causes short and long-term changes in the brain. ELS adverse effects are linked with the etiology of mental health disorders like major depression. Studies suggest that ELS impacts coping abilities and cognitive functions in patients with depression by altering the hypothalamic-pituitary-adrenal (HPA) axis response, increasing peripheral inflammation, and evoking structural brain alterations. Although the underlying causes are unknown, polymorphisms in the FK506-binding protein 5 (FKBP5) gene, an endogenous

regulator of stress-neuroendocrine response, interact with childhood adversities to increase vulnerability to depressive disorders. We hypothesized that high FKBP5 protein levels combined with early life stress (ELS) would model some features of depression in mice including altered HPA axis and brain as well depressive-like behavior. To test this, we exposed males and females of a mouse model overexpressing FKBP5 in the brain (rTgFKBP5 mice), or littermate controls, to maternal separation for 14 days after birth. We assessed neuroendocrine, behavioral, and hippocampal changes in young adult and aged mice. Lower basal corticosterone (CORT) levels were detected in rTgFKBP5 mice, especially in females, when compared to control group. Aged, but not young, rTgFKBP5 mice showed increased depressive-like behaviors. Moreover, hippocampal neuron density was reduced in aged rTgFKBP5 mice, while microglia expression was induced by high FKBP5. However, these effects were reversed by ELS. Overall, our findings demonstrate that overexpression of FKBP5 impacts basal CORT levels, depressive-like symptoms, and numbers of neurons as well as microglia in the hippocampus in an age-dependent manner.

Disclosures: L.J. Blair: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent on Transgenic Animal Model. **M. Criado-Marrero:** None.

Digital Abstract Session

P284. Mechanisms Contributing To Psychiatric Symptomatology: Insights From Animal Models

Program #/Poster #: P284.03

Topic: G.05. Mood Disorders

Title: Intergenerational Transmission of Existential Trauma (or Traumatic Stress): Reprogramming of the Brain Metabotranscriptome and Opportunity for Reversibility

Authors: *S. T. ALHASSEN, S. CHEN, A. D. SILVA, P. BALDI, G. ABBOTT, A. ALACHKAR;
Univ. of California, Irvine, Irvine, CA

Abstract: Traumatic stress transmission from pregnant mothers to the offspring increases the life-course susceptibility to depression and is a major risk factor for developing different neuropsychiatric disorders. Whether the intergenerational trauma transmission and its deleterious outcomes are a consequence of in-utero fetal neurodevelopment disruptions or from poor maternal care by traumatized mothers are still largely unknown. The purpose of this study was to further investigate the differences between the prenatal and postnatal mechanisms that are responsible for the effects on the offspring. On day 17 of gestation, female mice were exposed to a predator scent to induce stress to the animal. Pups of control (C) and stress (S) mice were raised by their biological mother (C/C and S/S) or were cross-fostered to a female of the other treatment (C/S and S/C for S pups with C mothers and C pups with S mothers respectively) within 48h of birth. The male mice were then selected for the study and underwent a series of

behavioral assays to understand the effects of the prenatal and postnatal stress conditions. We found that the exposure to traumatic stress during pregnancy induces in the offspring depressive-like behavior and social deficits. Metabolomics, transcriptomics and bioinformatics analyses reveal mechanisms that involve stress- and hypoxia-response energy metabolic pathways (mitochondrial ATP production). We also found that early pharmacological intervention, through the use of acetyl-L-carnitine (ALCAR) supplementation, was able to produce outlasting protection against the observed behavioral deficits. This study extends the knowledge on both predictive and protective values of ALCAR in vulnerable populations who are at high-risk to depression. Given ALCAR's unique features, such as the very high efficiency, multiple mechanisms of action, low cost, high safety profile, rapid onset of action, and outlasting antidepressant effects, it provides an innovative and unique prophylactic and therapeutic strategy, when administered in the right time of life.

Disclosures: S.T. Alhassen: None. **S. Chen:** None. **A.D. Silva:** None. **P. Baldi:** None. **G. Abbott:** None. **A. Alachkar:** None.

Digital Abstract Session

P284. Mechanisms Contributing To Psychiatric Symptomatology: Insights From Animal Models

Program #/Poster #: P284.04

Topic: G.05. Mood Disorders

Title: Mating and parenting experiences sculpture mood-modulating effects of oxytocin-MCH signaling

Authors: *L. ALHASSEN, J. PHAN, A. ARGELAGOS, W. ALHASSEN, B. VACHIRAKORNTONG, Z. LIN, O. CIVELLI, A. ALACHKAR;
Univ. of California, Irvine, Irvine, CA

Abstract: Oxytocin (OXT) and melanin concentrating hormone (MCH) are hypothalamic neuropeoptides that play a physiological role associated with maternal behavior and emotion. In this study, we illustrate the role of a hypothalamic circuit (OXT-MCH signaling) in regulating mood, parenting and mating, and depressive behavior. To understand how oxytocin-MCH signaling controls mood, we looked at maternal behaviors in an animal model that has OXT receptors deleted from MCH neurons in both sexually naïve females/males and postpartum mothers. A series of maternal and mood behaviors were carried out on both virgin and postpartum mothers, such as pups retrieval, maternal aggression, and forced swim. We show that deleting OXT receptors from MCH neurons increases and decreases depressive behavior in sexually naïve females and postpartum mothers. However, OXTR deletion from MCH neurons did not affect mood in naïve male. In spite of this, OXTR deletion from MCH neurons did not affect maternal behaviors, which is evidenced by the normal pups' retrieval, nest building, and maternal aggression in the OXTR-cKO mice. Overall, these findings suggest that this action is sexually dimorphic and dependent on maternal and mating experiences.To understand how

OXTR deletion from MCH neurons is associated with changes in neuronal activity, we looked at the alterations of Arc (activity-regulated cytoskeletal gene) expressions. Arc is an early gene that plays a crucial role in roles in neural plasticity in the frontal cortex involved in cognitive functions and emotions. We showed that there is an increase in Arc expression during late postpartum stage in various brain regions such as the LH, PVN, SON, VTA, and BLA, in both control and OXTR-cKO female mice. This is the first report on the remapping of Arc expression by maternal experience. Furthermore, this finding suggests that Arc expression can serve as a marker for mapping the neural substrates recruited by female brain to induce neuroplasticity and prepare females for parenthood. Together, the data suggests that the oxytocin-MCH pathway can serve as a potential therapeutic target for major depressive disorder and postpartum mood abnormalities.

Disclosures: L. Alhassen: None. **J. Phan:** None. **A. Argelagos:** None. **W. Alhassen:** None. **B. Vachirakorntong:** None. **Z. Lin:** None. **A. Alachkar:** None. **O. Civelli:** None.

Digital Abstract Session

P284. Mechanisms Contributing To Psychiatric Symptomatology: Insights From Animal Models

Program #/Poster #: P284.05

Topic: H.04. Executive Functions

Title: Investigating Lewis Rats as a Potential ADHD Animal Model for Cognitive Flexibility

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Abstract: If the mind were a TV, cognitive flexibility could be considered analogous to changing the channels, where each channel is a concept or stream of thought. Impairments in cognitive flexibility, where struggling to flip the channels occurs, are found in a wide range of psychiatric disorders. Attention deficit-hyperactivity disorder (ADHD) is a mental health disorder; classified by high levels of hyperactivity, impulsive behavior, and impaired decision-making. ADHD is one of the many dopamine (DA) linked mental health disorders that are implicated in cognitive flexibility impairments. Spontaneously hypertensive rats (SHRs) are the most common model of ADHD in animals, but some research has shown that Lewis rats show similar impairments in a variety of decision-making tasks (Panfil, In progress). Three different strains (Long-Evans, SHR, & Lewis) of rats were run through the attentional set-shifting task (ASST), which is the rodent equivalent of the Wisconsin Card Sorting Task and tests cognitive flexibility and attention (Birrell et al., 2000). To control for order effects, a Latin square counterbalancing procedure was used to ensure each pair of exemplars would be experienced in each phase across the rats (Tait et al., 2018). Data collection is still in progress, but a total of 18 rats have been run throughout the task thus far. Preliminary results indicate that SHR rats were

significantly impaired across the entire task, which replicates previous research with this strain (Cao et al., 2012). Lewis rats were not impaired across any phases of the task and even performed better than controls (Long-Evans) in some phases of the task. This offers evidence that the Lewis strain may be a good ADHD model for some timed decision-making tasks, but within the model of cognitive flexibility may not be an effective animal model of ADHD.

Disclosures: Z. McKinnell: None. **T. Maze:** None. **B. Challans:** None. **L. Parks:** None. **C. King:** None. **B. Plakke:** None.

Digital Abstract Session

P284. Mechanisms Contributing To Psychiatric Symptomatology: Insights From Animal Models

Program #/Poster #: P284.06

Topic: G.05. Mood Disorders

Support: NIH grant R01MH086828 (SMT)

Title: What does the Sucrose Preference Test tell us about reward behavior? An analysis of licking behavior during SPT

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Abstract: The Sucrose Preference Test (SPT) is widely used for studying hedonic behaviors and a loss of SPT is commonly used as a model for anhedonia, a key symptom of depression. In the SPT, the preference for a 0.5-2% sucrose solution over regular water, is tested by measuring volume drunk by experimental mice overnight. Despite its common use, we know little about how the volumetric sucrose preference is produced and if it is related to hedonic, metabolic, and/or memory processes. To get a better understanding of this, we measured the licking microstructure of mice as they underwent the SPT, using an in-house built capacitive lickometer. The lickometers were validated by a significant correlation between the licks at each bottle and the volume consumed overnight.
We found that stress-naïve mice preferred 1% sucrose when measuring the total number of licks, number of lick bouts, and the average number of licks-per-bout. There was a significant positive correlation between the lick preference and the volume preference. The significant correlation between the volume preference and the licks-per-bout preference is especially noteworthy because this measurement is related to the hedonic value of the consumed solution. This data supports the notion that the SPT does indeed measure hedonic processes in the test animals. In addition, we tested a small cohort of animals on the SPT, followed by a choice between 1% and 10% sucrose. Mice displayed a decrease in all licking behaviors for 1% sucrose, when given a choice of 10% sucrose, suggesting that licking behaviors and volume preference represents a relative hedonic value. We also found evidence that reward memory processes are involved in producing the SPT. First, on each night we found that drinking from the water bottle decreased,

while lick preference for sucrose increased, throughout the night, suggesting that the place of the sucrose bottle had to be memorized first. Furthermore, we found no evidence that mice would switch from the water bottle to the sucrose bottle during a session of drinking, further supporting the involvement of memory in producing the sucrose preference.
Finally, we subjected mice to chronic treatment with corticosterone, a stress-hormone known to produce anhedonia, and subsequent injection of psilocybin to determine how anhedonia and rapid-acting antidepressant treatment affected licking behavior during the SPT.
We conclude that the volumetric SPT does indeed measure hedonic processes, but also involves contributions from memory processes and possibly metabolic processes. Recording licking behaviors can thus be used as a supplement or substitution for the volumetric SPT.

Disclosures: A.B. Wulff: None. **S.M. Thompson:** None.

Digital Abstract Session

P284. Mechanisms Contributing To Psychiatric Symptomatology: Insights From Animal Models

Program #/Poster #: P284.07

Topic: G.05. Mood Disorders

Title: Investigating the neurobehavioral effects of high fat diet in male and female mice

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Abstract: Major depression (MD) and obesity are two highly comorbid disorders that pose a significant public health problem. Indeed, rodent studies have shown long-term consumption of a diet high in fatty-acids (HFD) to elicit a depressive-like phenotype. Further, evidence from both clinical and preclinical research suggests that the symptomology and neurobiology of MD are sex-specific. However, the majority of preclinical research into the neurobiological mechanisms underlying the link between MD and obesity has focused on males, and the role of sex in obesity-related depressive neurobehavioral responses remains understudied. Specifically, little is known about the neurochemical systems underlying the sexually-differentiated behaviors observed in the HFD model. Thus, the goal of the current study was to elucidate the sex-specific behavioral and neurochemical effects of long-term (i.e., 16 weeks) HFD consumption in C57BL/6J mice of both sexes. Interestingly, our results showed sex-differentiated depressive-like behavior in HFD-fed mice, as only male mice exhibited reduced grooming behavior in the splash test. Both sexes exhibited anxiogenic behavior in the open field test and novelty-suppressed feeding test, as well as anhedonia as assessed by sucrose consumption. HFD consumption also altered regional brain neurochemistry assessed *ex vivo* using high performance liquid chromatography (HPLC) in a sex-specific manner. Interestingly, these brain region-dependent neurochemical alterations extended to the serotonergic, dopaminergic and glutamatergic neurochemical systems. Taken together, present results are the first to establish a sex-specific

behavioral response to HFD-fed mice, in addition to elucidating underlying region-specific neurochemical abnormalities.

Disclosures: **B. Klocke:** None. **C. Thelen:** None. **S. Koeller:** None. **J. Coffey:** None. **P. Pitychoutis:** None.

Digital Abstract Session

P284. Mechanisms Contributing To Psychiatric Symptomatology: Insights From Animal Models

Program #/Poster #: P284.08

Topic: G.05. Mood Disorders

Support: NIDA RO1DA026854
NIDA R01DA046794
TAMU College of Liberal Arts

Title: Adolescent exposure to vicarious social defeat stress and western-style diets leads to physiological dysregulation, decreases in reward sensitivity, and reduced antidepressant efficacy

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Abstract: A dramatic increase in the prevalence of major depression and diet-related disorders in adolescents has been observed over several decades, yet the mechanisms underlying this comorbidity have only recently begun to be elucidated. Exposure to western-style diets (WSDs), high in both fats (45% kcal) and carbohydrates (35% kcal): i.e., high fat diet; HFD, has been linked to the development of metabolic syndrome-like symptoms and behavioral dysregulation in rodents, as similarly observed in the human condition. Because adolescence is a developmental period highlighted by vulnerability to both stress and poor diet, understanding the mechanism(s) underlying the combined negative effects of WSDs and stress on mood and reward regulation is critical. To this end, adolescent male C57 mice were exposed to vicarious social defeat stress (VSDS), a stress paradigm capable of separating physical (PS) versus emotional (ES) stress, followed by normal chow (NC), HFD, or a separate control diet high in carbohydrates (same sucrose content as HFD) and low in fat (LFD), while measuring body weight and food intake. These mice were subsequently tested for reward sensitivity and responsiveness to antidepressant treatment. No-stress control mice exposed to 5 weeks of NC or HFD showed no significant differences in body weight or social interaction. Interestingly, mice exposed to VSDS followed by HFD showed rapid weight gain 1week after initiation of WSD, with the ES-exposed mice showing significantly higher weight gain as compared to the PS-exposed and control mice. These mice also exhibited a reduction in saccharin preference, indicative of anhedonic-like behavior. To further delineate whether high fat was the major contributing factor to these deficits, LFD was introduced. The mice in the VSDS+HFD gained weight more rapidly than the VSDS+LFD

group. However, though LFD-exposed mice did not gain weight as rapidly as HFD-mice, both VSDS+LFD- and VSDS+HFD- mice exhibited attenuated response to chronic antidepressant fluoxetine. These data show that diets high in both fats and carbohydrates are responsible for both rapid weight gain and reduced reward sensitivity, and chronic LFD consumption, though it does not lead to rapid weight gain, both HFD and LFD-exposure after stress leads to reduced response to fluoxetine. Furthermore, findings indicate that exposures to WSDs and stress during adolescence leads to physiological and reward dysregulation, likely contributing to maladaptive behaviors, negative health outcomes, and reduced antidepressant efficacy in adulthood.

Disclosures: O.K. Sial: None. **T. Gnecco:** None. **A.M. Cardona:** None. **E.L. Vieregg:** None. **E. Cardoso:** None. **L.F. Parise:** None. **C.A. Bolanos-Guzman:** None.

Digital Abstract Session

P285. Pharmacology in Animal Models of Depression, Anxiety and Schizophrenia

Program #/Poster #: P285.01

Topic: H.13. Schizophrenia

Title: Phencyclidine-induced impairment of spine molecular plasticity shown in the AiCE-Tg mice

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Abstract: Phencyclidine (PCP), a well-known major hallucinogenic and dissociative drug of abuse, induces a broad range of schizophrenic-like symptoms. Pharmacologically, PCP is a dopamine D2 receptor agonist and an NMDA receptor blocker. We analyzed PCP effects on the molecular plasticity of dendritic spines in our new AiCE-Tg mouse line that expresses an enhanced green fluorescent protein (EGFP)-tagged CapZ marker. CapZ protein, which binds F-actin, has been shown previously to be increased in spines in LTP-induced regions, likely owing to rapid accumulation and/or regulated degradation. Plastic changes in EGFP-CapZ reflecting reorganization of spine proteins upon stimulation *in vivo* can be visualized directly in these transgenic mice. Previously, we showed that relative EGFP-CapZ signal intensification occurs in a small subset of dendritic spines located selectively in stimulated-side cortices of AiCE-Tg mice 20 min after unilateral visual or somatosensory stimulation. In this presentation, we present data (see Figure) showing that this stimulus-dependent right-left difference in EGFP-CapZ signal levels is abolished by PCP administration (~30 min). This inhibition may reflect an endophenotype of PCP-induced brain abnormality that has the potential to be utilized for development of novel therapeutic strategies for PCP abuse symptomatology and possibly for schizophrenia.

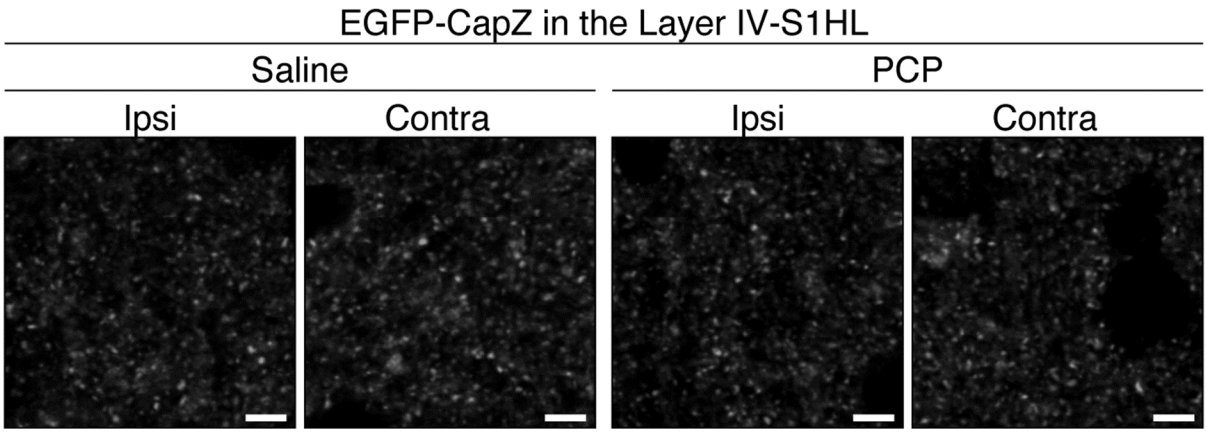

Figure: Contra-ipsi difference in spine EGFP-CapZ intensity was abolished by PCP. Spine-localized EGFP-CapZ looked generally similar in both sides, but for limited numbers of brighter puncta in the stimulated (contralateral) part of sensory cortex for the intact hind limb only in saline-injected AiCE-Tg. Quantitative analyses showed a significant right-left difference for the saline group, but not for the PCP group. Scale bars, 5 μm.
Ref: Kuboyama K. et al. *Scientific Reports* **10:** 15266 (2020). Kitanishi, T. et al. Genes to Cells **15**: 737-747 (2010)

Disclosures: K. Kuboyama: None. **M. Tawada:** None. **M. Yoshioka:** None. **T. Fujishima:** None. **M.K. Yamada:** None.

Digital Abstract Session

P285. Pharmacology in Animal Models of Depression, Anxiety and Schizophrenia

Program #/Poster #: P285.02

Topic: H.13. Schizophrenia

Title: The GABAA receptor Positive Allosteric Modulator, GT-002, has procognitive effects in rats

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Abstract: The $GABA_A$ receptor ($GABA_AR$) is an ionotropic receptor and ligand-gated ion channel. Its endogenous ligand is γ-aminobutyric acid (GABA), and GT-002 is a $GABA_AR$ positive allosteric modulator (PAM) which has been examined in several preclinical studies in vivo. The aim of the studies being to assess the potential antipsychotic effects of GT-002 in social interaction and novel object recognition (NOR) performed in male Sprague-Dawley rats treated with MK-801 and to compare the effects of GT-002 to the antipsychotic drug clozapine (as an animal model of schizophrenia) and to the procognitive drug used in Alzheimer's patients,

Aricept, in its generic form donepezil in NOR, as an animal model cognition in Alzheimer's disease, and to establish whether the $GABA_A$ PAM, GT-002 can reverse the associated cognitive impairments seen in this model. As expected, MK-801 (0.1 mg/kg sc) impaired social interaction decreasing the duration of interactions and the number of episodes to about 50% when compared to controls, corresponding to the negative symptoms in schizophrenia. GT-002 at a dose of 3 mg/kg increased social interaction in terms of both parameters, increasing the duration and the number of interactions. Furthermore, 3 mg/kg GT-002 partially reversed the MK-801 induced deficits. Clozapine, used as reference drug, administered at a dose of 1 mg/kg partially reversed MK-801-induced deficits in the social interaction test while its higher dose (3 mg/kg) was ineffective possible due to sedative properties of this drug. Similarly as expected, MK-801 (0.1 mg/kg sc) impaired novel object recognition with rats exploring both objects equally during the recognition session, whilst rats receiving clozapine (1mg/kg) or GT-002 (1mg/kg) performed better than controls when tested one hour later. In NOR studies where the recognition session was performed 24 hours later, GT-002 at 3 mg/kg or donepezil at 1 mg/kg or 3 mg/kg significantly improved performance with rats spending more time investigating the novel object than controls. There was a corresponding significant increase in recognition index following administration of these drugs.These results indicate that GT-002 promotes social interaction (an animal model of the negative symptoms in schizophrenia) and enhances cognitive performance in NOR with similar efficacy to donepezil (Aricept) which is given to Alzheimer's patients to improve memory.

Disclosures: **C.N. Ryan:** A. Employment/Salary (full or part-time):; A. **M. Witt:** A. Employment/Salary (full or part-time):; Gabather. **E. Lorenc-Koci:** None. **Z. Rogóż:** None. **S. Rehnmark:** A. Employment/Salary (full or part-time):; Gabather. **M.P. Nielsen:** A. Employment/Salary (full or part-time):; Gabather.

Digital Abstract Session

P285. Pharmacology in Animal Models of Depression, Anxiety and Schizophrenia

Program #/Poster #: P285.03

Topic: G.04. Emotion

Title: Time and dose-dependent effects of acute nicotine on light-enhanced startle

Authors: *R. C. BARNET;
Col. William & Mary.

Abstract: Two experiments using Light-enhanced startle (LES) examined dose-dependent and time-dependent effects of acute nicotine on anxiety. In Experiment 1 rats were exposed to saline, .15 mg/kg, or .40 mg/kg (i.p.) nicotine and 5 minutes later were behaviorally tested in LES. Nicotine at both doses reduced the magnitude of LES in males but not in females. In females, the higher nicotine dose, .40 mg/kg increased LES (was anxiogenic) during later portions of the test session. In both males and females, within-session variation in LES suggested LES generally increased in magnitude as time since nicotine administration increased. In Experiment 2, longer

drug-to-test intervals were examined in order to explore possible time-dependent increases in LES produced by nicotine. In Experiment 2, rats were exposed to saline or .40 mg/kg nicotine and were behaviorally tested 15 min or 35 min after nicotine administration in LES. Trends in the available data suggested an anxiogenic profile of nicotine when tested 15 min following drug administration but an anxiolytic profile when tested 35 min following drug administration. At the short 5 min drug-to-test interval used in Experiment 1, findings contradict those in other experiments using the social interaction test of anxiety. Collectively, results suggest Light-enhanced startle is sensitive to dose and time-dependent effects of nicotine on anxiety. Possible differences between reflexive and non-reflexive measures of fear are discussed.

Disclosures:

Digital Abstract Session

P285. Pharmacology in Animal Models of Depression, Anxiety and Schizophrenia

Program #/Poster #: P285.04

Topic: F.04. Stress and the Brain

Support: NEUROSCIENCE RESEARCH OPPORTUNITY TO INCREASE DIVERSITY program (NEURO-ID-UPR RIO PIEDRAS NIH-ENDURE R25NS080687-10).

Title: The effects of the endocannabinoid receptors 2 (CB2) and voluntary wheel running in depression-like behaviors in rats

Authors: A. P. RAMOS-ROLÓN[1], *P. A. MUNOZ RODRIGUEZ[2];
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Abstract: Theendocannabinoid system (eCB) has been implicated in the pathophysiology ofdepression. Studies have shown that CB2 receptor (CB2R) plays a role indepression-like behavior by modulating the serotonergic system. Other studiesrevealed that aerobic exercise is also involved in decreasing depression-likebehavior by upregulating 5-HT release. This project is aimed to observe if themanipulation of the CB2R would enhance the therapeutic effect of aerobicexercise in depression. In this set of experiments, we used the forced swimtest (FST) paradigm as a depression model in male Sprague Dawley rats. All ratshad access to 2hr of wheel running exercise (aerobic exercise model) for 5consecutive days. One group of rats received intraperitoneal (ip) injections of(+)-WIN 55,212-2 (1mg/kg), an agonist eCB receptors, and the other groupreceived the vehicle. On the 5th and last day, rats were submitted to the FSTbehavioral paradigm to measure depression-like behaviors. Behavioral resultsshowed no significant difference at the selected dose in exercise and sedentarygroups when compared to the controls. Further studies with higher doses will beconducted. In addition, biochemical analysis of the expression of CB2R withinthe medial prefrontal cortex and 5-HT 1A receptors within the hippocampus andamygdala will be conducted. It is expected that these findings will permit

abetter understanding of the neurocircuits underlining the depression-likeresponses and the interactions between exercise and the eCB.

Disclosures: A.P. Ramos-Rolón: None. **P.A. Munoz Rodriguez:** None.

Digital Abstract Session

P285. Pharmacology in Animal Models of Depression, Anxiety and Schizophrenia

Program #/Poster #: P285.05

Topic: G.04. Emotion

Support: HEC grant

Title: Clinical Trial of herbal tea on depressed associated with hyperlipidemia patients in Aga Khan University, Pakistan

Authors: *S. A. KHAN, A. KHAN, H. RAFIQ, H. MESIYA;
Aga Khan Univ., Karachi, Pakistan

Abstract: Abstract Objectives: Depression are one of the neuropsychological refers to constellation of sad mood, agitation, lack of interest and feelings of worthlessness. Many allopathic treatments are available in hospitality for the management of this disease to control their adverse effects. Although there is also the interest of developing countries to use alternative remedies for the treatment of psycho-neurological issues like depression. The present clinical trial was designed in the clinical trial unit of Aga Khan University, Karachi, Pakistan to determine the effect of chamomile and saffron tea bags as an adjuvant therapy in patients with mild to moderate depression using PHQ9 scale. **Methods**: 100 depressed patients visiting the outpatient psychiatry clinic at the Aga Khan University, Karachi, Pakistan after obtaining written consent were selected and randomly divided in control and test groups. The test subjects were given herbal tea bags, prepared with 20mg of chamomile and 1mg of saffron to be taken twice daily for a period of 4 weeks only as adjuvant therapy to their prescribed medicine. The controls received nothing and were advised to continue taking their prescribed medication. Depression in all the subjects was evaluated by the PHQ9 scale and serum samples were taken for evaluation of fasting lipid profile and serum tryptophan levels, both before the initiation and after one month of completion of the study. **Results:** Results revealed that one month of herbal tea intake as adjuvant therapy significantly reduced depression and fasting lipid profile in test subjects than controls. However, analysis of tryptophan levels analysis through HPLC method is to underway and results are pending. **Conclusions:** It is concluded from the present trial that the indigenous herbs have a potential to maintain blood lipid profile and alleviate of psycho-neurological deficits possibly through augmented tryptophan entry through blood brain barrier into brain; escalating serotonin synthesis. **Keywords**: neurophysiological deficits, herbal products, saffron, chamomile, PHQ-9. **Conflict of interest**: None to declare.

Disclosures: S.A. Khan: None. **A. Khan:** None. **H. Rafiq:** None. **H. Mesiya:** None.

Digital Abstract Session

P286. Amphetamines: Behavioral and Neural Mechanisms of Addiction

Program #/Poster #: P286.01

Topic: G.09. Drugs of Abuse and Addiction

Support: Canada Research Chairs program (Dr. Tyndale, the Canada Research Chair in Pharmacogenomics)
CIHR Foundation Grant (FDN-154294)
Centre for Addiction and Mental Health
The CAMH Foundation

Title: CYP2D-mediated methamphetamine metabolism in rat brain alters striatal dopamine and serotonin release and behavioral sensitization

Authors: *M. R. STOCCO[1,3], A. A. EL-SHERBENI[1,4], B. ZHAO[1,3], M. NOVALEN[1,3], R. F. TYNDALE[1,3,2];
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Abstract: Cytochrome P450 2D (CYP2D) enzymes metabolize many addictive drugs, including methamphetamine. Variable CYP2D metabolism in the brain may alter CNS drug and metabolite concentrations, consequently affecting drug response and chronic effects, including addiction liability and neuropsychiatric outcomes; components of these can be modeled by behavioral sensitization in rats. To investigate the role of CYP2D in the brain in methamphetamine-induced behavioral sensitization, adult male rats were pretreated centrally with a CYP2D irreversible inhibitor (or vehicle) 20 hr prior to each of 7 daily methamphetamine (0.5 mg/kg subcutaneous) injections; after 20 hr, CYP2D in the brain is inhibited, and the remaining inhibitor has been cleared from the brain. *In vivo* brain microdialysis was then used to assess brain drug and metabolite concentrations, and neurotransmitter release. CYP2D inhibitor (versus vehicle) pretreatment enhanced methamphetamine-induced stereotypy response sensitization. CYP2D inhibitor (versus vehicle) pretreatment also increased brain methamphetamine concentrations and decreased the brain *p*-hydroxylation metabolic ratio. With microdialysis conducted on day 1 and day 7 of the daily administration paradigm, CYP2D inhibitor (versus vehicle) pretreatment again exacerbated stereotypy sensitization, as well as enhanced dopamine and serotonin release in the dorsal striatum. Day 1 brain methamphetamine concentrations correlated with striatal dopamine and serotonin release, which in turn correlated with the stereotypy response slope across sessions (i.e. day 1 through day 7), used as a measure of sensitization. Thus, CYP2D-mediated methamphetamine metabolism in the brain was sufficient to alter brain drug concentrations, striatal dopamine and serotonin release, and behavioral sensitization. Moreover, acute (i.e. day 1) methamphetamine-induced neurotransmitter release may be an important predictor of subsequent behavioral sensitization. Together this suggests the novel contribution of CYP2D in the brain to methamphetamine-induced behavioral sensitization and suggests that the wide variation in

CYP2D6 in human brain may contribute to differential methamphetamine responses and chronic effects, including addiction liability and neuropsychiatric outcomes.

Disclosures: **M.R. Stocco:** None. **A.A. El-Sherbeni:** None. **B. Zhao:** None. **M. Novalen:** None. **R.F. Tyndale:** F. Consulting Fees (e.g., advisory boards); RFT has consulted for Quinn Emanuel and Ethismos Research Inc..

Digital Abstract Session

P286. Amphetamines: Behavioral and Neural Mechanisms of Addiction

Program #/Poster #: P286.02

Topic: G.09. Drugs of Abuse and Addiction

Title: Brain Region-specific molecular neuroadaptations in Male and Female Rats as Consequences of Methamphetamine Self-administration

Authors: *A. P. DAIWILE, S. JAYANTHI, J. L. CADET;
Mol. Neuropsychiatry Res. Br., NIH-National Inst. On Drug Abuse IRP, Baltimore, MD

Abstract: Brain Region-specific molecular neuroadaptations in Male and Female Rats asConsequences of Methamphetamine Self-administration Atul P. Daiwile, Subramaniam Jayanthi, and Jean Lud Cadet*Molecular Neuropsychiatry Research Branch, NIDA Intramural Research Program, Baltimore, MD 21224
Methamphetamine use disorder (MUD) is a neuropsychiatric disorder that inflicts significant health burdens throughout the world. Importantly, there are significant sex differences in use and relapse rates exist among human METH users. Some sex differences have also been reported in animal models of MUD. The molecular and biochemical bases for these behavioral differences remain to be determined. We have therefore begun to address some of these issues by using a METH self-administration model in order to identify potential differences in gene expression between female and male rats. Rats were trained to self-administer METH (0.1 mg/kg/injection, i.v.) using two 3-hours daily sessions (separated by a 30-minute off interval) for 20 days. Rats were then assessed for cue-induced drug seeking on withdrawal days (WD) 3 and 30. Rats were euthanized 24 hours after WD30. Prefrontal cortex (PFC) and hippocampus (HIP) were dissected to measure mRNA levels. We found that both female and male rats increased their METH intake during the 4 weeks of the experiment and showed incubation of METH seeking during withdrawal. Females had higher basal level expression of Hcrtr1 and Pdyn mRNAs in the PFC and HIP. Basal Crhr1, Crhr2, Hcrtr2, Oprk1 mRNA levels were higher in the PFC of females whereas Crh and Crhr1 mRNA levels were higher in the HIP of males. In females, METH caused significant decreases in Pdyn mRNA levels in the PFC and HIP whereas Pdyn mRNA expression was affected only in the HIP of males. There were METH-induced decreases in the expression of Oprd1, Oprk1, and Oprm1 in the female PFC, but increases in Oprk1 mRNA levels in the male PFC. In contrast, METH SA increased Crh and Crhr1 in the HIP of both sexes and Crhr2 only in the female HIP. Importantly only increased HIP Crh and Crhr1 mRNA levels correlated positively with incubation of METH craving in both sexes, supporting their potential

involvement and that of the hippocampus in the partial regulation of this phenomenon. When taken together, our results identified sexual dimorphic responses in rats that had self-administered METH and support the importance of understanding the molecular neurobiology of sex differences when developing therapeutic agents against METH use disorder.**Acknowledgement:** This work is supported Department of Health and Human Services/ NIH/ NIDA/ IRP.

Disclosures: **A.P. Daiwile:** None. **S. Jayanthi:** None. **J.L. Cadet:** None.

Digital Abstract Session

P286. Amphetamines: Behavioral and Neural Mechanisms of Addiction

Program #/Poster #: P286.03

Topic: G.09. Drugs of Abuse and Addiction

Title: The dopamine D1-like receptor antagonist, SCH-23390, suppresses compulsive methamphetamine taking in the presence of punishment: molecular neuroadaptations in in brain regions of Sprague-Dawley rats

Authors: *S. JAYANTHI, M. T. MCCOY, B. LADENHEIM, J. L. CADET;
NIDA-IRP, Baltimore, MD

Abstract: Methamphetamine use disorder remains a pervasive public health problem. Nevertheless, its molecular substrates remain to be elucidated. Rats were trained to self-administer methamphetamine using long (9 hours) access to the drug. Rats were then exposed to increasing intensity of contingent footshocks (0.18 - 0.42 mA). While still receiving footshocks, rats were injected intraperitoneally with the dopamine D1 receptor antagonist, SCH23390, prior to each self-administration (SA) session. Cue-induced methamphetamine craving was recorded after 2, 7, and 30 days of withdrawal (WD) from methamphetamine SA. Dorsal striatum (DStr), nucleus accumbens, and hippocampus were used in RT-qPCR analyses to measure mRNA expression. Pretreatment with SCH23390 before sessions suppressed lever pressing in rats that had escalated their methamphetamine intake and had been separated into shock-resistant and shock-sensitive methamphetamine phenotypes by contingent footshocks. Stopping of SCH23390 administration led to re-emergence of compulsive methamphetamine taking in resistant rats. Resistant rats also showed greater cue-induced METH craving than shock-sensitive at WD7 and WD30. RT-qPCR analyses revealed significant increases in the expression of all D1 and D2-like subtypes of dopamine receptors, Fra1, Fra2, and Nr4a3 mRNAs in the DStr of only shock-sensitive rats whereas JunD expression was increased in the DStr of only shock-resistant rats. Incubation of methamphetamine craving correlated positively with increased expression of striatal JunD and hippocampus Nr4a2 expression. Differential changes in gene expression observed mainly in the DStr of rats support the notion that this brain structure may mediate, in part, incubation of methamphetamine craving, a potential indicator of relapse potential.
Acknowledgement: This work is supported by DHHS/NIH/NIDA/IRP

Disclosures: **S. Jayanthi:** None. **M.T. McCoy:** None. **B. Ladenheim:** None. **J.L. Cadet:** None.

Digital Abstract Session

P286. Amphetamines: Behavioral and Neural Mechanisms of Addiction

Program #/Poster #: P286.04

Topic: G.09. Drugs of Abuse and Addiction

Support: R00DA041350 (Li)
NARSAD Young Investigator Award (Li)

Title: Gene alterations in striatonigral projection neurons after prolonged withdrawal from methamphetamine self-administration

Authors: *R. ALTSHULER, K. GARCIA, X. LI;
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Abstract: In both rodents and humans, methamphetamine (Meth) seeking progressively increases after withdrawal, a phenomenon termed incubation of Meth craving. We have previously demonstrated a critical role of the dorsal striatum (DS) in this incubation and associated gene alterations in both DS homogenate and relapse test-activated DS neurons. Here, we aimed to examine whether these gene alternations observed previously occur in a projection-specific manner and we focused on the major direct pathway, which projects from the DS to the substantia nigra (SN). We trained Sprague-Dawley male rats to self-administer Meth (0.1 mg/kg/infusion) or saline for 10 days (6 h/day). On withdrawal day 18, we injected Alexa Fluor 647-conjugated cholera toxin B (CTb, a retrograde tracer), bilaterally into the SN and collected the DS on withdrawal day 28. We then used fluorescence-activated cell sorting to isolate striatonigral projection neurons in the DS (CTb-positive neurons) and measured gene expressions of several candidate genes, including *Bdnf* and *Trkb*, glutamate receptors, epigenetic and ubiquitination enzymes. We found decreased mRNA expression of ubiquitin specific peptidase 7 (*Usp7)* in Meth rats compared to saline rats, with no changes of other candidate genes. These results demonstrate a projection-specific gene alterations associated with ubiquitin-proteasome system during incubation of Meth craving. Future studies will investigate the role of ubiquitin-proteasome system in the direct pathway in incubation of Meth craving.

Disclosures: **R. Altshuler:** None. **K. Garcia:** None. **X. Li:** None.

Digital Abstract Session

P286. Amphetamines: Behavioral and Neural Mechanisms of Addiction

Program #/Poster #: P286.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIMH-IRP Project MH002386

Title: Psychomotor stimulant-induced locomotor sensitization in mouse: association with cAMP-dependent D1 receptor signaling

Authors: H. ZHANG[1], T. SALMAN[3], W. XU[4], S. DAHLKE[4], C. R. GERFEN[5], S. Z. JIANG[4], ***L. E. EIDEN**[2];
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Abstract: Increased locomotor activity after repeated administration is a behavioral hallmark of psychomotor stimulants (PSs) such as methamphetamine (MA) and cocaine (COC) that elicit increased post-synaptic availability of dopamine in the nucleus accumbens (NAc). It has been hypothesized that cellular plasticity, especially in D1 dopaminoceptive medium spiny neuron (D1-MSNs), upon repeated psychomotor stimulant administration underlies the engagement of behavioral circuits leading to increased appetitive drive for PS preference/self-administration. Examination of downstream pathways activated by dopamine (DA) in D1-MSNs have established a convincing link between activation of the MAP kinase ERK, and both locomotor sensitization and development of preference for COC self-administration in mice (Pascoli et al., Biol. Psychiatr.**76**: 917, 2014). Using conditioned place preference (CPP) as a behavioral index of increased preference for PS self-administration, we have determined that both COC-induced locomotor sensitization, and CPP, are associated with NCS-RapGEF2-dependent activation of ERK in NAc D1-MSNs (Jiang et al., BioRxiv doi.org/10.1101/2020.07.02.184754). Here, we asked whether or not MA elicits ERK activation upon repeated administration, as in the case of COC, and examined whether or not this is associated with alterations in the NAc transcriptome as a function of repeated MA administration. We confirmed that a daily dose of 2 mg/kg of MA on two successive days elicited a similar increase in locomotor activity, and activation of pERK in NAc, as 20 mg/kg COC. Furthermore, ERK phosphorylation was increased in NAc slices incubated with MA (10 uM). Transcriptome analysis (Affymetrix 2.0 mouse microarray) of NAc from MA-treated mice one hour after initial administration after MA, and one hour after the final of six daily treatments with MA revealed a set of core transcripts up-regulated after both single and repeated-dose regimens. The immediate-early gene c-Fos, was up-regulated by both single and repeated MA administration, and similarly regulated after administration of COC. Gs-coupled GPCR activation, shown to activate ERK via NCS-RapGEF2 in the NS-1 neuroendocrine cell line also resulted in up-regulation of c-Fos. We hypothesize that RapGEF2-dependent activation of ERK, and of ERK-dependent IEGs in D1-MSNs, may represent the signaling that leads, initially, to locomotor sensitization and upon repeated administration, to altered plasticity of D1-MSN output neurons leading ultimately to activation of a behavioral program for PS preference and self-administration.

Disclosures: H. Zhang: None. **T. Salman:** None. **W. Xu:** None. **S. Dahlke:** None. **L.E. Eiden:** None. **C.R. Gerfen:** None. **S.Z. Jiang:** None.

Digital Abstract Session

P286. Amphetamines: Behavioral and Neural Mechanisms of Addiction

Program #/Poster #: P286.06

Topic: G.09. Drugs of Abuse and Addiction

Support: American Foundation for Pharmaceutical Education
University of Utah Health Sciences

Title: Effects of methamphetamine induced dopamine toxicity on striatal plasticity

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Abstract: Methamphetamine (METH) is a highly addictive psychostimulant. Cognitive deficits are apparent in individuals with a history of METH abuse, and targeting cognitive function may be an efficacious approach to managing METH abuse and addiction. However, in order to develop a successful treatment for METH abuse, the consequences of METH-induced neurotoxicity must be better understood. METH exposure is known to be associated with damage to central monoamine systems, particularly dopamine signaling. Rodent models of such damage have revealed a decrease in the amplitude of phasic dopamine signals and significant striatal dysfunction, including changes in molecular, systems, and behavioral functions of the striatum. Importantly, phasic dopamine signals activate dopamine D1 receptors, which are involved in striatal synaptic plasticity, particularly long-term potentiation (LTP) in D1 receptor-expressing striatal medium spiny neurons. We therefore hypothesize that METH-induced dopamine neurotoxicity will diminish D1 receptor-dependent striatal plasticity in mice. To test this hypothesis, mice were treated with a METH binge regimen (4 x 10 mg/kg *d,l*-methamphetamine, s.c.). This binge regimen provides a reliable rodent model that recapitulates all of the known METH-induced neurotoxic effects observed in humans, including dopamine toxicity. Three weeks later, plasticity was assessed using white matter high frequency stimulation (HFS) and striatal field recordings. Under these conditions, LTP was induced in the dorsomedial striatum of saline-pretreated, but not METH-pretreated mice. Further, the LTP observed in saline-pretreated mice was abolished by the dopamine D1 receptor antagonist SCH23390, indicating that this LTP is D1 receptor-dependent. Finally, acute *in vivo* treatment with bupropion (50mg/kg i.p.) can restore LTP in striatal slices from METH-pretreated mice. Together these studies suggest that METH-induced neurotoxicity impairs D1-dependent LTP within the dorsomedial striatum, and that LTP can be restored with the FDA-approved drug bupropion. As a behavioral correlate to this plasticity deficit, experiments are currently ongoing to determine if METH-induced neurotoxicity impairs striatally-dependent motor learning, and if this deficit can also be rescued with acute bupropion treatment.

Disclosures: **A.S. Gibson:** None. **P.J. West:** None. **K.A. Keefe:** None.

Digital Abstract Session

P286. Amphetamines: Behavioral and Neural Mechanisms of Addiction

Program #/Poster #: P286.07

Topic: G.09. Drugs of Abuse and Addiction

Support: DA009621

Title: Incubation of methamphetamine craving and associated synaptic plasticity in the nucleus accumbens core: comparison of male and female rats

Authors: *J. R. FUNKE, E.-K. HWANG, A. M. WUNSCH, M. E. WOLF;
Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: One of the intractable features of methamphetamine (METH) substance use disorder is the high rate of relapse, which occurs even after prolonged abstinence. Relapse can be induced by exposure to drug-paired cues leading to craving and subsequent relapse; this craving progressively intensifies during abstinence, a phenomenon termed 'incubation of drug craving'. In our incubation paradigm, rats undergo extended-access self-administration (SA) of METH for 6 hr/day for 10 days. A light cue is paired with each METH infusion. Rats then undergo cue-induced seeking tests during abstinence in which rats nose poke for presentation of the METH-paired light cue but do not receive drug. Previously, we found that cue-induced METH seeking increases during the first week of abstinence and remains elevated through Withdrawal Day (WD) 45 (Scheyer et al., Biological Psychiatry 2016). Furthermore, the accumulation of high conductance calcium (Ca^{2+})-permeable AMPARs (CP-AMPARs) in medium spiny neurons (MSN) of the nucleus accumbens (NAc) core mirrors this time course, and activation of these receptors is required for the expression of incubated craving. The overall goal of this project is to establish the rising and falling phases of METH incubation in both male and female rats, as well as CP-AMPAR accumulation during these time periods. Using a between-subject design, male and female rats undergo METH self-administration as described above and then undergo cue-induced METH seeking tests on WD1, WD7, WD30, and WD100. Estrous cycle is assessed in the female rats after each seeking test. Current data show that females follow the same trajectory as males during the rising phase of METH incubation, through WD30; tentatively, estrous cycle stage appears to have no impact on seeking. Similar to what we previously reported in male rats (Scheyer et al., 2016), after incubation of METH craving female rats showed increased synaptic CP-AMPAR levels in the majority of MSNs in the NAc core. Thus, this neuroadaptation is not sex-specific. Furthermore, there were no changes in presynaptic function as measured with paired pulse ratios. Future experiments will investigate the status of incubation on WD100, as well as CP-AMPAR levels in late withdrawal. Incubation of craving also occurs in abstinent human METH users, so it is important to define its time course and underlying mechanisms to develop strategies for relapse prevention in a clinical environment.

Disclosures: J.R. Funke: None. **E. Hwang:** None. **A.M. Wunsch:** None. **M.E. Wolf:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R01DA044761
NIMH Grant R01MH115604
Ministerio de Ciencia e Innovación Grant PGC2018-095980-B-I00

Title: Cerebellum-infralimbic anatomical map

Authors: *J. GUARQUE-CHABRERA, M. MIQUEL, K. KHODAKHAH;
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Several findings support the existence of reciprocal loops between the medial prefrontal cortex (mPFC) and the cerebellum. Dysfunction of both areas underlie the phenotypes of several neuropsychiatric disorders, and may operate as a risk factor for drug addiction.
In rodents, the mPFC includes, among others, two areas that have shown to exhibit opposite roles in drug-related behaviors. The prelimbic cortex (PL) is involved in initiating cocaine-seeking, while the infralimbic cortex (IL) is responsible for the inhibitory control over drug-seeking. Miquel's lab has reported that either a cerebellar lesion in lobule 8 (L8) or IL deactivation facilitated the establishment of conditioned preference for cocaine-related cues. However, this effect was not replicated by PL deactivations. Cerebellum-IL functional relationships appear to be compensatory because simultaneous cerebellum-IL deactivations abolished the facilitative effect of separate deactivations. Moreover, unpublished results showed that IL but not PL deactivations during a repeated cocaine experience upregulates neuronal activity in the posterior vermis in rats. Additionally, we have observed disynaptic projections from the deep cerebellar nuclei (DCN) to the mPFC using transneuronal labeling. In fact, one of the relays for the cerebellum to modulate mPFC functions is the ventral tegmental area (VTA). Indeed, optogenetic manipulation of the cerebellar outputs targeting VTA were able to modulate reward-related behaviors.
Anatomical projections from the IL to the cerebellum have been poorly described and it is far to be clear through which brain regions the IL might regulate cerebellar activity. Neither, whether IL projections are monosynaptic or involve disynaptic pathways. Therefore, our goal is to characterize the cerebellum-IL loop in mice to create an anatomical map of this circuit. To establish the anatomical cerebellum-IL loop we used a combination of monosynaptic and transneuronal viral and classic tracing agents. Our results show that for the cerebellum-IL ascending pathway, L8 might contact the IL through the projections from the interposed DCN to the VTA. For the IL-cerebellum descending pathway, the IL might reach L8 through its projections to both interposed DCN and inferior olive.

Disclosures: J. Guarque-Chabrera: None. **M. Miquel:** None. **K. Khodakhah:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.02

Topic: G.09. Drugs of Abuse and Addiction

Support: BSF 2017252

Title: Anatomical, physiological and behavioral differences between four ventral pallidal projections

Authors: *N. BERNAT, Y. M. KUPCHIK;
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Abstract: The ventral pallidum (VP) is a central brain region in the reward system, implicated in many behaviors, but mostly as mediator of motivated behavior and addiction. Despite its involvement in major psychiatric pathology, it is yet to be explored in a projection-specific manner. In this research, we systematically characterize projection neuron subpopulations in the VP, defined by their projection sites: ventral tegmental area (VTA), medial dorsal thalamus (MDT), lateral hypothalamus (LH) and lateral habenula (LHb), as well as determine the involvement of relevant subpopulations in cocaine conditioning. To characterize each of these VP four subpopulations, we delineated anatomical patterns by employing rabies tracing methods, tissue clearing techniques and light sheet fluorescent microscopy. We also describe physiological characteristics of these projections by using retrograde tracing, whole-cell patch clamp and optogenetics. Finally, we use the cocaine conditioned place preference (CPP) behavioral model in combination with chemogenetic (DREADDs) tools to examine the relevance of each of the VP projections to positive reinforcement, driven by cocaine reward. We show that the four different VP projection neurons are largely non-overlapping populations, that receive different patterns of inputs from brain-wide sources and show different physiological traits. We also show that inhibiting the VP→VTA projection increases cocaine CPP, while inhibition of the VP →LH/LHb decreases cocaine CPP, compared with control. In summary, we identify specific functions to the different projections of the VP in cocaine conditioning. We also established differences in traits between the different subpopulations which emphasize the heterogeneity of the VP. This work would lay the foundations for future research on the VP and functional neuronal microcircuits in reward research and addiction.

Disclosures: **N. Bernat:** None. **Y.M. Kupchik:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R21 DA037744

Title: Monosynaptic retrograde tracing from prelimbic neuron subpopulations projecting to either nucleus accumbens core or rostromedial tegmental nucleus

Authors: *A. M. CRUZ[1], T. H. KIM[1], R. J. SMITH[2];
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Abstract: The prelimbic (PL) region of medial prefrontal cortex (PFC) has been implicated in both driving and suppressing cocaine-seeking behaviors. These seemingly opposing functions may be mediated by different efferent targets of PL projections, such as the nucleus accumbens (NAc) core and rostromedial tegmental nucleus (RMTg), which have contrasting roles in reward-seeking behaviors. We used conventional retrograde tracers to reveal distinct subpopulations of PL neurons projecting to NAc core vs. RMTg in rats, with very little overlap. To examine potential differences in input specificity for these two PL subpopulations, we then used Cre-dependent rabies virus (EnvA-RV-EGFP) as a monosynaptic retrograde tracer and targeted specific PL neurons via injections of retrograde CAV-Cre in either NAc core or RMTg. We observed a similar catalog of cortical, thalamic, and limbic afferents for both NAc- and RMTg-projecting populations, with the primary source of afferent information arising from neighboring prefrontal neurons in ipsilateral PL and infralimbic cortex (IL). However, when the two subpopulations were directly compared, we found that RMTg-projecting PL neurons received a greater proportion of input from ipsilateral PL and IL, whereas NAc-projecting PL neurons received a greater proportion of input from most other cortical areas, mediodorsal thalamic nucleus, and several other subcortical areas. NAc-projecting PL neurons also received a greater proportion of contralateral cortical input. Our findings reveal that PL subpopulations differ not only in their efferent target, but also in the input specificity from afferent structures. These differences in connectivity are likely to be critical to functional differences of PL subpopulations.

Disclosures: A.M. Cruz: None. **T.H. Kim:** None. **R.J. Smith:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.04

Topic: G.09. Drugs of Abuse and Addiction

Support: Medical Research Council Programme Grant MR/N02530X/1

Title: Optogenetic mapping of the circuit by which the basolateral amygdala influences the activity of dorsolateral striatum medium spiny neurons

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Abstract: Although the basolateral amygdala (BLA) does not project directly to the anterior dorsolateral striatum (aDLS), it has been shown to exert control over its function. The BLA bidirectionally gates the response of subpopulations of medium spiny neurons (MSNs) in the aDLS to their major glutamatergic input from the cortex. The remote control whereby associative information processed in the amygdala can influence the function of the aDLS, the neural locus of control of habits, has far-reaching implications as it may subserve a mechanism by which the amygdala gates the progressive devolvement of control over the aDLS habit system over the course of the development of drug addiction. Our recent investigations of the neural mechanisms supporting this functional control have shown that, in drug-naive rats, electrical stimulation of the BLA applied 100 to 300 milliseconds before that of the motor cortex 1 (M1) elicits a bidirectional regulation of spike probability of the aDLS MSNs. We further showed that the control exerted by the BLA over the activity of aDLS MSNs depends on a polysynaptic network that involves antecedent glutamatergic mechanisms in the core of the nucleus accumbens (AcbC) to which the BLA projects. Here we used a projection specific expression of channelrhodopsin to investigate the role of the BLA-AcbC pathway in mediating the control of the BLA over aDLS MSNs activity. In anaesthetised rats, we combined the optogenetic stimulation of the specific BLA-AcbC pathway with extracellular in vivo recordings of the activity of the MSNs in the aDLS evoked by electrical stimulation of M1. Photostimulation of the terminals of BLA neurons in the AcbC was sufficient to bidirectionally modulate the activity of aDLS MSNs. As previously reported using electrical stimulation of the BLA, aDLS MSNs were shown either to up or down regulate their spike probability following photostimulation of the BLA-AcbC pathway. These data demonstrate that the BLA can exert control over the physiology of the aDLS indirectly through its direct projections to the AcbC. Further experiments will establish whether the AcbC mediates this influence on the aDLS via the ascending dopamine-dependent spiralling circuitry that has been shown to be necessary for the functional recruitment of aDLS dopamine dependent drug seeking habits.

Disclosures: **A. Belin-Rauscent:** None. **B.J. Everitt:** None. **D. Belin:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.05

Topic: G.09. Drugs of Abuse and Addiction

Support: The Israeli Science Foundation Grant 1381/15
The Faye & Max Warshafsky Medical Research Scholarship

Title: Abstinence from cocaine strengthens the excitatory input from the mPFC and BLA to VP-projecting accumbal D1-MSNs but not D2-MSNs

Authors: *K. INBAR, L. A. LEVI, Y. M. KUPCHIK;
Dept. of Med. Neurobio., Fac. of Medicine, The Inst. for Med. Res. Israel-Canada, The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Background:
Withdrawal from cocaine induces synaptic plasticity at glutamatergic inputs to nucleus accumbens (NAc) medium spiny neurons (MSNs). This plasticity is believed to control cocaine seeking by modulating two competing pathways arising from the NAc - 1) the direct pathway that promotes cocaine seeking and originates in D1 dopamine receptor-expressing MSNs (D1-MSNs) projecting to the ventral mesencephalon; and 2) the indirect pathway that decreases cocaine seeking and consists of ventral pallidum (VP)-projecting MSNs expressing the dopamine receptor D2 (D2-MSNs). Recent findings have identified D1-MSNs that project in the indirect pathway to the VP (iD1-MSNs) and promote drug seeking. Thus, the indirect pathway consists of both D1- and D2-MSNs. This therefore calls for a reexamination of the cocaine related synaptic changes along it. In this work, we ask whether abstinence from repeated cocaine exposure differentially alters the excitatory input from the medial pre-frontal cortex (mPFC) and the basolateral amygdala (BLA) to VP-projecting D1- and D2- MSNs in the NAc core and shell.
Results:
To this end we injected the VP of D1-tdTomato mice with RetroBeads and either the mPFC or BLA with ChR2. Mice received either cocaine or saline for 2 weeks in a conditioned place preference paradigm (CPP). After two weeks of abstinence, we recorded the AMPA to NMDA (A/N) ratio from mPFC-NAc or BLA-NAc synapses in VP-projecting D1- or D2-MSNs using the whole-cell patch clamp electrophysiology. We found that cocaine CPP and abstinence increased A/N ratio only in iD1-MSNs and did not affect the A/N ratio measured in D2-MSNs. While the A/N ratio recorded in BLA-iD1-MSN synapses was elevated in both the NAc core and shell compartments, the A/N ratio in mPFC-iD1-MSN synapses was increased only in the NAc shell. The increase of A/N ratio in mPFC-iD1-MSN but not BLA-iD1-MSN synapses was accompanied by a change in AMPA current rectification index.
Conclusions:
Our results suggest that even when focusing only on the MSNs projecting to the VP, D1- and D2-MSNs still undergo different synaptic changes following cocaine use and abstinence. This may be part of the mechanism allowing these two inputs to the VP to have opposite influence on behavior.

Disclosures: K. Inbar: None. **L.A. Levi:** None. **Y.M. Kupchik:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.06

Topic: G.09. Drugs of Abuse and Addiction

Support: F30- DA048575
T32-DA041349
P50-DA006634

Title: Functional interactions between the dopamine transporter and kappa opioid receptor modulate the reinforcing efficacy to self-administer cocaine

Authors: *P. M. ESTAVE[1], L. B. KUIPER[1], K. M. HOLLERAN[1], A. K. KARKHANIS[2], S. R. JONES[1];
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Abstract: Chronic cocaine exposure leads to compensatory alterations of the mesolimbic dopamine system and kappa opioid receptor (KOR) system, which influence the reinforcing effects of cocaine. After extended cocaine exposure, KOR activity in the nucleus accumbens is augmented, and there is a decrease in cocaine's ability to bind to the dopamine transporter (DAT). Furthermore, literature suggests that the DAT and KOR form a physical complex, and that KOR activation promotes DAT function. Thus, this study focuses on how the DAT and KOR are functionally linked, and how these interactions may have influenced our earlier results showing that the dopamine releaser phenmetrazine and the KOR antagonist norBNI both decrease cocaine-motivated behaviors. Using *ex vivo* fast scan cyclic voltammetry, we investigated dopamine release and uptake kinetics in Sprague Dawley rats following 5 days of cocaine exposure (1.5 mg/kg/inf, FR 1 x 40), followed by 14 days of a progressive ratio (PR) schedule of reinforcement (0.1875 mg/kg/infusion). At baseline, there was lower electrically stimulated dopamine release and slower dopamine uptake in cocaine exposed animals compared to naïve controls. Next we examined DAT and KOR function through bath application of cocaine or the KOR agonist U50,488, respectively. We found that cocaine exposure attenuated cocaine potency at the DAT and increased KOR-mediated inhibition of dopamine release. To study the interaction between KOR and DAT in naïve animals, the IC50 dose of the KOR agonist U50,488 was applied to the slice, followed by a cocaine concentration response curve. Acute KOR activation lead to a decrease in cocaine potency at the DAT, similar to cocaine-exposed animals. A separate cohort of animals were treated with norBNI (10 mg/kg; i.p.) prior to cocaine exposure. Animals with norBNI treatment did not develop tolerance to cocaine's inhibitory effects at the DAT, appearing to be more similar to their naïve counterparts than animals with equivalent cocaine exposure. These studies suggest the potential benefit of dual-targeting the DAT and KOR for treatment of cocaine use disorder, which may allow for lower doses of dopamine agonists to be effective, decreasing abuse liability. Further studies are necessary to elucidate changes in complex formation after cocaine self-administration and treatment with KOR antagonists and dopamine releasers.

Disclosures: P.M. Estave: None. **L.B. Kuiper:** None. **K.M. Holleran:** None. **A.K. Karkhanis:** None. **S.R. Jones:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant T32 DA7281
NIH Grant R01 DA044204

Title: Effects of Repeated I.P. Cocaine Injections on Locomotor Sensitization and Glutamatergic Plasticity in the Nucleus Accumbens

Authors: *A. M. FRANCE[1], T. L. FETTERLY[1], A. M. NIETO[1], T. E. ROBINSON[2], C. R. FERRARIO[1,2];
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Abstract: The development and persistence of addiction is mediated in part by drug-induced alterations in the function of the nucleus accumbens (NAc), a region that mediates drug-craving and seeking behaviors. AMPA-type glutamate receptors provide the main source of excitatory drive to the NAc, and enhancement in transmission of calcium-permeable AMPAR (CP-AMPARs) within the NAc mediate cue-triggered drug-seeking. Previous work has shown that cocaine treatment regimens that result in psychomotor sensitization enhance subsequent drug-seeking and drug-taking behaviors. Furthermore, cocaine-induced locomotor sensitization followed by 14 days of withdrawal is accompanied by increases in the surface expression of GluA1 and GluA2 AMPAR subunits (consistent with increases in non-CP-AMPARs) and increases in AMPA/NMDA ratio in males. However, potential effects of cocaine-induced sensitization on AMPAR expression and synaptic transmission in females are poorly understood, and few studies have evaluated potential effects on CP-AMPAR transmission following sensitization regimens. Therefore, here male and female rats were given repeated systemic cocaine injections to induce locomotor sensitization (15 mg/kg, i.p, 1 injection/day, 8 days). Controls received saline (1 mL/kg, i.p.). After 14-16 days of withdrawal (WD), tissue from the NAc was biotinylated and western blotting was used to measure changes in the surface expression of GluA1 and GluA2. In addition, brain slices were prepared from separate sets of rats and whole-cell patch clamp approaches were used to measure effects on spontaneous EPSC frequency and amplitude, and sensitivity to the CP-AMPAR antagonist Naspm (200 μM). Repeated cocaine injections resulted in a progressive enhancement in locomotor activity in both males and females. We found slight increases in GluA1, but not GluA2 surface expression in males, and initial results show an increase in CP-AMPAR transmission and sEPSC frequency. In contrast in females, NAc AMPAR subunit expression in cocaine and saline treated groups was similar. Ongoing studies are examining potential changes in synaptic transmission in females. However, biochemical results suggest that although cocaine produced robust locomotor sensitization in both sexes, NAc AMPAR plasticity differs between males and females.

Disclosures: A.M. France: None. **T.L. Fetterly:** None. **A.M. Nieto:** None. **T.E. Robinson:** None. **C.R. Ferrario:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA033436

NIH Grant DA037270

Title: Involvement of prefrontal cortex in the ability of ceftriaxone to attenuate cue-primed reinstatement of cocaine seeking

Authors: *J. R. MESA[1], V. HODGES[1], C. LOGAN[1], A. BECHARD[2], L. KNACKSTEDT[1]; [1]Univ. of Florida, Gainesville, FL; [2]SUNY Geneseo, Geneseo, NY

Abstract: Cocaine use disorder is a chronic condition marked by relapse even after months or years of abstinence. The intravenous self-administration model is used to model relapse in rodents. After establishing self-administration, the instrumental response made to obtain cocaine is then extinguished and can be reinstated by exposure to drug-associated cues and other stimuli. The antibiotic ceftriaxone shows therapeutic potential as it consistently attenuates cue-induced reinstatement to cocaine seeking. Ceftriaxone increases nucleus accumbens (NAc) glutamate transporter activity and NAc GLT-1 expression is necessary for ceftriaxone to attenuate reinstatement. Yet, overexpression of GLT-1 transporter is insufficient to prevent reinstatement to cocaine seeking. Thus, we hypothesize that ceftriaxone may also reduce the glutamate efflux that drives cue-primed reinstatement by altering activation of prefrontal cortex (PFC) glutamatergic efferents to the NAc. Here, we aim to measure the effects of ceftriaxone on reinstatement-induced expression of immediate early gene product Fos. Male Sprague Dawley rats underwent 12 days of cocaine self-administration followed by 2 weeks of instrumental extinction training during which time half of the rats received ceftriaxone (200 mg/kg IP, 6d). Rats then underwent cue-primed reinstatement testing before perfusion. Fos immunohistochemistry was used to measure neuronal activity in the prelimbic (PL) and infralimbic (IL) cortices. We found that ceftriaxone attenuated cue-induced reinstatement but had no effect on PL and IL fos expression. This is in contrast to our published findings in a model that utilized abstinence without instrumental extinction, in which ceftriaxone reduces cue-primed relapse and PL fos expression. Thus, the ability of ceftriaxone to attenuate NAc glutamate release following extinction is not due to overall decrease in PFC neuronal activation. Future directions will test whether ceftriaxone is able to reduce activity of specific NAc-projecting PFC neurons.

Disclosures: J.R. Mesa: None. **C. Logan:** None. **L. Knackstedt:** None. **V. Hodges:** None. **A. Bechard:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DAO31900

Title: The effects of Hypocretin Receptor 1 blockade on Incubation of Cocaine Craving and Associated Dopamine Terminal Adaptations

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Abstract: Relapse remains a major obstacle to recovery from drug dependence. Across all major drugs of abuse, drug craving has been reported to intensify during periods of abstinence. This phenomenon, called *incubation of craving,* is thought to promote drug seeking, thereby increasing the risk of relapse. Despite recent progress, there continues to be a scarcity of treatment options for those suffering from persistent drug cravings. Furthermore, although dopamine signaling is known to regulate drug craving, dopamine's involvement in incubation of craving remains elusive. To investigate whether incubation of cocaine craving is associated with adaptations in dopamine neurotransmission, we trained rats to self-administer cocaine on an intermittent access (IntA) schedule of reinforcement followed by one week of abstinence. We found that IntA followed by abstinence produced robust incubation of cocaine craving. Using *ex vivo* fast scan cyclic voltammetry (FSCV), we then showed that incubation of craving was tied to higher cocaine potency at the dopamine transporter (DAT) within the nucleus accumbens. Next, we examined whether treatment with a hypocretin receptor 1 antagonist early in the abstinence could prevent incubation of cocaine craving and increased cocaine potency. We again trained animals to self-administer cocaine on an IntA schedule followed by one week of abstinence. On the first day of abstinence, rats were treated with a single dose of the hypocretin receptor 1 antagonist, RTIOX-276, or vehicle. We found, that vehicle-treated rats show robust incubation of cocaine craving whereas rats treated with RTIOX-276 did not incubate their craving. In addition, we show with *ex vivo* FSCV that cocaine potency at the DAT was reduced following RTIOX-276 treatment. Together, these data suggest that antagonism of hypocretin receptor 1 early in abstinence from cocaine may block incubation of cocaine craving as well as the dopamine terminal adaptations that may promote drug seeking.

Disclosures: P.J. Clark: None. **R.A. España:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.10

Topic: G.09. Drugs of Abuse and Addiction

Support: F31DA049458
R01DA031900

Title: Hypocretin receptor 1 knockdown on dopamine vs GABA neurons in the ventral tegmental area differentially modulates processes underlying cocaine use disorder.

Authors: *E. M. BLACK, B. M. O'CONNOR, J. R. BARSON, R. A. ESPAÑA;
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Abstract: Dopamine is a typical target for treatments of cocaine use disorder, however these treatments have proven to be ineffective, intolerable, and often pose abuse potential themselves.

As such, a different treatment target is needed for cocaine use disorder. The hypocretin system has been shown to support cocaine use through actions on hypocretin receptor 1 in the ventral tegmental area and is thought to be a promising therapeutic avenue for cocaine abuse. To date, however, hypocretin-based treatment approaches have limited efficacy due to the lack of cell-specific manipulations of hypocretin receptor 1. This is particularly problematic for understanding the therapeutic potential of these treatments given that hypocretin receptor 1 are present on both dopamine and GABA neurons in the ventral tegmental area. In the present study we employed a combinatorial viral approach to selectively knockdown hypocretin receptor 1 on dopamine or GABA neurons allowing for the selective examination of hypocretin manipulations on cocaine abuse. Data demonstrate that specific knockdown of hypocretin receptor 1 on dopamine neurons leads to decreased dopamine dynamics in the nucleus accumbens core at baseline and in response to cocaine while knockdown of hypocretin receptor 1 in GABA neurons enhances the effects of cocaine. Further, the behavioral implications of specific hypocretin receptor 1 knockdown are being examined through cocaine self-administration studies. These findings provide a more comprehensive understanding of hypocretin modulation of dopamine neurotransmission in the nucleus accumbens core and may uncover potential targets for future development of treatment strategies for cocaine use disorder.

Disclosures: **E.M. Black:** None. **B.M. O'Connor:** None. **J.R. Barson:** None. **R.A. España:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.11

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute of General Medical Sciences (2SC1GM084854)
National Center for Research Resources (5R25GM061838-15, 2G12-RR003051)
National Institute on Minority Health and Health Disparities (8G12-MD007600)
NSF Partnerships in International Research and Education (PIRE) program
Neural Mechanisms of Reward & Decision (OISE-1545803)
Research initiative for Scientific Enhancement RISE (5R25GM061151-18)

Title: I_h blocker (ZD 7288) reduces acute cocaine-induced firing patterns on putative dopaminergic neurons of the Ventral Tegmental Area

Authors: *K. Y. BOSQUE CORDERO[1], R. VAZQUEZ-TORRES[2], C. CALO-GUADALUPE[2], D. CONSUEGRA-GARCIA[2], G. R. FOIS[3,4], F. GEORGES[3,4], C. A. JIMENEZ-RIVERA[2];
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Abstract: The hyperpolarization-activated cation current (I_h) is a determinant of intrinsic neuronal excitability in various cells, including dopaminergic neurons (DA) of the ventral tegmental area (VTA). In contrast to other cellular conductances, the I_h activates through hyperpolarizing voltage steps to potentials negative to -55mV and depolarizes the membrane potential. Our laboratory demonstrated that cocaine sensitization, a chronic cocaine behavioral model, significantly reduces I_h amplitude in VTA DA neurons (Arencibia-Albite et al., 2012). After the development of cocaine sensitization, the spontaneous firing of VTA DA cells remains similar to control groups. Suggesting that I_h reduction could reduce cocaine-induced excitability as a homeostatic adaptation for neuronal excitability, although this relationship is still poorly understood. Through in vivo anesthetized single-unit extracellular electrophysiology, we explore the contribution of the I_h on VTA DA neurons spontaneous firing patterns. A key feature of evaluating spontaneous excitability is the detection of bursting activity. Bursting is defined as trains of two or more spikes occurring within a short interval and followed by a prolonged period of inactivity. Burst formation increases the reliability of information transfer. Rhythmic burst firing in thalamic relay neurons arises mainly from the interaction of two dominant currents, I_h and T type Calcium Channels (I_t) (McCormick & Pape, 1990). The I_h activation depolarizes the membrane potential towards the threshold activating I_t and generating a low-threshold Ca^{2+} spike. The generation of this Ca^{2+} spike activates a burst of fast Na^+ and K^+ dependent action potentials. This provides a possible mechanism on how I_h could influence burst firing in VTA DA neurons. To elucidate the contribution of I_h on VTA DA neurons spontaneous firing patterns, we perfused I_h blocker (ZD 7288, 8.3 μM) and evaluated its effect. I_h blockade significantly reduced firing rate, bursting frequency, and percent of spikes within a burst on VTA DA neurons. Using whole-cell patch-clamp, we determine the progressive reduction of the I_h after acute and chronic cocaine administration (15mg/k.g). In addition, I_h blockade significantly reduced acute cocaine-induced firing rate, bursting frequency, and percent of spikes within a burst on the low firing/high bursting VTA DA neuron group. These data suggest that the progressive reduction of I_h could serve as a homeostatic regulator of cocaine-induced spontaneous firing patterns related to VTA DA excitability.

Disclosures: R. Vazquez-Torres: None. **C. Calo-Guadalupe:** None. **D. Consuegra-Garcia:** None. **G.R. Fois:** None. **F. Georges:** None. **C.A. Jimenez-Rivera:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.12

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA021955

Title: The mTOR inhibitor Everolimus blocks incubated cocaine-craving via inhibition of Akt1 in vmPFC subregions

Authors: *L. L. HUERTA SANCHEZ, A. S. CHIU, M. C. KANG, K. K. SZUMLINSKI;
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Abstract: A time dependent increase in PI3K/Akt/mTOR signaling, and a reduced Group 1 metabotropic glutamate receptor expression (mGlu1), in the ventromedial prefrontal cortex (vmPFC) has been found to be critical for the incubation of cocaine-craving during protracted withdrawal. In this study, immunoblotting was conducted in order to further elucidate the changes in withdrawal-induced protein expression within the prelimbic (PL) and infralimbic (IL) subregions of the vmPFC following cocaine addiction when given an acute oral pretreatment of the FDA-approved mTOR inhibitor, Everolimus. Rats self-administered IV cocaine for 10 days in which each cocaine delivery was paired with a light-tone stimulus complex and at 3- or 30-days withdrawal, rats underwent a 2-h test for cue-reinforced responding. Then, the IL and PL were dissected upon test completion and immunoblotting was conducted. Previously, we have shown that single oral dosing with Everolimus dose-dependently blocked incubated cocaine-seeking in rats. Here, immunoblotting demonstrated that rats in protracted withdrawal, when compared to rats in early withdrawal, showed an increase in the expression of Homer2a/b and phosphorylated Akt1, P70-rpS6 kinase, and rpS6 within the PL. In the IL, there was an increase in expression of p-Akt1 and p-P70-rpS6 kinase when compared to rats in early withdrawal. Therefore, we conclude that the "anti-incubation" effect of Everolimus were associated with the withdrawal-induced increase in Akt1 activity. These data further demonstrate Everolimus can be repurposed as a viable strategy to interrupt cocaine-craving and aid addiction recovery in protracted withdrawal.

Disclosures: L.L. Huerta Sanchez: None. **A.S. Chiu:** None. **M.C. Kang:** None. **K.K. Szumlinski:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.13

Topic: G.09. Drugs of Abuse and Addiction

Support: T32 DA041349

Title: Synaptogyin-3 prevents cocaine-induced dopaminergic adaptations and promotes behavioral resiliency

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Abstract: Synaptogyrin-3 (SYG3) is a synaptic vesicle protein highly expressed in dopamine-containing neurons that directly interacts with the dopamine transporter (DAT), suggesting a role in synaptic dopamine dynamics. We tested the hypothesis that chronic exposure to cocaine will disrupt SYG3 function, leading to alterations in DAT that drive subsequent excessive cocaine

taking. Rats were trained to self-administer cocaine and, after successful acquisition, rats were tested on a progressive ratio (PR) schedule of reinforcement. Western blots indicated a significant positive correlation between relative SYG3 and DAT protein levels in the ventral tegmental area, and a significant negative correlation between SYG3 and PR breakpoint. We then overexpressed SYG3 in the VTA of naive rats to assess alterations in baseline behavior and terminal dopamine dynamics. Anxiety-like behavior was assessed using the novel open field test, elevated plus test, and social interaction test. In a separate cohort of rats, nucleus accumbens dopamine terminal function was assessed using *ex vivo* fast-scan cyclic voltammetry (FSCV). Overexpression of SYG3 in the VTA resulted in greater time spent in the center of the open field, as well as augmented dopamine release and reuptake kinetics in brain slices containing the nucleus accumbens. Together, these data suggest that SYG3 is a powerful modulator of dopamine kinetics and dopamine-related behavior, indicating this as a fruitful potential target for pharmacotherapeutics to treat cocaine use disorder.

Disclosures: E.G. Peck: None. **S.R. Jones:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.14

Topic: G.09. Drugs of Abuse and Addiction

Title: Acetylcholine Signaling Genes are Required for Cocaine-Stimulated Egg-Laying in C. elegans

Authors: *S. D. EMERSON[1], M. HAY[2], M. SMITH[1], R. EL BEJJANI[1];
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Abstract: Despite the toxicity and addictive liability associated with cocaine abuse, the mode of action of the drug is not completely understood, and effective pharmacotherapeutic interventions remain elusive. The cholinergic effects of cocaine are becoming increasingly appreciated, but the direct effects of cocaine on acetylcholine receptors, synthetic enzymes, and degradative enzymes have been the focus of relatively little empirical investigation. Due to its genetic tractability and anatomical simplicity, the egg-laying circuit of the hermaphroditic nematode, *Caenorhabditis elegans*, is a powerful model system to precisely examine the genetic and molecular targets of cocaine *in vivo*. Here, we report a novel cocaine-induced behavioral phenotype in *Caenorhabditis elegans*, cocaine-stimulated egg-laying. In addition, we present the results of an *in vivo* candidate screen of synthetic enzymes, receptors, degradative enzymes, and downstream components of the intracellular signaling cascades of the main neurotransmitter systems that control *Caenorhabditis elegans* egg-laying. Our results show that cocaine-stimulated egg-laying is dependent on genes involving in cholinergic neurotransmission, but not those involved in neurotransmitter systems known to control the behavior. In addition, our data suggest that

cocaine's effect on *Caenorhabditis elegans* egg-laying may intersect with its known effects on locomotion.

Disclosures: S.D. Emerson: None. **M. Smith:** None. **R. El Bejjani:** None. **M. Hay:** None.

Digital Abstract Session

P287. Cocaine: Circuitry and Neurophysiology of Addiction

Program #/Poster #: P287.15

Topic: G.09. Drugs of Abuse and Addiction

Support: CONACYT FOSISS No. 0260971
CONACYT No. 253072

Title: Clinical and functional connectivity outcomes of 5-Hz repeated transcranial magnetic stimulation as treatment in cocaine use disorder

Authors: *E. A. GARZA-VILLARREAL[1], S. FERNANDEZ-LOZANO[2], R. ALCALA-LOZANO[3], E. MORELOS-SANTANA[3], A. DAVALOS[3], V. VILLICAÑA[4], S. ALCAUTER[1], F. X. CASTELLANOS[5], J. J. GONZALEZ-OLVERA[3];
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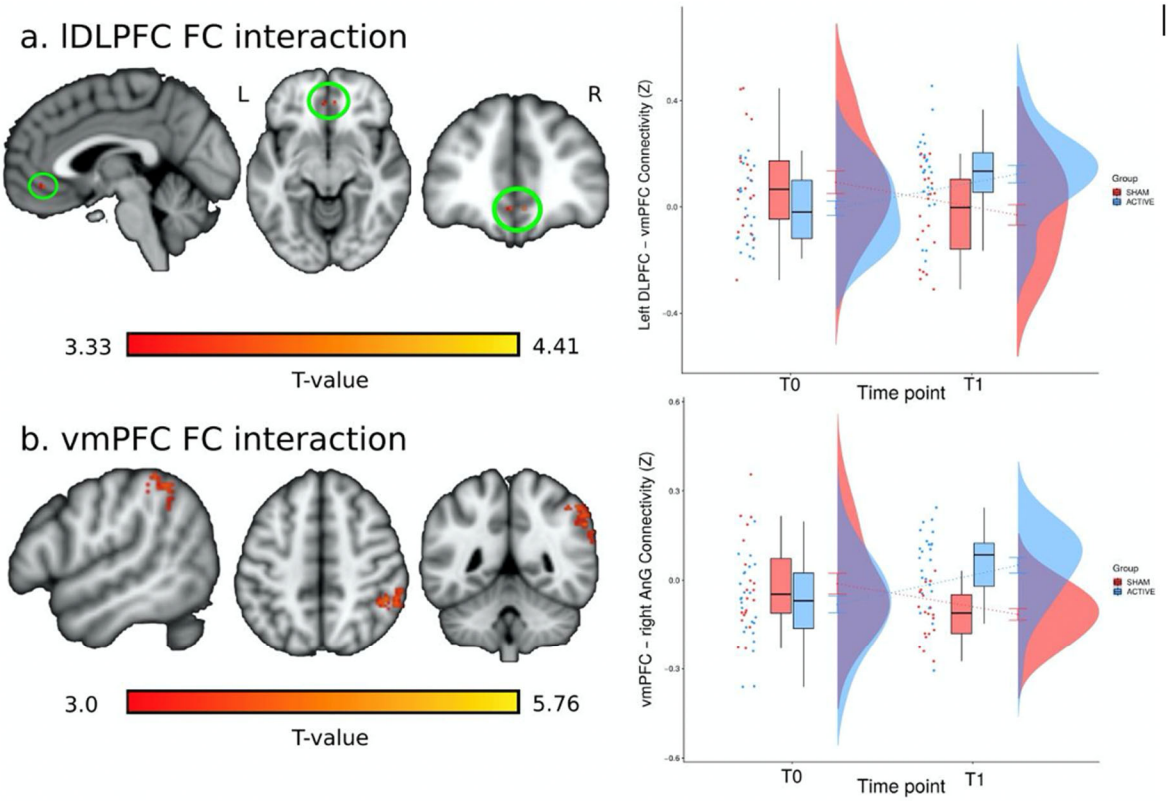
Abstract: Cocaine use disorder (CUD) is a global condition lacking effective treatment. Repeated magnetic transcranial stimulation (rTMS) is a technique that has shown promise by reducing craving and frequency of cocaine use, but little is known about its efficacy and physiological brain effects. Using a double-blind placebo-controlled randomized clinical trial (RCT) [NCT02986438], we sought to elucidate short- and long-term clinical benefits of 5-Hz rTMS as an add-on to standard treatment in CUD patients and discern underlying functional connectivity effects using magnetic resonance imaging. Forty-four randomly assigned CUD patients completed the 2-week double-blind acute phase [Sham (n=20, 2 female) and Active (n=24, 4 female)], in which they received 2 daily sessions of rTMS (5,000 pulses) on the left dorsolateral prefrontal cortex (lDLPFC). Subsequently, n=20 CUD patients continued to open-label maintenance (2 weekly sessions for up to 6 months). Measures were acquired at baseline, 2 weeks, 3 months and 6 months. Overall, 5-Hz rTMS plus standard treatment for 2 weeks significantly reduced craving (Sham group: baseline (T0), 2.6 SD: 2.8; 2 weeks (T1), 2.3 SD: 2.5 | Active group: T0, 3.9 SD: 3.6; T1, 1.5 SD: 2.4, p = 0.013, d=0.77) and impulsivity (Sham: T0, 60.8 SD: 17; T1, 59.8 SD: 21.4 | Active: T0, 64.8 SD: 16.8; 53.1 SD: 17.4, p = 0.011, d=0.79) in the Active group; decreased impulsivity correlated with increases in functional connectivity in lDLPFC-ventromedial PFC (vmPFC) (r=-0.35, p=0.02) and vmPFC-right angular gyrus (r=-0.34, p=0.03). Clinical and functional connectivity effects were maintained for 3 months but regressed

by 6 months of maintenance rTMS. We did not observe reduction of positive cocaine urine tests, however, self-reported frequency and grams consumed for 6 months were reduced.

Figure 1. Functional connectivity results of the acute phase.



a. lDLPFC-vmPFC significant interaction cluster; b. vmPFC-rAnG significant interaction cluster; right column shows the raincloud plots at each time point, where each data point represents the cluster's mean Z-value for each subject; lDLPFC = left dorsolateral prefrontal cortex; vmPFC = ventromedial prefrontal cortex; rAnG = right angular gyrus; FC = functional connectivity.

Disclosures: E.A. Garza-Villarreal: None. **S. Fernandez-Lozano:** None. **R. Alcala-Lozano:** None. **E. Morelos-Santana:** None. **A. Davalos:** None. **V. Villicaña:** None. **S. Alcauter:** None. **F.X. Castellanos:** None. **J.J. Gonzalez-Olvera:** None.

Digital Abstract Session

P288. Developmental Effects of Addictive Drugs: Prenatal & Adolescence Impacts

Program #/Poster #: P288.01

Topic: G.09. Drugs of Abuse and Addiction

Support: Departmental Startup Funds (Li)

Title: Adolescent-onset oxycodone self-administration is associated with attenuated incubation of oxycodone craving in male rats

Authors: *K. T. GARCIA, R. D. ALTSHULER, X. LI;
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Abstract: Relapse is a major obstacle to curb the ongoing epidemic of prescription opioid abuse. We have previously demonstrated that oxycodone seeking in adult male rats progressively increases after abstinence from oxycodone self-administration (incubation of oxycodone craving). Although the onset of oxycodone use often occurs during adolescent, little is known about the effect of adolescent-onset opioid use on subsequent relapse behavior in adulthood. Here, we examined incubation of oxycodone craving in rats after adolescent-onset oxycodone self-administration. We trained adolescent (postnatal day 35 at start) and adult (postnatal day 77 at start) Sprague Dawley rats to self-administer oxycodone on a fixed ratio 1 schedule (0.1 mg/kg/infusion, 6 h/d for 10 d). Next, we tested both groups for relapse on abstinence day 1 and 15 after the last session of oxycodone self-administration. We found that adolescent rats self-administered less oxycodone during training, and exhibited decreased oxycodone seeking behavior on both abstinence day 1 and 15 compared with adult rats. These results demonstrated that incubation of oxycodone craving is attenuated in rats with adolescent-onset oxycodone self-administration, which is possibly due to decreased oxycodone intake during training. We are currently replicating these behavioral findings and will explore the neurobiological basis underlying these differences between adolescent and adult rats.

Disclosures: K.T. Garcia: None. **R.D. Altshuler:** None. **X. Li:** None.

Digital Abstract Session

P288. Developmental Effects of Addictive Drugs: Prenatal & Adolescence Impacts

Program #/Poster #: P288.02

Topic: G.09. Drugs of Abuse and Addiction

Title: The Effects of Adolescent Sucrose Exposure on Ethanol Self-Administration in Male and Female Adult Rats

Authors: J. WATSON[1], M. CRAWFORD[2], R. PARMAR[3], C. APPLING[4], L. EVANS[1], C. ALONSO[1], M. HOCHSTETLER[1], *A. M. GANCARZ[1];
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Abstract: Over the last 60 years, there has been a dramatic increase in the amount of sucrose that is consumed by all age groups. However, the greatest increase in sucrose consumption is seen in adolescents (Guthrie & Morton, 2000). Adolescence is a critical time of development that has been shown to influence adult drug use (Burke & Miczek 2014). Sucrose has been shown to activate the mesolimbic dopamine circuitry which is the reward circuity that is activated by drugs

of abuse (Volkow et al., 2012). Previous work from our lab and others indicates that exposure to sucrose in adolescence increases vulnerability to cocaine self-administration in adult male rats. Here, we sought to determine if adolescent sucrose exposure increases vulnerability to other drugs of abuse. We hypothesized both male and female rats that are exposed to sucrose in adolescence will self-administer more ethanol compared to water controls. To test this, rats were given noncontingent access to 10% sucrose solution or water (PND 28-42). Once the rats reached adulthood (PND 60), they were tested for ethanol self-administration for 16 days in 30-minute sessions. Active responses resulted in the delivery of 100 ul/presentation of 10% ethanol. Both male and female rats that were exposed to sucrose in adolescence administer a significantly greater number of infusions and emit significantly more active responses, but not inactive responses, compared to water controls. Taken together, these findings have important implications to the human condition, suggesting exposure to sucrose during the adolescent period may increase susceptibility to non-natural rewards in adulthood.

Disclosures: J. Watson: None. **M. Crawford:** None. **R. Parmar:** None. **A.M. Gancarz:** None. **L. Evans:** None. **C. Alonso:** None. **M. Hochstetler:** None. **C. Appling:** None.

Digital Abstract Session

P288. Developmental Effects of Addictive Drugs: Prenatal & Adolescence Impacts

Program #/Poster #: P288.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01 DA041529

Title: Developmental impact of perigestational morphine exposure on the alcohol reward system of Sprague Dawley rats

Authors: *C. SEARLES, A. Z. MURPHY, H. HARDER, L. HANUS;
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Abstract: Approximately every fifteen minutes, a baby is born in the US experiencing neonatal opioid withdrawal syndrome (NOWS). NIDA reports that since 2004, the rate of NOWS has increased 5-fold. Remarkably, the long-term consequences of early life exposure to morphine are largely unknown. Clinical studies have established intrauterine exposure to drugs of abuse as a risk factor for adverse health outcomes in adult life, including propensity for drug use. Despite extensive knowledge about common mechanisms of action in the neural circuitry of reward that drive opioid and alcohol use, there is no data on the risks that those born with NOWS incur with alcohol use later in life. Here, we investigate the impact of perigestational opioid exposure on the mesolimbic reward system of male and female Sprague Dawley rats at postnatal and adolescent ages. To do so, our lab has developed a clinically relevant model for morphine exposure during early development spanning pre-conception to postnatal day 7. Using this model, we report age-specific changes in key components of the reward system, including mu opioid receptor (MOR) and potassium-chloride cotransporter (KCC2) expression. In addition, we explore microglia-

related expression and morphology in the VTA that have previously been shown to precipitate these changes. We also show changes in self-regulated alcohol consumption in perigestationally-exposed rats that is not a consequence of hyperactivity, as reflected in operant conditioning and open field testing.

Disclosures: C. Searles: None. **A.Z. Murphy:** None. **H. Harder:** None. **L. Hanus:** None.

Digital Abstract Session

P288. Developmental Effects of Addictive Drugs: Prenatal & Adolescence Impacts

Program #/Poster #: P288.04

Topic: G.09. Drugs of Abuse and Addiction

Support: K99DA049908
R21DA049253

Title: Maternal opioid exposure heterogeneously impairs offspring cognition and executive function in mice

Authors: *B. L. SMITH, C. J. LAAKER, A. FORD, A. HASSLER, T. M. REYES;
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Abstract: With the current U.S. opioid crisis, there is an epidemic of pregnant women using opioid drugs and a subsequent surge in the number of infants exposed to these drugs *in utero*. Opioid use during pregnancy has increased by more than 60% in the past decade. In humans, children born to mothers who reported opioid use during pregnancy often have cognitive delays, cognitive deficits, attentional problems, poor inhibitory control, and are more frequently diagnosed with ADHD. An animal model is required to understand the long-term consequences, develop interventions, and control for confounds in the clinical literature. To this end, we developed a maternal morphine model in mice and studied behavior and executive function in the offspring in 3 separate cohorts to enhance rigor. We treated female mouse dams with either saline (SAL, n = 7-11) or morphine (MO, n = 7-13) (10 mg/kg daily s.c.) starting 7 days prior to breeding, through gestation and lactation, until offspring were weaned on postnatal day (P)21. In the third cohort, we added a non-injected group of dams to control for the stress of daily injections (CON, n = 7). Experimenters were blinded for all offspring assessments.
To study executive function in adult offspring, we used the operant-based 5 choice serial reaction time task (5CSRTT). Male MO exposed offspring exhibited decreased motivation, decreased accuracy or correct responding, and a reduction in impulsive errors. Female MO offspring did not show these effects, but had increased impulsive errors and reduced sensitivity to the locomotion-depressing effects of MO when challenged as adults. One of our male MO cohorts did not have deficits in the 5CSRTT, but instead displayed a pattern of novelty-seeking behavior. We observed variability between cohorts, indicating that prenatal opioid exposure may induce heterogeneous effects between cohorts or serve as a subthreshold stimulus for inducing behavioral deficits. This makes it important to study other factors frequently present in opioid

using mothers.
Finally, we assessed gene expression after behavioral testing and in behaviorally-naïve cohorts at P1 and P21. In MO exposed offspring, we found evidence of microglial activation with increased expression of the Complement Receptor 3 (CR3) gene and toll like receptor genes in the prefrontal cortex at P21 and in the amygdala at P1. At P21, we found that maternal MO increased expression of the PSD95 gene, COMT, and PNOC in the prefrontal cortex, but not in the nucleus accumbens, amygdala, or ventral tegmental area. This is important because the COMT and PNOC genotype are implicated in NAS in humans, and COMT is related to executive function and ADHD.

Disclosures: B.L. Smith: None. **C.J. Laaker:** None. **A. Ford:** None. **A. Hassler:** None. **T.M. Reyes:** None.

Digital Abstract Session

P288. Developmental Effects of Addictive Drugs: Prenatal & Adolescence Impacts

Program #/Poster #: P288.05

Topic: H.04. Executive Functions

Support: 2T32AA014127
2P50AA022534-06

Title: Prenatal Alcohol Exposure Reduces Power in a Translational Neurophysiological signal during Rodent Touchscreen Tasks

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Abstract: Although it is well established that alcohol consumption during pregnancy can lead to poor outcomes in offspring, Fetal Alcohol Spectrum Disorders (FASD) remain the most common type of neurodevelopmental syndrome. The National Institutes of Mental Health (NIMH) has promoted the Research Domain Criteria (RDoC) initiative, which emphasizes the quantification of behavior more likely to reflect a specific neurophysiological circuit. Two domains that have been reported to be impaired in individuals with FASD include cognitive control utilizing a 5-Choice Continuous Performance Task (5C-CPT) and reward learning via probabilistic learning tasks (PLT). Importantly, these tasks can be integrated with electroencephalography (EEG) recordings in humans and dura-resting EEG-like recordings in rodents. Here, we integrated touchscreen analogs of these tasks with EEG-like recordings in a rodent prenatal alcohol exposure (PAE) model. Male and female mice were obtained from the NMARC where PAE is established via a drinking-in-the-dark model (10% EtOH with 0.066% saccharine for 4hrs/day throughout gestation; BAC: ~90mg/dL; controls=0.066% sweetened water, “SAC”). Beginning at 8-10 weeks, mice trained to touch white square target stimuli. Once responding rapidly and accurately mice underwent stereotactic surgery and fitted with dura-resting skull screws targeting

medial prefrontal, parietal, and motor cortices. After recovery and reminder to criterion, non-target trials (5 stimuli presented; withholding of response rewarded) were added at a 2:1 ratio for 5 days followed by recording at a 5:1 ratio for 12 days. Animals recorded on PLT for 5 days which consisted of 3 blocks of 20 trials with decreasing probability of reward (90/10, 80/20, and 70/30). Additionally, animals were recorded for 1 day on an additional task, progressive ratio breakpoint (PRBP), to examine the physical effort domain wherein PAE animals had to touch a single stimuli for progressively more responses in order to receive reward. We found that PAE impaired the ability to withhold responding to non-target stimuli during 5C-CPT and altered PLT performance at the most ambiguous probabilities. Importantly, when PAE animals displayed deficits in behavior, this tracked with decreases in power in relevant *a priori* regions of interest in the EEG-like signal. These results utilizing dura-resting EEG-like recording suggests that alterations in cortical activity can be used as a potential biomarker of PAE.

Disclosures: S.L. Olguin: None. **J.L. Brigman:** None.

Digital Abstract Session

P289. Tolerance, Dependence, and Toxicity of Addictive Drugs

Program #/Poster #: P289.01

Topic: G.09. Drugs of Abuse and Addiction

Support: VA/SLVHCS Merit Review Award to JEZ

Title: Tolerance, dependence, and anti-addiction potential of endomorphin analog ZH853 following systemic administration in male rats

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Abstract: Opioids continue to be the gold standard in pain treatment, but their long-term use is limited by side effects including addiction potential, tolerance, and drug dependence. We previously demonstrated the reduced tolerance of endomorphin analog ZH853 following chronic intrathecal infusion relative to that induced by morphine. Here, we expand our assessment of tolerance through a more clinically relevant model of systemic administration in male Sprague-Dawley rats. Dependence liability of ZH853 was also examined by assessing weight changes and withdrawal-induced mechanical hyperalgesia throughout 14 days of drug exposure and during spontaneous withdrawal. Chronic intravenous infusion led to reduced tolerance in ZH853-treated as compared to morphine-treated animals. Twice-daily injection of morphine inhibited normal weight gain, while ZH853 treatment had no effect on weight as compared to vehicle controls. Following cessation of drug administration, both morphine- and ZH853-treated rats exhibited weight loss, peaking at 48 hours into spontaneous withdrawal. Morphine-treated animals also exhibited withdrawal-induced mechanical hyperalgesia lasting several days, while no hyperalgesia was observed in ZH853-treated rats. These data further support the reduced

tolerance and dependence induced by ZH853 as compared to morphine. Lastly, current FDA approved opioid agonist therapies for opioid use disorder (OUD) are used to treat withdrawal symptoms during detoxification. The potential use of ZH853 as a treatment for OUD was investigated by measuring the ability of ZH853 administration to alleviate somatic signs of withdrawal in morphine-dependent male rats. Pretreatment of ZH853 led to a reduction in somatic signs of morphine withdrawal relative to vehicle-treated controls. Taken together, these findings support the potential use of ZH853 as a novel reduced side effect treatment for OUD.

Disclosures: **A.T. Amgott-Kwan:** None. **J.E. Zadina:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder.

Digital Abstract Session

P289. Tolerance, Dependence, and Toxicity of Addictive Drugs

Program #/Poster #: P289.02

Topic: G.09. Drugs of Abuse and Addiction

Support: DA25267
DA48353

Title: The Effects of Mitragynine, the Primary Mitragyna Speciosa (Kratom) Alkaloid, on Withdrawal from and Tolerance to Morphine in Rats.

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Abstract: *Mitragyna speciosa* (kratom) has been used traditionally for centuries in Southeast Asian countries. Anecdotal evidence suggests that kratom is used to treat opioid dependence. However, scientific evidence to support the medical use of kratom is not firmly reported. The aim of the study was to assess preclinical efficacy of mitragynine (MG) against the expression and development of tolerance to and withdrawal from morphine (MOR) in Sprague-Dawley rats (eight rats per group). On day 1, a dose-effect function of MOR-induced antinociception (cumulative 1.0-56 mg/kg, i.p., every 15 min) was assessed using a hotplate assay at 52°C. On days 2-5, MOR tolerance and naltrexone induced withdrawal were produced by increasing MOR doses 10 mg/kg/day (10-40 mg/kg, b.i.d., every 12 hours). On day 6, a second dose-effect function reassessed MOR's potency to induce antinociception. On day 7, the presence of diarrhea as a withdrawal sign was assessed prior to and 30 min after an injection of the opioid antagonist naltrexone (10 mg/kg, i.p.). MOR tolerance development was demonstrated by a 7.9-fold rightward shift to the antinociceptive potency of MOR. Concomitant administration of 32 mg/kg mitragynine with MOR during the tolerance/withdrawal development regimen, decreased the rightward shift by 2.1 fold. Spontaneous withdrawal was not observed following the withdrawal

development regimen of MOR alone; however, diarrhea was induced by naltrexone in 100% of dependent rats. In MOR tolerant/dependent rats, MG (32 and 56 mg/kg) on days 6 and 7 did not affect the expression of antinociceptive tolerance to MOR but dose dependently decreased the expression of naltrexone-induced diarrhea by up to 50%. The effects of MG on the expression of tolerance to and withdrawal from MOR were further compared to those of FDA-approved medications for opioid dependence and withdrawal (buprenorphine and lofexidine). Buprenorphine, the μ-opioid receptor partial agonist, (1.0 mg/kg, i.p.) produced a 3.5-fold increase in tolerance to the MOR-induced antinociception and decreased naltrexone-induced diarrhea by 25%. In addition, lofexidine, the α_2-adrenergic agonist, (1.0 mg/kg, i.p.) produced a 4.0-fold decrease in tolerance to the MOR-induced antinociception and fully blocked naltrexone-induced diarrhea. Concomitant administration of MG with MOR effectively attenuated the development of MOR tolerance. Similarly to lofexidine, acute MG decreased the expression of naltrexone induced diarrhea. The results support the potential of MG as a medication to attenuate the development of opioid tolerance and treat the symptoms of opioid withdrawal.

Disclosures: **J.D. Zuarth Gonzalez:** None. **A. Patel:** None. **R.C. Patel:** None. **M.A. Patel:** None. **L.R. Gamez-Jimenez:** None. **N.P. Ho:** None. **F. Leon:** None. **M. Mottinelli:** None. **C.R. McCurdy:** None. **L.R. McMahon:** None. **T. Hiranita:** None.

Digital Abstract Session

P289. Tolerance, Dependence, and Toxicity of Addictive Drugs

Program #/Poster #: P289.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NBIO T32
Foundation of Hope

Title: The effect of chronic cocaine administration on allopregnanolone levels in male and female rat brain and serum

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Abstract: Neurosteroids are compounds synthesized within the brain that influence neuronal activity, in part, via actions at γ-aminobutyric acid type A ($GABA_A$) receptors. Altered brain and serum levels of neurosteroids are associated with multiple psychiatric disorders. Furthermore, the endogenous GABAergic steroid (3α,5α)-3-hydroxy-5-pregnan-20-one, or allopregnanolone, a metabolite of progesterone, has emerged as a clinically beneficial therapeutic for the treatment of different diseases, which include post-partum depression, as well as substance use disorders. Although allopregnanolone has been shown to reduce cocaine-seeking and reinstatement in rats,

the underlying mechanism remains unclear. Additionally, increasing allopregnanolone levels via progesterone administration in cocaine-dependent individuals resulted in reduced cocaine craving and stress-induced arousal. Furthermore, studies have shown that chronic cocaine use was correlated with decreased levels of pregnenolone, a precursor to allopregnanolone. In this study, we hypothesized that chronic cocaine exposure in male and female rats would result in decreased levels of allopregnanolone in serum and brain regions involved in dopaminergic transmission and neurosteroidogenesis, such as olfactory tubercle, prefrontal cortex, dorsal and ventral striatum, and midbrain. To determine whether chronic cocaine use would decrease allopregnanolone levels, we administered 15mg/kg cocaine or saline to Sprague-Dawley rats daily for 14 days. We recorded locomotor activity and stereotyped behavior each day to assess for cocaine sensitization. We extracted allopregnanolone from brain tissue and serum and measured concentration with ELISA. Preliminary analysis (n=4 rats/sex/group) revealed no differences in allopregnanolone levels between cocaine and saline groups in any brain regions that were analyzed. However, females showed higher levels of allopregnanolone in serum and all brain regions examined except for ventral and dorsal striatum (main effect of sex, $p<0.05$). Furthermore, cocaine-exposed males presented no significant differences in serum allopregnanolone levels compared to saline controls (n=8 rats/sex/group) ($p<0.05$). These findings revealed that allopregnanolone levels vary across brain regions and by sex, which may play a part in sex differential responses to stress.

Disclosures: **M.H. McFarland:** None. **M.M.F. Machado:** None. **G. Boero:** None. **T.K. O'Buckley:** None. **D.L. Robinson:** None. **G.M. Sansbury:** None. **A. Morrow:** None.

Digital Abstract Session

P289. Tolerance, Dependence, and Toxicity of Addictive Drugs

Program #/Poster #: P289.04

Topic: G.09. Drugs of Abuse and Addiction

Title: The effect of socialization to recover from the lasting effects of early exposure to methylphenidate in male rats.

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Abstract: Methylphenidate (MPD) is the main treatment for the attentional deficit and hyperactivity disorder (ADHD), but also it is widely misused as a psychostimulant. Most of the users usually start consuming during adolescent years when brain development continues. It has been reported several negative effects because of early exposure to psychostimulants. The lasting effects include behavioral impairments such as sensitization and obsessive drug-seeking,

cognitive impairments, mainly in memory and executive functions, and psychiatric disorders such as depression and anxiety. Socialization has been linked to cognitive improvement, increases neurogenesis and as a protector for developing psychiatric disorders. Our study aimed to evaluate the effect of socialization on these lasting negative effects of early exposure to methylphenidate. Twenty-eight male rats (34 days-old) were divided into 4 groups, socialization + methylphenidate, socialization + saline, isolated + methylphenidate, and isolated + saline. All four groups received methylphenidate (1.5 mg/kg) or saline (2ml/kg) intraperitoneal administration for 20 days twice a day. Animals were housed isolated or in a group depending on their group condition. All rats remained undisturbed until 90 days-old when memory and anxiety-like were evaluated. We hypothesize there will be significant differences between socialization and individual groups treated with MPD, and between individual conditions but there will not be significant differences between both socialization groups.

Disclosures: **A. Tapia-De Jesus:** None. **M.H. Buenrostro-jauregui:** None. **E. Castaneda:** None. **L. Rodriguez-Serrano:** None. **M. Chavez Hernandez:** None. **M. Jesus:** None.

Digital Abstract Session

P289. Tolerance, Dependence, and Toxicity of Addictive Drugs

Program #/Poster #: P289.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01 DA046457

Title: The role of habitual behavior in punishment resistance differs for food and cocaine self-administration

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Abstract: Addiction is characterized by compulsive drug use that persists even when faced with negative consequences. In animal models of addiction, some rats display a similar type of compulsive drug use and continue to self-administer IV cocaine despite receiving footshock. Recent work from our lab has demonstrated that punishment resistance is associated with increased utilization of a habitual response strategy, whereas punishment sensitivity is associated with increased utilization of a goal-directed strategy. Here, we wanted to investigate whether punishment would induce similar patterns of response-strategy switching in rats self-administering food. Male Sprague Dawley rats were trained on a seeking-taking chained schedule of reinforcement, in which presses on a seeking lever gave access to a separate taking lever. The seeking link of the chain required completion of a random ratio (RR20) or random interval (RI60) schedule, and the taking link was reinforced with either a food pellet or IV cocaine (0.5 mg/kg) under a fixed ratio (FR1) schedule. Following at least 12 days on the final seeking-taking reinforcement schedule, animals were exposed to 4 days of punishment testing, in which footshock (0.4 mA, 0.3 s) was delivered randomly on one-third of trials following

completion of seeking and prior to extension of the taking lever. Both before and after punishment testing (4 days prior to punishment, and then 4 days after rats had resumed self-administration), response strategy was assessed using outcome devaluation via satiety. Satiety was induced via pre-session access to food pellets (for food self-administering rats) or noncontingent IV cocaine (for cocaine self-administering rats). We found increased punishment resistance in rats trained to self-administer food, as compared to cocaine. Interestingly, punishment resistance for food self-administration was not associated with increased utilization of a habitual response strategy, unlike what we observed for cocaine self-administration. Therefore, punishment resistance in food self-administering rats appears to be goal-directed, while punishment resistance in cocaine self-administering rats is associated with increased habitual behavior. Together, these data indicate that punishment learning may be different for cocaine and food rewards, and that persistent habits may be a unique feature of compulsive cocaine seeking.

Disclosures: **B. Jones:** None. **R. Smith:** None.

Digital Abstract Session

P290. Neural and Molecular Mechanisms of Addictive Drugs

Program #/Poster #: P290.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA007359
NIH Grant DA047233

Title: The Role of ΔFosB in the Development of Drug Addiction: Identifying ΔFosB Transcriptional Targets in Nucleus Accumbens

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Abstract: Drug addiction exacts devastating impact on drug users and a tremendous burden on their family members, the economy, and the nation's public health. Although different in chemical structures and initial mechanisms affecting the brain, all classes of drugs of abuse induce the expression of the transcription factor ΔFosB in nucleus accumbens (NAc), the central node of the reward circuit. Genetically overexpressing ΔFosB in mouse NAc neurons increases cocaine-elicited compulsive behaviors, suggesting that the expression of ΔFosB in NAc is involved in the development of addiction. However, the molecular mechanisms of ΔFosB remain incompletely understood. Here, we extend early work on revealing ΔFosB transcriptional targets with chromatin immunoprecipitation (ChIP)-chip methodology, which interrogated promoter regions only, by leveraging the newer ChIP method, CUT&RUN (cleavage under targets and release using nuclease), to locate genomic loci with ΔFosB coverage genome-wide upon chronic cocaine exposure. Male and female mice ~8 to 10 weeks of age underwent 7 days of intraperitoneal cocaine or saline injections, and bilateral punches of NAc were collected 24 hours

after the last administration. Nuclei were isolated from NAc tissue with discontinuous sucrose gradients and subjected to CUT&RUN procedures. Thousands of ΔFosB binding sites were revealed through this approach, and, interestingly, one third of the loci are positioned at distal intergenic regions while less than 10 percent of the peaks are placed within 1 kilobase from known transcription start sites, suggesting that a primary function of ΔFosB is coordinating distal regulatory elements with transcription machinery. NAc is composed mainly of medium spiny neurons (MSNs) expressing either dopamine receptor D1 or D2; ΔFosB is prominently induced in D1-type MSNs after most drugs (only opioids induce ΔFosB in both cell types), and directs differential synaptic plasticity in these two types of neurons. Further extension of this CUT&RUN approach to D1- and D2-type MSNs within NAc will set the groundwork for understanding distinct roles of MSNs, and their adaptations to chronic drug exposure, in drug addiction.

Disclosures: S. Yeh: None. **M.S. Estill:** None. **C.K. Lardner:** None. **L. Shen:** None. **E.J. Nestler:** None.

Digital Abstract Session

P290. Neural and Molecular Mechanisms of Addictive Drugs

Program #/Poster #: P290.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R21DA 048633
NIH Grant R21DA 050821

Title: Drug-induced glutamate-to-GABA neurotransmitter switching impacts behavior

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Abstract: Repeated exposure to drugs of abuse influences brain activity and induces maladaptive neuroplastic changes that have been consistently associated with the appearance of drug-induced behavioral alterations. Some of these changes (e.g. altered neuronal activity in the prefrontal cortex) can be induced by drugs that have different targets but similar behavioral consequences. Neurotransmitter switching is a form of environmentally-induced neuroplasticity in which a subset of neurons loses its neurotransmitter and/or gains a different one. It is commonly triggered by sustained changes in neuronal activity and has been shown to modify behavior. Here we investigated whether exposure to drugs induces neurotransmitter switching and whether this causes drug-induced behavioral changes. We focused our experiments on two substances: phencyclidine (PCP) and methamphetamine. Although PCP is an NMDA receptor antagonist and methamphetamine increases monoamine signaling, both drugs acutely produce an increase in neuronal activity in the prefrontal cortex and cause cognitive deficits as well as locomotor sensitization. We used mice that express a Cre-dependent H2B-mCherry reporter and

a Cre recombinase under the promoter of the vesicular glutamate transporter 1 (vGluT1) to permanently label glutamatergic neurons. This allowed us to discover that both PCP and methamphetamine induce a subset of glutamatergic neurons in the prelimbic cortex (PrL) to acquire a GABAergic phenotype. Indeed, these neurons gain the expression of GABA, its synthetic enzyme GAD67, and its vesicular transporter, while at the same time showing a reduced expression level of vGluT1 compared to other glutamatergic neurons in this region. We then focused on PCP-treated mice to determine if this transmitter switch plays a role in PCP-induced changes in behavior. To prevent glutamatergic neurons from gaining GABA upon PCP exposure, we stereotaxically injected a Cre-dependent AAV expressing GAD1sh-interference-RNA in the PrL of vGluT1::Cre +/- mice. Remarkably, this prevented the appearance of PCP-induced cognitive deficits in the novel object recognition test, the spontaneous alternation task, as well as PCP-induced locomotor sensitization. These results identify a causal link between the gain of GABA in glutamatergic pyramidal neurons and PCP-induced behavioral alterations. We are now using a chemogenetic approach to selectively activate parvalbumin-positive PrL interneurons during PCP treatment, to test if manipulation of neuronal activity can suppress PCP-induced neurotransmitter switching and behavioral deficits. Supported by NIH R21DA 048633 and R21DA 050821

Disclosures: **M. Pratelli:** None. **N.C. Spitzer:** None.

Digital Abstract Session

P290. Neural and Molecular Mechanisms of Addictive Drugs

Program #/Poster #: P290.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIMH MH115409
NIMH MH120637
NIDA

Title: Behavioral variability in response to chronic stress and morphine in BXD and parental mouse lines

Authors: C. MOREL[1], Y. VAN DER ZEE[2], L. F. PARISE[2], M. CAI[2], O. ISSLER[2], C. BROWNE[2], K. LECLAIR[2], R. W. WILLIAMS[3], M. K. MULLIGAN[3], S. J. RUSSO[2], E. J. NESTLER[2], M.-H. HAN[2];
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Abstract: Drug addiction is a multifactorial syndrome in which genetic predispositions and environment stress exposure constitute major risk factors for early onset, escalation and relapse of addictive behaviors. While it is well known that both social and non-social stressors play a key role in drug addiction, the genetic factors that make certain individuals particularly sensitive

to stress and thereby more vulnerable to become addicted are unknown. In an effort to map a complex set of G x E interactions—specifically *Gene x Chronic Stress*—here we leveraged a systems genetics resource: BXD recombinant inbred mice (BXD5, BXD8, BXD14, BXD22, BXD29 and BXD32) in addition to their C57BL/6J and DBA/2J parental lines. Utilizing the chronic social defeat stress paradigm (CSDS), we first showed that DBA/2J and BXD22 male and female mice are more susceptible to chronic social stress than C57BL/6J mice, as evidenced by stronger social avoidance and anxiety-like behaviors. Further, we observed sexual dimorphisms in response to CSDS amongst the BXD5, BXD8, BXD14, BXD29 and BXD32 lines. To investigate the interaction between genetics and vulnerability to prolonged exposure to non-social stressors, we exposed C57BL/6J, DBA/2J, BXD8, BXD22 and BXD29 male and female mice to the chronic variable stress paradigm (CVS). We observed that DBA/2J female mice are more sensitive to CVS when compared to C57BL/6J female mice (i.e., strong decrease in sucrose preference). Confirming the stress vulnerability of BXD22 mice observed after CSDS, BXD22 male and female mice after CVS displayed higher levels of anxiety-like measures than C57BL/6J mice. Interestingly, while BXD29 mice behaved like C57BL/6J after CSDS, both BXD29 female and male mice developed a higher anxiety profile following CVS when compared to C57BL/6J mice. Finally, we identified that DBA/2J and C57BL/6J mice pre-exposed to CSDS displayed differences in morphine sensitivity. Our results support the hypothesis that genetic variations in predispositions to stress responses influence sensitivity to morphine and *in fine* regulate the development of drug addiction. Characterization of the genetic, neurobiological, social and environmental factors that mediate addiction risk will fundamentally improve our understanding of individual variations in responses to drugs of abuse, and provide highly useful information for the development of new treatment strategies and eventually prevention measures.

Disclosures: **C. Morel:** None. **Y. Van der Zee:** None. **L.F. Parise:** None. **M. Cai:** None. **O. Issler:** None. **C. Browne:** None. **K. Leclair:** None. **R.W. Williams:** None. **M.K. Mulligan:** None. **S.J. Russo:** None. **E.J. Nestler:** None. **M. Han:** None.

Digital Abstract Session

P290. Neural and Molecular Mechanisms of Addictive Drugs

Program #/Poster #: P290.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA007359

Title: Phosphodiesterase 1b is an Upstream Regulator of a Key Gene Network in the Nucleus Accumbens Associated with Addiction-like Behaviors

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Abstract: Cocaine use disorder (CUD) is a highly prevalent neuropsychiatric disease characterized by compulsive drug taking and repeated relapse. Despite the public health burden of CUD, there remains no FDA-approved medications for this brain disease. Identifying effective treatments for CUD is hindered by an incomplete understanding of which genes are most important in mediating addiction pathophysiology. In this study, we performed unbiased transcriptional network analysis on a published RNA sequencing (RNA-seq) dataset from 6 different brain regions of animals that underwent cocaine self-administration followed by prolonged (30 d) withdrawal plus relapse to identify gene networks associated with individual differences in addiction-like behavior. We ranked modules by their fold enrichment in genes whose expression is significantly correlated with an "addiction index" (AI), a composite score developed by machine learning to capture maladaptive, addiction-like behaviors during cocaine self-administration. We identify phosphodiesterase 1b (*Pde1b*), a Ca^{2+}/calmodulin-dependent enzyme that catalyzes the hydrolysis of cAMP and cGMP, as the strongest hub gene in module 504 (arbitrary number) in nucleus accumbens (NAc), the module with the strongest association with the AI of all gene modules in this brain region. To investigate the role of *Pde1b* in modulating addiction pathophysiology, we will pair clustered regularly interspaced short palindromic repeats (CRISPR)-based transcriptional regulation tools with viral delivery methods to selectively regulate *Pde1b* expression in the NAc. Our ongoing studies seek to validate that the CRISPR tools achieve robust and selective *Pde1b* regulation *in vitro* and *in vivo*. In future studies, we will investigate the effects of *Pde1b* regulation on addiction-like behaviors in mouse models of CUD. Given successful drug discovery efforts focused on other PDE isoforms, this work raises a novel therapeutic approach for this illness.
Supported by NIDA

Disclosures: C.D. Teague: None. **X. Zhou:** None. **D.M. Walker:** None. **L.M. Holt:** None. **B. Zhang:** None. **E.J. Nestler:** None.

Digital Abstract Session

P290. Neural and Molecular Mechanisms of Addictive Drugs

Program #/Poster #: P290.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant MH107507
NIH Grant DA033934

Title: Electrophysiological characterization of muscarinic excitation of dopamine neurons in the ventral tegmental area

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Abstract: Dopaminergic (DA) neurons in the ventral tegmental area (VTA) play a crucial role in reward and motivational behaviors, including the development of drug addictions. VTA DA neurons receive excitatory cholinergic inputs. Blockage of the M5 muscarinic receptor in DA neurons has been shown to attenuate drug-induced DA release and abuse-related behaviors, but the molecular mechanism is unknown. In this study, experiments were designed to identify the electrophysiological effects of muscarinic agonism involved in the modulation of action potential kinetics and firing patterns in VTA DA neurons. Whole-cell current-clamp and cell-attached patch-clamp experiments were performed on acute midbrain slices prepared from C57BL/6 mice (P14-P31). VTA DA neurons were identified by anatomical location, cell morphology, and electrophysiological properties. Muscarinic receptors were stimulated or inhibited by bath perfusion of the non-selective cholinergic agonist carbachol and the muscarinic antagonist atropine, respectively. In the presence of tetrodotoxin (TTX), carbachol depolarized membrane potential (by 2.68 ± 0.38 mV, n = 32) and decreased input resistance. Moreover, carbachol increased tonic firing frequency (by 1.72 ± 0.34 Hz, n = 12) without affecting firing regularity, frequency-current relationship, and HCN sag ratio in VTA DA neurons. Together with the depolarization, carbachol altered the shape of action potentials: this effect was reversed by restituting the original membrane potential using the clamping circuit. Atropine preapplication inhibited carbachol effects in all experiments. In addition, carbachol had no effects on sIPSCs in VTA DA neurons. Collectively, the current evidence suggests that activation of HCN channels, inhibition of potassium channels, and changes in inhibitory synaptic inputs do not mediate the carbachol-induced depolarization in VTA DA neurons, but points toward the participation of TTX-insensitive cation channels in this muscarinic receptor-mediated excitation.

Disclosures: **R.Y. Chen:** None. **J.M. Eltit:** None. **A.R. McQuiston:** None.

Digital Abstract Session

P290. Neural and Molecular Mechanisms of Addictive Drugs

Program #/Poster #: P290.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NeuroChoice, an Initiative of the Wu Tsai Neurosciences Institute at Stanford University
Author LT is funded through an Early PostDoc.Mobility Fellowship from the Swiss National Science Foundation

Title: Structural coherence of white-matter projections to the nucleus accumbens predicts relapse to stimulant drug use in humans

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Abstract: Nucleus Accumbens (NAcc) activity can predict relapse to stimulant use. Whether such functional associations are subserved by differences in the coherence of white-matter connections projecting to and modulating activity in the NAcc, however, is less clear. Previously, we found coherence metrics of ascending dopaminergic midbrain projections to the NAcc to be related to diagnosis of stimulant use disorder (SUD), but not relapse (MacNiven et al., 2020). Here, we tested whether the structural coherence of glutamatergic projections connecting the Medial PreFrontal Cortex (MPFC), Anterior Insula (AIns), and Amygdala (Amy) to the NAcc might predict relapse to stimulant use.
We analyzed Diffusion-Weighted Imaging data from a sample of 40 healthy controls (57% male, 32.7±11.8 years old) and 70 patients with SUD (81% male, 41.4±11.7 years). At 6 months follow-up, we retained comparable sub-samples of individuals who had abstained (n = 30, 80% male, 39.5±12.5 years) and who had relapsed (n = 28, 86% male, 42.3±10.9 years). To address methodological challenges such as crossing fibers and inter-subject anatomical variability, we fit a constrained spherical deconvolution model and conducted probabilistic tractography in individuals' native space. This method successfully resolved all of the sample's white-matter tracts. We subsequently tested if structural properties indicative of tract coherence (i.e., inverse radial diffusivity (1-RD)) could predict relapse 6 months after treatment release.
Correcting for multiple comparisons via permutation testing, lower 1-RD (i.e., lower coherence) in a cluster along the right AIns tract predicted relapse (OR = 0.41, z = -2.54, p = 0.01). Additionally, lower 1-RD in a cluster along the left Amy tract also predicted relapse (OR = 0.52, z = -2.12, p = 0.03). In contrast, MPFC-NAcc tract coherence did not predict relapse (but declined with age). Our results hold when controlling for age, gender and motion, as well as for other coherence metrics. Crucially, none of these coherence metrics were associated with SUD diagnosis.
Our findings suggest that structural properties of specific white-matter tracts are selectively associated with relapse to stimulant use but not diagnosis, and thus identify novel targets for prediction of relapse. The implication of structural projections from the AIns to the NAcc points toward incentivized inhibition as a potential mechanism for relapse, rather than aberrant value integration commonly associated with MPFC-NAcc projections. Future research may test these predictions by linking structural tract properties to functional brain activity and risky behavior in the context of relapse.

Disclosures: L. Tisdall: None. **K.H. Macniven:** None. **J.K. Leong:** None. **B. Knutson:** None.

Digital Abstract Session

P290. Neural and Molecular Mechanisms of Addictive Drugs

Program #/Poster #: P290.07

Topic: G.09. Drugs of Abuse and Addiction

Title: Long-term Withdrawal from Compulsive Oxycodone Taking Results in Dose-Dependent Changes in Glutamate Receptor Expression in the Hippocampus

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Abstract: People suffering from opioid use disorder (OUD) exhibit cognitive dysfunctions. Cognitive dysfunctions have been shown to be accompanied with altered expression of glutamate receptors in the brain. AMPA, NMDA, and metabotropic glutamate receptors are important for many types of synaptic plasticity in the hippocampus. They are important for consolidation of memory and are thus promising targets for understanding drug seeking and relapse following long-term withdrawal. Yet, it is still unclear if altered expression of these receptors might occur in those who suffer from opioid use disorder. As a first step towards testing that possibility, we measured the expression of glutamate receptors in the hippocampi of Sprague-Dawley rats that had undergone oxycodone self-administration (SA). Rats were euthanized at 2 hours or 30 days after the last SA session. RNA was extracted from the hippocampi of rats and used in quantitative polymerase chain reaction (qPCR) analyses. We found that rats given long-access (LgA) to oxycodone took significantly more drug than short-access (ShA) rats. We were able to separate LgA rats into two separate phenotypes of high oxycodone intake (LgA-H) and low intake (LgA-L) rats. Quantitative PCR revealed that LgA rats exhibited increased mRNA expression of *GluA1-3, GluN2a-c, mgluR3, mGluR5, mGluR6,* and *mGlur8* subunits of glutamate receptors after 31 days but not after 2 hours of stopping the SA experiment. These results suggest that prolonged withdrawal from oxycodone SA may be associated with significant changes in glutamate receptor protein compositions. These studies offer additional insight into the potential basis of oxycodone-associated changes in learning and memory observed in some individuals who suffer from OUDs. These results suggest the need for more post-mortem studies focused on the hippocampus of patients who met diagnostic criteria for these disorders.

Disclosures: A.J. Salisbury: None. **C.A. Blackwood:** None. **J.L. Cadet:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant U19 NS113201
NIH Grant NS 108740
Simons Collaboration on Global Brain
Japan Society for the Promotion of Science
AMED JP20dm0207069

Title: A gradual backward shift of dopamine responses during associative learning

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Abstract: It has been proposed that the activity of dopamine neurons approximates temporal difference (TD) prediction error, a teaching signal developed in reinforcement learning theories. However, whether this similarity holds true during learning remains elusive. In particular, some TD learning models predict that the error signal gradually shifts backward in time from reward delivery to a reward-predictive cue. However, such a shift has not been demonstrated experimentally. In this study, we examined conditions in which such a shift can be detected. We sought to optimize our experimental conditions such that we can observe even a small signal on a trial-by-trial basis. To this goal, we used the methods that integrate signals from a large population of neurons (fiber fluorometry) and across time (i.e. slow measurements using calcium indicator or dopamine sensor). To resolve signals over time, we included a relatively long delay (e.g. 3 sec) relative to the sensors. Using these methods, we were able to detect a small "bump" between cue and reward on a trial-by-trial basis. As a result, we reliably observed a gradual backward shift of dopamine activity in the ventral striatum during learning of novel odor-reward association in naïve as well as in well-trained mice (naïve: 3.9±0.3 ms/trial, $p = 0.16 \times 10^{-4}$, n = 7 mice; well-trained: 9.4±0.9 ms/trial, $p = 0.65 \times 10^{-3}$, n = 5 mice) or reversal learning (nothing to reward: 27.1±1.6 ms/trial, $p = 0.37 \times 10^{-2}$, n = 3; air puff to reward: 30.2±4.8 ms/trial, $p = 0.33 \times 10^{-2}$, n = 5 mice). Our modeling confirms that integrating signals over time using slow measurements can indeed facilitate a detection of such a small and time-spread activity. These results establish that, at least in some conditions, a gradual backward shift of dopamine responses, a long-sought link between TD learning and dopamine activity, can be observed during associative learning. The shared dynamics of TD error and dopamine activity provide a critical evidence supporting the TD account of dopamine signals.

Disclosures: **R. Amo:** None. **A. Yamanaka:** None. **K.F. Tanaka:** None. **N. Uchida:** None. **M. Watabe-Uchida:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH DA046522
P20GM121310
NIH DA 040004
NIH DA003906
NIH DA12513

Title: Reward-specific ensembles in the nucleus accumbens core.

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Abstract: Poorly regulated reward seeking is a central feature of substance use disorder. Recent research shows that rewarding drug-related experiences induce synchronous activation of a discrete number of neurons in the nucleus accumbens that are causally linked to reward-related contexts. Here we comprehensively characterize the specific ensemble of neurons built through experience that are linked to seeking behavior. We additionally address the question of whether or not addictive drugs usurp the neuronal networks recruited by natural rewards by evaluating cocaine- and sucrose-associated ensembles within the same mouse. We used $Fos^{CreERT2/+}$/Ai14 transgenic mice to tag cells activated by and potentially encoding cocaine and sucrose seeking. We tagged ~1% of neurons in the core subregion of the accumbens (NAcore) activated during cue-induced seeking for cocaine or sucrose. The majority of tagged cells in the seeking ensembles were D1-MSNs, and specifically activated during seeking, not during extinction or when mice remained in the home cage. To compare different reward-specific ensembles within the same mouse, we used a dual cocaine and sucrose self-administration protocol allowing reward-specific seeking. Using this model, we found ~70% distinction between the cells constituting the cocaine- compared to the sucrose-seeking ensemble. Establishing that cocaine recruits an ensemble of NAcore neurons largely distinct from neurons recruited into an ensemble coding for sucrose seeking suggest a finely tuned specificity of ensembles. The findings allow further exploration of the mechanisms that transform reward-based positive reinforcement into maladaptive drug seeking.

Disclosures: **A. Bobadilla:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; DA046522, SBC support P20GM121310. **E. Dereschewitz:** None. **L. Vaccaro:** None. **J. Heinsbroek:** None. **M.D. Scofield:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; DA040004. **P.W. Kalivas:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; DA003906, DA12513.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.03

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Ventral Pallidal GABAergic Neuronal Activity Encodes Instrumental Reward Cues and Reward Anticipation

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Abstract: Environmental cues are powerful modulators of motivated behavior. The ventral pallidum (VP) is a key neural node contributing to cue-driven motivated behaviors. Many VP neurons are excited by instrumental reward cues and the strength of this response in a subset of neurons predicts reward seeking behavior. However, the specific VP neuronal populations (cell-type and output pathways) involved in instrumental cue-driven reward seeking are unknown. Recent recordings from VP during a Pavlovian task found that cue- and reward-excited neurons are largely GABAergic, whereas cue- and reward-inhibited neurons are glutamatergic. Therefore, we hypothesized that cue-evoked responses in VP GABA neurons encode the vigor of cue-elicited reward-seeking in an instrumental task. To test this, we recorded calcium activity of VP GABA neurons using *in vivo* fiber photometry during an instrumental reward seeking task, the discriminative stimulus (DS) task. Male and female Long-Evans rats (n=6 per sex) underwent stereotaxic surgery a month before behavioral training started. VP GABA neurons were targeted using a mixture of one virus expressing cre recombinase under the control of the GAD1 promoter along with a cre-dependent virus for the genetically encoded calcium indicator GCaMP6f. Bulk calcium-dependent and independent signals were recorded via optic fiber implants that were inserted during surgery. Rats were trained to perform the DS task, where an auditory cue (the DS) is associated with availability of a 10% sucrose solution. Sucrose is delivered if the animal makes a port entry during the DS. A neutral stimulus (NS) is introduced after initial DS training and is never associated with reward delivery. As expected, we found that DS cues increased VP GABA neuronal calcium activity relative to the NS control cue. Additionally, these responses emerged across training as animals learned the task. VP GABA neurons also developed a post-port entry increase in calcium activity, at the time that reward is normally delivered. Interestingly, post-port entry responses were present even on trials where reward delivery is omitted. This suggests that this post-port entry VP GABA neuron response encodes reward anticipation or expectation rather than consumption or hedonic evaluation. Altogether, these data show VP GABA neurons encode information imperative for instrumental reward-seeking behaviors. Further research will examine downstream targets of VP GABA neurons and the functional role this population plays in reward seeking and consummatory behavior.

Disclosures: A. Scott: None. **D. Palmer:** None. **J. Richard:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.04

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant F32MH117973
NIH Grant R01MH118451

Title: Stress effects on prefrontal cortical function and effortful reward-seeking behavior

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Abstract: Title: Stress effects on prefrontal cortical function and effortful reward-seeking behavior
Deficits in effort valuation (EV), a cost-benefit analysis comparing the magnitude of anticipated rewards with effort expenditure required, are associated with psychiatric conditions including depression and schizophrenia. Exposure to chronic stress serves as a risk factor for the development of depressive symptoms such as anhedonia, which is characterized by impairments in reward processing and diminished EV. Prefrontal cortical function is critical to support EV however distinct projection pathways may encode varying aspects of learned reward and effort expectations to modulate behavior. In order to define the circuit mechanisms contributing to effortful reward seeking, mice were trained in a novel head-fixed EV task which allowed for simultaneous in vivo 2-photon calcium imaging. Corticostriatal neurons were targeted for imaging and found to exhibit statistically significant reward-predictive cue encoding, as expected. A comparatively smaller proportion of corticostriatal neurons were activated in response to an effort-predictive cue. High-dimensional encoding of reward-predictive cues was also determined by accurate decoding from population activity, while effort cue decoding remained largely at chance level. In order to model anhedonia-relevant behaviors, mice were exposed to chronic social defeat stress (CSDS) for 10 days and tested again in the EV task. Overall, mice showed reduced motivational and consummatory effortful reward seeking behavior following stress, with notable individual variability. The effects of CSDS on EV were uncorrelated with social interaction scores. CSDS also reduced the accuracy of decoding rewarded trials from corticostriatal population activity specifically during the reward consumption period. Ongoing studies are focused on comparing the roles of different PFC projection pathways in reward- and effort-predictive cue encoding and the effect of chronic stress on these circuits.

Disclosures: P.K. Parekh: None. **J. Kaminsky:** None. **J. Roshgadol:** None. **S. Johnson:** None. **C. Xia:** None. **C. Liston:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA Grant R01DA044761

NIMH Grant R01MH115604

Title: Motor and reward-related computation on the cerebellar-nigral pathway

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Abstract: The cerebellum and substantia nigra pars compacta (SNc) contribute to motor control, and their dysfunction contributes to abnormal motor function (e.g., ataxia and Parkinson disease). We have recently found that the deep cerebellar nuclei (DCN, the main output of the cerebellum), send monosynaptic, excitatory projections to the SNc (DCN-SNc). The nature of the "information" that the cerebellum provides to the SNc remains to be established. As a first step to address this question, we measured calcium as a proxy for neuronal activity in DCN axons within the SNc, while the animal moved a lever or, as it performed a Pavlovian task. We found that 1) the DCN-SNc projections were bilaterally active when the animal unilaterally moved the lever, 2) In a simple Pavlovian task where reward was delivered at a same time as a sensory stimulus, the DCN-SNc projections showed robust activity with reward delivery, but not when reward was omitted, 3) In a random reward Pavlovian task where the value of the reward was changed concomitant with the same sensory stimulus, the DCN-SNc activity was correlated with the reward value, 4) The higher activity of the DCN-SNc projections when higher value rewards were delivered could not be solely attributed to difference in motor performance. Our findings suggest that in addition to conveying motor-related information, the cerebellum to the substantia nigra pathway may also contain information regarding reward, and its value. How and whether this information is used in reward processing, or in modulating movement vigor requires further scrutiny.

Disclosures: J. Yoshida: None. **S. Washburn:** None. **M. Oñate:** None. **V. Lovallo:** None. **F. Nadim:** None. **K. Khodakhah:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.06

Topic: G.02. Reward and Appetitive Learning and Memory

Support: DA14241
DA037566

Title: Cholinergic signaling in the basolateral amygdala during reward learning

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Abstract: The ability of animals to learn to associate environmental stimuli with the outcomes they predict is crucial for survival. The basolateral amygdala (BLA) is important for forming associations between predictive stimuli and either positive or negative outcomes. Release of the neuromodulator acetylcholine (ACh) is known to be related to learning and the BLA receives an abundance of cholinergic input, especially from the nucleus basalis of Meynert (NBM). During a cue-reward learning task, in which mice were trained to nose poke following an auditory tone to receive food reward, we recorded cholinergic signaling in the BLA. Convergent fiber photometric results using a genetically encoded fluorescent ACh sensor and a calcium indicator in NBM cholinergic terminal fibers in the BLA (NBM-BLA) showed that BLA ACh was released following reward-related events, and eventually shifted to the reward predictive tone as mice acquired the cue-reward contingency. Incorrect responses that resulted in punishment initially elicited BLA ACh release as well, but diminished as behavioral performance improved. Given the time-locking and plasticity observed in BLA ACh signaling in response to salient events during cue-reward learning, we hypothesized that manipulating cholinergic signaling would affect learning. Optogenetic stimulation of NBM-BLA cholinergic terminal fibers during learning enhanced acquisition rate and decreased incorrect responses compared to controls. Interestingly, this improvement in learning was observed when stimulation was contingent and non-contingent with behavior. Nicotine administration also led to a modest improvement in performance. We combined optogenetic stimulation with fluorescent ACh sensor recordings to compare differences in endogenous and evoked ACh release. In conclusion, BLA ACh signaling is important for cue-reward learning but the precise relationship between timing of release and the effect in the BLA of this cholinergic signaling remains to be determined.

Disclosures: R.B. Crouse: None. **K. Kim:** None. **H.M. Batchelor:** None. **E.M. Girardi:** None. **R. Kamaletdinova:** None. **J. Chan:** None. **P. Rajebhosale:** None. **S.T. Pittenger:** None. **L.W. Role:** None. **D.A. Talmage:** None. **M. Jing:** None. **Y. Li:** None. **X. Gao:** None. **Y.S. Mineur:** None. **M.R. Picciotto:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.07

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDDK Grant R01DK106229

Title: Trans-synaptic Investigation of Upstream Regulators of the Mesolimbic Pathway

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Abstract: The mesolimbic pathway is well-characterized as an important mediator of motivated behavior for substances of abuse, highly palatable food, and other reinforcers. However, it remains unclear which specific neurons project onto the mesolimbic pathway. Typically, mono-synaptic (i.e., region A to region B) viral tracing techniques have been utilized to investigate mesolimbic neurocircuitry. Utilizing novel trans-synaptic viral techniques, in which at least three brains regions can be mapped concurrently (i.e., region A to region B to region C), we aim to trace upstream brain regions acting as regulators of the ventral tegmental area (VTA) to nucleus accumbens (NAc) mesolimbic pathway. We used stereotaxic surgical methods to microinject a retrograde adeno-associated virus (AAV) 6 expressing the avian derived receptor TVA (CMV AAV6 TVA mcherry) into the nucleus accumbens of 11-week-old C57BL/6 male mice. After adequate expression of AAV6, we injected a G-protein deleted rabies virus enveloped by the avian sarcoma leukosis virus receptor (EnvA G deleted GFP Rabies) into the VTA. Utilizing immunohistochemical studies, we observed appropriate mcherry AAV6 expression at the primary site of infection, the NAc, and expression of GFP rabies in the VTA. Rabies expression was also identified in the lateral hypothalamus, periventricular region of the hypothalamus, sub paraventricular region of the hypothalamus, nucleus accumbens, and infralimbic cortex indicating that these brain regions project directly onto the VTA to NAc pathway. These results demonstrate successful neuro-tracing of inputs into the mesolimbic pathway. This study highlights neuroanatomical evidence of upstream regions that may have modulatory effects specific for directing mesolimbic activity and function. This viral-tracing strategy supports the elucidation of inputs more broadly into neural pathways instead of individual brain regions.

Disclosures: K.O. Ogunseye: None. **J. Kasper:** None. **J. Hommel:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.08

Topic: G.02. Reward and Appetitive Learning and Memory

Support: FWO research grant 1519516N

Title: Deletion of the glycine alpha 2 receptor enhances dopaminergic modulation of striatal MSN activity.

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Abstract: The cortico-mesolimbic circuit comprises cortical glutamatergic and midbrain dopaminergic projections to the striatum, and integration of these inputs underlies adequate reward-motivated behaviour. We have earlier demonstrated that the homomeric glycine alpha 2

receptor (GlyRa2) plays a crucial role in the development of the cortico-mesolimbic circuitry: GlyRa2 knockout mice exhibit impaired proliferation and migration of cortical layer 5 projection neurons as well as striata medium spiny neurons, a shift in the excitation/inhibition balance of cortical projection neuron activity, and altered cortical input to the striatum at adult age. We next demonstrated that dopamine neuron basal as well as burst firing is unaltered in GlyRa2 knockout animals. Nonetheless, we also described changes is cortico-mesolimbically orchestrated behaviour. More specifically, we showed an exaggerated appetitively motivated response and enhanced increase in motor activity in response to d-amphetamine in GlyRa2 knockout mice, while leaving baseline motor activity unaltered. This led us to hypothesise that the GlyRa2 is crucial to integration of cortical and midbrain inputs to the striatum. To address this hypothesis, we performed whole cell patch clamp recordings on striatal medium spiny neurons. We elicited cortical currents using electrical stimulation, and triggered dopamine release to medium spiny neurons by optogenetic stimulation of dopaminergic terminals. We report enhanced dopaminergic modulation of activity of putative D1 MSNs in GlyRa2 knockout animals, compared to wildtype littermates. Next, to verify whether this enhanced dopaminergic modulation induces an increase in striatal activity in vivo, we measured expression of immediate early gene cFos, a commonly used indicator of neuronal activity, in response to d-amphetamine treatment. In agreement with the electrophysiological recordings do we report that d-amphetamine increases cFos-positive cells to a greater extent in GlyRa2 knockout animals. Moreover, 3-D processing revealed alignment of c-Fos-positive cells in strings that were longer after amphetamine stimulation. Taken together, we demonstrate a crucial role for GlyRa2 in signal integration in the adult striatum, which renders it an interesting target for the treatment of pathologies that are characterized striatal signal integration impairment, such as psychosis and drug addiction.

Disclosures: J. Devoght: None. **J. Rigo:** None. **E. Piccart:** None. **M. Van de Ven:** None. **B. Brone:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.09

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Wellcome 202831/Z/16/Z
Wellcome 214314/Z/18/Z
Wellcome 215198/Z/19/Z

Title: Internal models of task structure shape mesolimbic and dorsomedial striatal dopamine

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Abstract: The world is in continuous change, requiring animals to flexibly adapt their actions to new situations. It is thought that dopamine activity shapes flexible behaviour by conveying a reward prediction error (RPE) signal, used to update value estimates when predicted and actual outcomes differ. However, a key open question concerns what sources of value information inform dopaminergic RPE's. Specifically, we wanted to determine 1) whether whether dopamine activity is shaped by model-based computations using knowledge of the task structure in tandem with model-free updates, and 2) whether these signals are transmitted uniformly across projection targets in striatum. To address these questions, we trained mice on a probabilistic multi-step decision making task and recorded calcium activity in dopamine neuron cell bodies and axons, and dopamine release in striatal subregions, using fibre photometry (18 mice, 512 sessions, 184,645 trials). Mice chose between two first-step actions which led probabilistically to two second-step states where reward could be obtained. Reward probabilities in the second-step state were anticorrelated. We observed signatures of task structure knowledge in both mouse choice behaviour and dopamine signalling. When animals made a first-step choice, dopamine in the ventral tegmental area (VTA) and the nucleus accumbens (NAc) were modulated by both a 'model-free' component that directly reflected the previous outcome for a prior first-step choice, and a 'model-based' component that respected both the anti-correlated reward probabilities and transition probabilities mapping first-step actions to second-step states. At the same time, dopamine activity in dorsomedial striatum (DMS) was shaped by the model-based component, but not by the model-free one. When the second-step state was revealed, dopamine activity and release in the three regions was not only positively modulated by previous reward from the same second-step, but also negatively modulated by previous reward from the other second-step. This was consistent with behavioural evidence that the mice inferred a single latent variable that controlled both reward probabilities (i.e., that if one second-step state had high reward probability, then the other had a low probability); surprisingly, though, they updated this estimate using only rewards, and not reward omissions. In addition, dopamine signalling was influenced at different timepoints and timescales by other task factors such as reward rate, state transition likelihood and movement. Together, our results demonstrate heterogeneous influences on dopamine activity and release across projection regions.

Disclosures: **M. Blanco-Pozo:** None. **T. Akam:** None. **T.E.J. Behrens:** None. **M.E. Walton:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.10

Topic: G.02. Reward and Appetitive Learning and Memory

Support: RO1-DK111475
NIH Grant P30-DK020572

Title: Divergent effects of Orexin and Melanin Concentrating Hormone optogenetic stimulation on cue potentiated feeding

Authors: *J. R. LEE, L. RAYCRAFT, N. RUSSELL, A. JOHNSON;
Michigan State Univ., East Lansing, MI

Abstract: Divergent Effects of Orexin and Melanin Concentrating Hormone Optogenetic Stimulation on Cue Potentiated Feeding

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Environmental factors such as food related cues can stimulate eating in the absence of metabolic need, a behavior characterized as cue potentiated feeding (CPF). The feeding signals, Orexin (ORX) and Melanin Concentrating Hormone (MCH) are expressed in the lateral hypothalamic area (LHA) and play a critical role in regulating appetite. Chronic pharmacological or genetic knockdown of these neuropeptides prevents CPF, however the protracted timeframes underlying these perturbations constrain resultant interpretations. In the current study, we used acute optogenetic stimulation to determine with high temporal and spatial resolution, the role of ORX and MCH in CPF. Male and female transgenic ORX or MCH Cre-expressing mice were bilaterally injected with a Cre-dependent Channelrhodopsin virus into the LHA, thus permitting cell-specific activation with blue light stimulation. Mice were initially trained in Pavlovian Conditioning sessions to associate one auditory CS+ with the delivery of a sucrose solution and a different auditory CS- with no reward. Following satiety treatment, mice underwent CPF, during which they had unrestricted access to sucrose and received separate presentations of the CS+ and CS- cues, under stimulated (i.e., laser on) and non-stimulated (i.e., laser off) conditions. Primary results indicate that optogenetic stimulation in MCH-Cre mice facilitated CPF as indicated by increased rate of licking specifically during CS+ trials. Surprisingly, optogenetic stimulation in ORX-Cre attenuated licking for sucrose during CPF. However, an increase in food-seeking behavior was observed as indicated by elevated rate of entry to the area where sucrose was delivered. Thus, using acute optical stimulation we revealed ORX is critical for facilitating food-seeking and inhibiting overeating, whereas MCH promotes overeating behaviors during CPF. Therefore, these cell populations may facilitate the invigoration of feeding behavior through contrasting mechanisms. These results are discussed within a context of dynamic interactions between ORX and MCH LHA cells, which allow for rapid transitions between different appetitive states and could provide insight as to the neural mechanisms of overeating in our current obesogenic environment.

Disclosures: J.R. Lee: None. **L. Raycraft:** None. **N. Russell:** None. **A. Johnson:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.11

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R01 DA046457

Title: Afferents to dorsomedial and dorsolateral striatum in the rat

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Abstract: The dorsal striatum is known for its role in learning and motor processes. Within the dorsal striatum, there are two functionally distinct subregions: dorsomedial striatum (DMS) and dorsolateral striatum (DLS). DMS is integral for guiding goal-directed behavior, while DLS is recruited for habitual behavior. Given the divergence in functions of these subregions, we wanted to fully characterize the brain regions that project to DMS versus DLS with the aim of comparing their circuitry. Specifically, we examined striatal afferents by injecting retrograde tracers (cholera toxin β-subunit or Fluorogold) into DMS or DLS of male Sprague Dawley rats. We characterized the resulting retrograde labeling qualitatively as heavy, medium, or light based on the amount of labeled neurons per brain structure. We observed that both DMS and DLS received heavy input from cortex and thalamus, but there were notable differences in the pattern of labeling within these areas. Cortical afferents to DMS were primarily restricted to the medial prefrontal cortex, including medial and ventral orbitofrontal cortex (OFC), prelimbic cortex, and cingulate cortex. In contrast, cortical afferents to DLS were primarily restricted to lateral areas in frontal cortex, including frontal association cortex, primary and secondary motor cortices, and lateral OFC. DLS also received cortical inputs from primary and secondary somatosensory cortices and insular cortex. In terms of thalamic afferents, both DMS and DLS received input from central medial thalamic nucleus, paracentral thalamic nucleus, parafascicular nucleus, and portions of the ventral thalamic nuclei. Thalamic inputs differed for DMS and DLS in terms of density and topography across medial-lateral and anterior-posterior axes. Finally, both DMS and DLS received medium to light input from amygdala, substantia nigra, and dorsal raphe. Altogether, these data reveal that DMS and DLS share some common inputs, but that there are striking differences in cortical and thalamic inputs to DMS and DLS that may be critical to their distinct roles in goal-directed and habitual behavior.

Disclosures: S.N. Handel: None. **R.J. Smith:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.12

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R00 DA042895
NIH Grant T32 DA007234

Title: The role of superior colliculus inputs to the ventral tegmental area in appetitive Pavlovian conditioning

Authors: *C. L. POISSON, C. R. HERUBIN, B. T. SAUNDERS;
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Abstract: Appetitive Pavlovian conditioning relies on associations made between sensory stimuli ("cues") and rewarding outcomes. In our previous studies, we have shown that optogenetic activation of dopamine (DA) neurons in the ventral midbrain in the presence of a neutral visual cue is sufficient to create a Pavlovian association that promotes conditioned behavior in response to that cue (Saunders et al., 2018). Critically, the circuit mechanisms underlying the integration of cue-related sensory input into DA centers to form Pavlovian associations remain unclear. One candidate structure that could provide visual information about cues is the superior colliculus (SC), which receives direct retinal input, and sends projections to DA neurons in the ventral tegmental area (VTA). The SC is known to be necessary for cue-evoked neural activity in DA neurons, but the behavioral function of SC-VTA neurons is unclear. Here, we characterize the SC-VTA circuit and use optogenetics to modulate this pathway's activity during Pavlovian conditioning in rats. First, we used a dual-virus approach to determine which SC neurons innervate the VTA. This revealed projections from deep-layer SC that largely innervate the dorsal and lateral VTA. To manipulate activity of the SC-VTA pathway, we injected viruses expressing excitatory (ChR2) or inhibitory (eNpHR) opsins into deep layer SC and implanted an optic fiber over VTA. We next trained rats in a Pavlovian conditioning procedure wherein visual cues were paired with optogenetic manipulation of SC-VTA terminals. Behavioral analysis revealed that excitation of these neurons failed to drive conditioned (i.e., learned) behavior towards the cue, in contrast to our previous studies targeting DA neurons directly. Notably, a subset of ChR2 rats demonstrated turning behavior. Turning was contralateral to stimulation hemisphere, and time-locked to laser duration. This suggests that the SC-VTA neurons can drive movement invigoration, presumably via their activation of DA neurons, but do not directly cause Pavlovian learning. In ongoing experiments, we are testing the working hypothesis that subsets of SC inputs to different DA populations may invigorate actions, and/or alter attention to relevant sensory information during learning.

Disclosures: C.L. Poisson: None. **C.R. Herubin:** None. **B.T. Saunders:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.13

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA Grant R01DA044761
NIMH Grant R01MH115604

Title: Neuroanatomical characterization of cerebellar inputs to the dopaminergic system

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Abstract: The emerging view of the cerebellum is in agreement with it prominent role in cognition in addition to motor control. Indeed, cerebellar impairment is implicated in a wide

variety of disorders, ranging from motor dysfunction such as dystonia or ataxia, or mental disorders like schizophrenia and autism. Recent work of our laboratory have described two underappreciated cerebellar pathways by which the cerebellum sends monosynaptic excitatory projections to the midbrain dopamine system; the substantia nigra pars compacta (SNc), and the ventral tegmental area (VTA). We have found that the cerebellum drives the activity of SNc and VTA neurons, producing dopamine release in downstream regions in the dorsal striatum and medial prefrontal cortex (mPFC), respectively; suggesting that the cerebellum might play a role in motor and cognitive functions through dopamine modulation. Despite the physiological characterization of these cerebellar pathways, detailed characterization of the connectivity of the neuronal sub-populations involved remains to be established. Using a combination of intersectional approaches with anterograde and retrograde tracers we characterized the neuroanatomical distribution, and the heterogeneity of the sub-circuits involved in these pathways. We injected AAV.Cre virus in the deep cerebellar nuclei (DCN) of *RCE* mice (harboring a R26R CAG-boosted EGFP reporter allele with a *loxP*-flanked STOP cassette upstream of the EGFP gene), to identify all the postsynaptic targets of the cerebellum through EGFP expression. Histological analysis of midbrain showed that the cerebellum targets TH-positive (presumed dopaminergic) and TH-negative neurons along the entire SNc and VTA. To identify the spatial distribution of these cerebellar inputs, we performed retrograde tracing using G-deleted rabies virus in DAT-Cre and GAD-Cre mice. We found that all three cerebellar nuclei (lateral, interposed and medial) contribute to these projections. Similar experiments complementing viral tracing with retroAAV virus from mPFC showed that a sub-population of the neurons in the VTA receive cerebellar inputs, and project to cortical areas, supporting the presence of a disynaptic cerebellar projection to the mPFC via the VTA. Characterization of the neuroanatomy and connectivity of the sub-circuits is essential if we are delineating the function and contribution of the cerebellum to dopamine release and cognitive processes.

Disclosures: **M. Oñate:** None. **V. Lovallo:** None. **N. Wazeed:** None. **J. Vera:** None. **K. Khodakhah:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.14

Topic: G.02. Reward and Appetitive Learning and Memory

Support: RF1AG060778

Title: Investigating contributions of the ventral tegmental area to age differences in risky decision-making

Authors: *W. S. PYON, S. L. BLAES, C. A. ORSINI, R. J. DRAGONE, B. SETLOW, J. L. BIZON;
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Abstract: Decision-making is a complex cognitive operation that relies on the concerted effort of multiple brain regions. As-of-yet unidentified changes in these circuits as a result of age will eventually cause many of us to undergo a fundamental shift in the way we make decisions, shifting us away from riskier choices towards safer outcomes. This alteration in decision-making has been further corroborated in rats performing a risky decision-making task, in which given the option between a small, "safe" food reward or a large food reward accompanied by varying probabilities of a mild footshock, aged rats are more risk-averse than their young adult counterparts. To begin to determine the mechanisms underlying this age-related shift toward risk-aversion, and more specifically, the contributions of dopamine signaling from the ventral tegmental area (VTA), we took a two-part approach. First, to determine how VTA dopamine neurons contribute to risky decision-making, we employed optogenetics in young rats to selectively inhibit VTA neurons during performance of the risky decision-making task. Optogenetic inhibition of VTA dopamine neurons specifically during receipt of the large reward on trials when it was unpunished caused young rats to exhibit more risk-averse behavior in future trials. Second, to begin to determine how age differences in VTA activity might contribute to risky decision-making, we investigated age differences in functional connectivity among various brain regions, including the VTA. Young (9 months old; n = 15) and aged (24 months old; n = 9) rats were trained on the risky decision-making task. After reaching stable performance, rats were anesthetized and placed in an 11.1 Tesla Bruker MRI Scanner in which anatomical and resting state functional MR imaging was performed. Preliminary analyses comparing functional connectivity in young and aged rats indicate a significant reduction in correlated activity between the VTA and the basolateral amygdala in aged rats. Ongoing analyses are determining relationships between individual risk preference and functional connectivity in these and other brain regions implicated in risky decision-making.

Disclosures: W.S. Pyon: None. **S.L. Blaes:** None. **C.A. Orsini:** None. **R.J. Dragone:** None. **B. Setlow:** None. **J.L. Bizon:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.15

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH R15 MH114026

Title: Sex- and duration-dependent neural circuit control of voluntary exercise behavior

Authors: *M. K. TANNER[1], K. BONAR[2], N. A. MOYA[1], A. A. HOHORST[1], J. K. P. DAVIS[2], J. JAIME[2], E. C. LOETZ[2], B. N. GREENWOOD[2];
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Abstract: Incidence rates of stress-related psychiatric disorders are increasing and affect females at nearly twice the rate of males. Exercise can protect against the development of stress-related

disorders, however, participation in regular exercise is decreasing. Dopamine (DA) and the dorsal striatum (DS) are critical for movement, however, the specific DA-striatal circuits that motivate voluntary exercise are unknown. Identifying the neural circuits that motivate voluntary exercise in both sexes could lead to novel strategies to increase exercise participation and reduce the effects of stress. Rats given access to running wheels demonstrate robust voluntary exercise behavior that follows a pattern of two phases consisting of an acquisition phase, during which nightly running escalates, followed by a maintenance phase, during which running distance plateaus. The goal of this study is to identify the DA-striatal circuits that support the different phases of voluntary wheel running in both male and female Long-Evans rats. Using a pharmacological inactivation technique, we temporarily inactivated two DS subregions during both phases of exercise. In males and females during phases of the estrous cycle other than proestrus, inactivation of the dorsomedial striatum (DMS), a region important for goal-oriented behavior, reduced running during the acquisition phase, but not the maintenance phase. In contrast, inactivation of the dorsolateral striatum (DLS), which is a region important for habit formation, reduced running during the maintenance phase, but had no effect on running during the acquisition phase. Interestingly, we observed that females in proestrus, the phase of the estrous cycle when estrogen is the highest, rely on the DLS to support the acquisition of exercise behavior, rather than the DMS. Using fluorescent in situ hybridization, we quantified the activation of DMS and DLS neurons expressing D1 receptors and found that females activate the DLS during the acquisition phase of exercise, while males do not. These data suggest that the neural circuits supporting voluntary exercise behavior depend on both the phase of exercise and sex. Different striatal subregions could be targets for manipulations aimed at enhancing the initial acquisition vs. long-term maintenance of exercise behavior. Additionally, we used immunohistochemistry to stain for cFos+ cells to verify our ability to successfully inhibit only our region of interest and also to quantify neural activity in the DMS and DLS during the maintenance of exercise. Preliminary data suggests that inactivating the DLS of females during the maintenance of exercise decreases cFos+ cells only in the DLS.

Disclosures: **M.K. Tanner:** None. **K. Bonar:** None. **N.A. Moya:** None. **A.A. Hohorst:** None. **J.K.P. Davis:** None. **J. Jaime:** None. **E.C. Loetz:** None. **B.N. Greenwood:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.16

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Differential activation of dopaminergic signaling pathways following right and left vagus nerve stimulation

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Abstract: Vagus Nerve Stimulation (VNS) is an FDA approved therapy for the treatment of epilepsy and major depression. Vagus nerve cuff electrodes are traditionally placed around the left cervical branch of the vagus nerve, due to concerns that stimulation of the right vagus nerve is more likely to lead to adverse cardiac effects. Preclinical studies, however, suggest that cardiac side effects are no more likely to result from right VNS than from left VNS.
A recent study by Han et al. (2018) suggests that stimulation of the right vagus nerve is likely to produce a pattern of activation within the CNS that is distinct from that produced by left VNS. The authors report that optogenetic stimulation of gut-projecting vagal neurons in the right nodose ganglion (NG) led to greater activation of noradrenergic neurons in the locus coeruleus (LC) than did left NG stimulation, and only right NG stimulation led to activation of midbrain dopaminergic neurons in the substantia nigra pars compacta (SNc). Based on this work, we hypothesized that right cervical VNS delivered using traditional cuff electrodes should similarly result in stronger engagement of midbrain dopaminergic circuits compared to left cervical VNS. To begin to test this hypothesis, we examined whether rats would self-stimulate for VNS delivered on either the right or the left side. Fourteen adult female Long-Evans rats were randomly assigned to either the right (Group 1, n = 7) or left (Group 2, n = 7) VNS treatment group. After an initial acclimation session in which lever pressing was paired with food reward, rats received 5 days of self-stimulation sessions in which lever pressing was paired with a brief (0.5 sec) train of VNS pulses (0.8 mA, 30 Hz) and a visual cue. Rats then received 5 extinction sessions in which neither cues nor VNS were delivered. Group 1 rats pressed significantly more than Group 2 rats across all 5 days of self-stimulation and during the first two days of extinction. Extinction was followed by one session of cue-only reinstatement (no VNS), and then one final session in which both the cue and VNS delivery were reinstated. During these sessions, cue and VNS delivery for Group 2 rats was yoked to rats in Group 1, so that both groups received equal amounts of stimulation. Animals were sacrificed 90 minutes after the final stimulation session. Brain slices containing the LC, SNc, and ventral tegmental area (VTA) were made and stained for *cfos* expression. Preliminary data from ongoing analyses suggest that right VNS may result in increased TH+ VTA neuron activation compared to left VNS. Taken together these findings suggest a unique role of right cervical vagal fibers in activating dopaminergic signaling pathways.

Disclosures: J. Brougher: None. **C.A. Thorn:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.17

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R37 MH0588833
NSF PIRE IPAN AWARD 15-PAF06754

Title: Prelimbic neurons signal approach-avoidance conflict

Authors: G. ROJAS-BOWE, A. CABAN-MURILLO, *H. BRAVO-RIVERA, S. N. AYALA-ROSARIO, J. PEREZ-TORRES, V. P. VALENTIN-VALENTIN, A. RIVERA-RIVERA, C. BRAVO-RIVERA, G. J. QUIRK;
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Abstract: In nature, animals must carefully evaluate when to approach rewards or avoid threats in risky environments. The neural mechanisms that underlie the resolution of approach/avoidance conflict remain unknown. We recently developed an approach/avoidance conflict task (Bravo-Rivera et al., in review) that pits food reward approach against threat (foot-shock) avoidance. In brief, male rats learn to avoid a tone-signaled foot-shock by stepping onto a platform and to seek a light-signaled reward by pressing a lever (located far from the platform). When the tone and light cues are co-presented, rats initially engage in lever pressing and then move onto the platform at the late phase of the trial to timely avoid the foot-shock. Pharmacological inactivation of the prelimbic (PL) cortex accelerated cue-induced avoidance, suggesting that PL delays avoidance to accommodate food-seeking under conflict conditions ($t_{(18)} = 2.71$, $p = 0.014$). Single-unit recordings revealed that populations of PL neurons distinguished the combined tone and light (conflict trials) stimulus from the tone-alone and light-alone (non-conflict) stimuli: 26.5% of all responsive cells (n = 207) responded exclusively to conflict trials, whereas 21.4% exclusively responded to the tone-alone trials, and 15% responded to the light-alone trials exclusively. Moreover, a subset of PL neurons signaled lever presses during the conflict trials, but not during the light-alone trials, whereas another subset displayed opposite responses. Whereas 28.8% of all responsive cells (n = 208) responded exclusively to pressing within the conflict trials, 38.0% exclusively responded to pressing within the light-alone trials. Furthermore, whereas a subset of PL neurons (47.4% of 78 responsive cells) signaled platform mounts occurring exclusively under conflict trials, another subset (37.2%) exclusively responded to mounting under the tone-alone trials. These findings suggest that PL signals conflict-relevant stimuli and the appropriate behavioral response.

Disclosures: G. Rojas-Bowe: None. **A. Caban-Murillo:** None. **H. Bravo-Rivera:** None. **S.N. Ayala-Rosario:** None. **J. Perez-Torres:** None. **V.P. Valentin-Valentin:** None. **A. Rivera-Rivera:** None. **C. Bravo-Rivera:** None. **G.J. Quirk:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.18

Topic: G.02. Reward and Appetitive Learning and Memory

Support: CIHR FDN-147473

Title: Diet induced obesity induces neuroinflammation in the lOFC

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Abstract: The lateral orbitofrontal cortex (lOFC) receives sensory information about food and integrates these signals with expected outcomes to guide future actions, and thus may play a key role in a distributed network of neural circuits that regulate feeding behaviour. An obesogenic diet induces microglial activation and inflammatory markers in hypothalamus and hippocampus[1] and induces cognitive dysfunction. However, so far, no studies have explored the effects of diet-induced or obesity induced neuroinflammation in the OFC. We hypothesized that access to an obesogenic diet produces neuroinflammation and synaptic changes in the OFC. Mice had either a 7-day, a 3-month exposure to a high fat diet (HFD) or chow. We used immunohistochemistry to identify markers of microglia and astrocytes and used slice electrophysiology to record neuronal activity in the OFC. Weight gain was significantly greater in mice exposed to HFD for 3 months compared to 7days. The expression of the microglial marker, Iba-1 was increased in the lOFC of 3-month, but not 7-day exposed mice. Furthermore, the number of cells expressing Iba-1 was significantly greater in 3-month than 7-day HFD exposed or chow fed mice. Expression and number of cells expressing of the astrocyte marker, S100, was increased in 3-month but not 7-day HFD exposed or chow fed mice. Both 7-day and 3-month exposure to a high fat diet suppressed inhibitory synaptic transmission onto lOFC neurons and increased their excitability. Finally, while short term exposure to a high-fat diet does not alter goal-directed behaviour, goal directed behaviour was impaired in obese mice. Taken together, synaptic changes in the lOFC can occur early on in diet exposure, inflammatory mechanisms appear at later stages of diet exposure. Thus, future work will assess how inflammatory mechanisms in the lOFC during obesity influence goal directed behaviours.

Disclosures: L.T. Seabrook: None. **T. Kenney:** None. **T. Tayyab:** None. **M. Sobey:** None. **M. Qiao:** None. **S. Borgland:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.19

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Natural Sciences and Engineering Research Council of Canada Discovery Grant 2018-04401 to QY
Faculty of Medicine Dean's Fellowship Award 2019 to KP

Title: Firing mode-specific functions of the locus coeruleus: heterogeneity within the basolateral amygdala

Authors: *K. POWER, A. GHOSH, T. OMOLUABI, T. SEPAHVAND, C. REINHARDT, S. TORRAVILLE, Q. YUAN;
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Abstract: The locus coeruleus (LC) is a noradrenergic brainstem nuclei with widespread projections that allow it to be involved in a variety of functions such as wakefulness, arousal, learning, and the stress response. The LC has two modes of activity (firing), phasic and tonic, which have traditionally been understood to be involved in optimal task performance and disengagement from tasks respectively. The LC has long been thought to operate as a homogeneous unit that is activated as a whole in order to release noradrenaline in a spatially non-specific manner. Recent advances in technology have allowed us to develop a more nuanced understanding of LC function, indicating the role of heterogeneity or modular organization at both the LC and its downstream projection sites. Recent work has implicated the role of phasic and tonic LC activity in the signaling of positive and negative valence respectively. The basolateral amygdala (BLA) receives dense innervation from the LC and is likewise involved in positive and negative valence via two distinct subpopulations. We hypothesized an interaction between modes of LC activation and the activation of these two subpopulations of the BLA; more precisely, we hypothesized that phasic and tonic LC activation would bias activation towards the nucleus accumbens (NAc)- and central amygdala (CeA)-projectors of the BLA respectively. Adult tyrosine hydroxylase-Cre rats underwent surgeries to receive the light-sensitive ion channel channelrhodopsin-2 (ChR2) via viral vector AAV-DJ-EF1a-hChR2(H134R)-mCherry in the LC, an optic cannula in the LC for optostimulation, as well as the retrograde tracer cholera toxin subunit-B (CTB) in the NAc and CeA for the labelling of subpopulations within the BLA. Next, rats underwent phasic, tonic, or no optostimulation in the presence of a neutral odor before sacrifice and transcardial perfusion. Tissue from the BLA underwent cFos immunohistochemistry and was imaged for cFos and CTB overlap via fluorescence microscopy. Phasic and tonic LC optostimulation biased activation towards BLA NAc- and CeA-projectors respectively, while the no light control group showed no bias of activation towards either subpopulation. Further, analysis of the ventral tegmental area (VTA) revealed that phasic LC optostimulation caused greater activation of the VTA as well as greater activation of VTA NAc-projectors than tonic and no light control groups. We are currently investigating adrenergic receptor distributions in the BLA that might account for its LC firing mode-specific activation patterns. This work is furthering our understanding of the mechanisms underlying the firing mode-specific functions of the LC.

Disclosures: K. Power: None. **A. Ghosh:** None. **T. Omoluabi:** None. **T. Sepahvand:** None. **S. Torraville:** None. **Q. Yuan:** None. **C. Reinhardt:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.20

Topic: G.02. Reward and Appetitive Learning and Memory

Support: FRQS
CIHR
Concordia University
Center for Studies in Behavioral Neurobiology
Canada Research Chairs
FRQNT
Brain & Behavior

Title: Distinct neuronal ensembles within the central nucleus of the amygdala regulate extinction learning

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Abstract: Correlational data from histochemical and physiological studies suggest that the central nucleus of the amygdala (CN) is involved in learning when expected events are omitted. Attempts at delineating the causal contribution of CN neurons to this learning have targeted the entire nucleus indiscriminately, disrupting the function of neurons. Recent research using selective approaches have uncovered that not all neurons within a brain area are recruited during learning. Rather, a specific neuronal ensemble supports learning with distinct subsets of neurons likely having different functional roles. We sought to determine the functional role of CN neurons that have been explicitly activated by the omission of an expected reward. c-Fos is a widely used marker for neuronal activity and here, we used the Daun02 inactivation procedure to assess the causal role of *activated* c-fos-expressing CN neurons in updating reward expectations during extinction. In the present study, male *fos-lacZ* transgenic rats were trained to expect the delivery of a food reward upon the presentation of an auditory cue. Subsequently, rats received non-reinforced exposure to the reward-associated cue to generate conditions of reward omission, that is extinction, and examine the effect of this on learning. Cell inactivation with Daun02 took place ninety minutes following the start of the non-reinforced session, presumably when the neurons that detected the reward omission were activated and the corresponding c-fos levels were at peak. This led to disruption in behaviour indicative of impaired retrieval of the extinction memory compared to rats that received a vehicle infusion, which left those neurons intact. Additional data show that further extinction learning was retarded in the absence of the neuronal ensemble in the CN. Moreover, inactivating these extinction-responsive CN neurons resulted in greater spontaneous recovery and reinstatement. Lastly, this disruption in behaviour was specific to the CN and not due to drug diffusion into the basolateral amygdala.

Disclosures: B.P. Lay: None. **M.D. Iordanova:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.21

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIMH grant R01MH119511

Title: The Psychometric Properties of the Pavlovian Instrumental Transfer Task in an Online Adult Sample

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Abstract: How we decide to act is governed not only by the expected value of an action but also by the valence of its expected outcome (Guitart-Masip et al., 2014). Pavlovian biases are evident in our tendency to approach reward and inhibit action in the face of punishment, and variability in their expression is implicated in multiple psychiatric disorders including anxiety, schizophrenia, and addiction. Previous research (Moutoussis et al. 2018) has suggested that a popular measure of Pavlovian bias, the Pavlovian-instrumental transfer (PIT) task has unacceptable psychometric properties (e.g. low test-retest reliability), calling into question the task's utility for studying individual differences. However, this work measured behavior on the task in an adolescent sample over an 18-month period, raising the question whether the previous results reflect unreliability of the task or development-related changes in the sample.
To resolve this question, we conducted a longitudinal study of Pavlovian bias in an adult sample. We recruited 103 participants from Amazon Mechanical Turk to complete the PIT task in four separate sessions across 28 days. Participant retention in our sample was high with more than 85% of our sample completing at least 3 sessions. We used a hierarchical Bayesian framework (Rouder & Haaf, 2019) to estimate both the within-session stability and between-session test-retest reliability of the parameters from a standard reinforcement learning model (Guitart-Masip et al., 2014) of behavior on the task.
We replicated the classic behavioral findings such that participants on average exhibited greater accuracy in the Pavlovian congruent conditions (go to win and no-go to avoid losing) and worse accuracy in the Pavlovian incongruent condition (no-go to win and go to avoid losing). We found that the within-session stability across parameters was acceptable (mean = 0.689, range = [0.291, 0.852]). We also found that between-session test-retest reliability of parameters was unacceptable (mean = 0.197, range = [0.121, 0.325]). Test-retest reliability was unacceptable irrespective of session, and was driven by strong practice effects after the first session. Corroborating previous findings, we found that the commonly used Pavlovian Instrumental Transfer task has unacceptable test-retest reliability over a short timescale (4 weeks). Our results suggest that the task's poor psychometric properties are largely driven by practice effects. We conclude by proposing a new version of the task with a trial structure that should preclude task-set learning and thereby improve test-retest reliability.

Disclosures: **N. Paredes:** None. **S. Zorowitz:** None. **Y. Niv:** None.

Digital Abstract Session

P291. Neural Systems Implicated in Addiction

Program #/Poster #: P291.22

Topic: G.09. Drugs of Abuse and Addiction

Support: DHHS/NIH/NIDA/IRP

Title: Compulsive oxycodone intake increases phosphorylation of proteins of the mitogen-activated protein kinase/ mitogen-stress-activated (MAPK-MSK) signaling cascade

Authors: *M. T. MCCOY[1], C. A. BLACKWOOD[2], B. LADENHEIM[2], J. L. CADET[3]; [1]DHHS/NIH/NIDA/IRP, Baltimore, MD; [2]NIH/NIDA, Baltimore, MD; [3]Nih/nida/Intramural Res., Baltimore, MD

Abstract: The abuse of oxycodone leads to opioid use disorder (OUD). However, very little is known about the neurobiological effects that might lead to increased prevalence of addiction in humans. Previous reports from our lab and those of others have demonstrated that the potential involvement of the striatal mitogen-activated protein kinase/ mitogen-stress-activated (MAPK-MSK) signaling pathway in the biochemical effects of several drugs of abuse. As a first step towards identifying the molecular mechanisms involved in OUD, we have measured potential changes in the phosphorylation states of proteins involved in the MAPK-MSK pathway in the dorsal striatum of rats during early withdrawal from escalated oxycodone self-administration (SA). Male Sprague-Dawley rats were trained to press a lever to self-administer oxycodone or saline for one 3h training sessions during the first week followed by 6h sessions during the second week then 9h for the remainder of the SA experiment. At the end of the first week, animals were split into 3 groups: saline SA rats, short access (ShA) rats that took oxycodone for 3h, and long access takers (LgA) rats that took the drug for 6h-9h. At the end of oxycodone SA we isolated tissue from the dorsal striata to perform postmortem biochemical and molecular analyses. We found that rats given long access, but not short access, to oxycodone escalated their drug intake to differential degrees, with some rats (LgA-H) taking very large amounts of oxycodone whereas others (LgA-L) consumed significantly less drug. Biochemical analyses revealed that the LgA-H animals showed increased MSK1/2 protein phosphorylation. We also found increased phosphorylation of CREB and histone H3 proteins that are targets for MSK proteins. Moreover, quantitative PCR studies revealed increased striatal expression of AMPA receptor subunits. When taken together, these observations suggest that the consumption of high amounts of oxycodone can result in MSK1/2-dependent histone phosphorylation that is associated with increased mRNA expression of AMPA receptors. The identification of MSK1/2 signaling pathway as a target for oxycodone exposure may open up potential avenues for pharmacological interventions against opioid use disorder.
This work is supported by DHHS/NIH/NIDA/IRP.

Disclosures: M.T. McCoy: None. **C.A. Blackwood:** None. **J.L. Cadet:** None. **B. Ladenheim:** None.

Digital Abstract Session

P292. Physiology of Addiction Related Systems

Program #/Poster #: P292.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: TCU/SERC grant G-180614
TCU/SERC grant UG-180534

Title: Chemogenetic inhibition and excitation of globus pallidus neurons in reward downshift and open field tasks: Motor or emotional effects?

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Abstract: The role of the external globus pallidus (GPe) in the emotional and motor components of reward downshift (RD) and open field (OF) tasks was studied using chemogenetic manipulations. The RD task is a model of frustrative nonreward that suppresses licking after a downshift in sucrose concentration. The OF task assesses locomotion in anxiety-inducing illuminated arenas. Groups of rats received bilateral infusion of the inhibitory hM4D(Gi) or excitatory hM3D(Gq) designer receptors into the GPe. After recovery, animals received 5-min access to either 32% sucrose (inhibitory treatment) or 12% sucrose (excitatory treatment) on RD sessions 1-10, and 2% sucrose on sessions 11-14. On session 15, rats were exposed to a 15-min OF test. Vehicle or clozapine N-oxide (CNO) was administered 30 min prior to RD sessions 11-14 and OF test. Consistent with either a motor or an emotional effect on licking, GPe inhibition reduced licking after a 32-to-2% sucrose downshift, whereas GPe excitation increased licking after a 12-to-2% sucrose downshift in the RD task. However, even though total distance traveled in the OF test was not affected by either GPe inhibition or excitation, the typically higher activity in the periphery relative to the central area of the OF was either reduced under GPe inhibition or eliminated under GPe excitation. These results are more consistent with an emotional function, rather than a motor function of GPe neurons in these tasks.

Disclosures: **M.R. Papini:** None. **S. Guarino:** None. **C. Hagen:** None. **Q. Nguyen:** None.

Digital Abstract Session

P292. Physiology of Addiction Related Systems

Program #/Poster #: P292.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NSERC Discovery grant to XC (261384-2008)
NSERC Discovery grant to QY (RGPIN-2018-04401)

Title: Locus Coeruleus activation patterns differentially modulate exploratory behavior and olfactory discrimination learning

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Abstract: Locus coeruleus (LC) is the main source of forebrain norepinephrine. LC neurons are known to spike in phasic and tonic patterns. Theories suggest distinct functional roles for two activity patterns- high frequency phasic activity (10-20 Hz) is related to focused attention and cortical encoding of salience, whereas tonic activity is related to quiet wakefulness at low frequency (0.5-2 Hz) and anxiety at high frequency (5-10 Hz). In this study we address how different LC activation patterns differentially modulate associative learning in a food-reward based go/no go odor discrimination paradigm. We also examine the roles of LC activation patterns in general exploratory behavior. We infused TH-cre rats with AAV-DJ-EF1a-DIO-hChR2(H134R)-mCherry in the LC (control rats were infused with AAV-DJ-EF1a-DIO-mCherry), followed by bilateral optical cannular implantation 3 weeks later. Photostimulation of the LC with 10-Hz phasic (either 300 msec on, 1700 msec off; or 10 sec on, 20 sec off), 10 Hz tonic and 25 Hz tonic patterns was carried out during behavioral tests. Our results show that exploratory behavior was enhanced by phasic stimulation and greatly reduced by 25 Hz tonic photostimulation. Both phasic patterns facilitate discrimination learning of similar odors, but not dissimilar odors, in a food retrieval paradigm. This is congruent with previous finding that NE enhances pattern separation and plasticity in the piriform cortex (PC). Pharmacological blockade of adrenoceptors (with phentolamine and alprenolol) in PC impaired similar odor discrimination learning regardless of photostimulation. In contrast, D1 receptor antagonist (SCH23390) infusion in the PC prevented LC photostimulation induced learning facilitation. Similar results were observed when ventral tegmental area (VTA) was blocked with local lidocaine infusion, indicating an LC-VTA-PC dopamine circuitry being responsible for the phasic pattern mediated facilitation. 10 Hz tonic photostimulation did not change odor discrimination learning. VTA immunohistochemical experiments showed higher cFos activation in TH+ neurons following phasic, but not tonic stimulation, further supporting the involvement of the LC-VTA dopamine circuit. Overall, our results indicate that LC firing patterns critically influence general and discrimination behavior through engaging differential down-stream circuitry.

Disclosures: **A. Ghosh:** None. **F. Massaeli:** None. **K.D. Power:** None. **T. Omoluabi:** None. **S.E. Torraville:** None. **J.B. Pritchett:** None. **V.D. Strong:** None. **C. Reinhardt:** None. **X. Chen:** None. **G.M. Martin:** None. **C.W. Harley:** None. **Q. Yuan:** None.

Digital Abstract Session

P292. Physiology of Addiction Related Systems

Program #/Poster #: P292.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: DGAPA-PAPIIT IA201420

Title: Functional role of insular cortex in the establishment of taste preference conditioning

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Abstract: Taste recognition is essential for animal's survival, this ability allows them to identify between nutritious and toxic substances. The establishment of taste preferences has an important adaptive role, since the organisms have to recognize tastants that are related to positive postingestive effects. There is scarce information about the brain structures involved in exteroceptive and interoceptive information processing necessary for the establishment of conditioned taste preference (CTP). However, it has been proposed that the insular cortex (IC) processes information about bodily states, including visceral and taste modalities. Importantly, the IC also plays a key role in the acquisition and consolidation of recognition memories through glutamate-dependent mechanisms. In this regard, the aim of this study is to assess whether the IC has a functional role during the establishment of taste preference memories. We evaluated the effects of NMDA receptor antagonist (APV) into the insular cortex on CTP establishment. Accordingly, bilateral guide cannulae were implanted on the IC in male Wistar rats. After recovery, rats were water deprived for 24 hours and baseline consumption were calculated for three days. On the acquisition day, the animals were given two bottles of 30 mL of saccharin (0.3%) and allowed to drink for 15 minutes, 30 minutes later, APV (0.1 µl) or SS was injected into the IC and afterwards animals were injected i. p. with glucose (350 mg/kg). Long-term memory was evaluated 72 hours later in a two-bottle session test. Our results demonstrate that NMDA receptors blockade within the IC hinders CTP establishment. Additionally, we evaluated whether the blockade of NMDA receptors impairs memory acquisition or consolidation processes; to achieve this, we administered APV or SS after the acquisition session; specifically, animals were allowed to drink saccharin (0.3%) from two bottles of 30 mL and injected i.p. with glucose, 30 minutes later APV or SS was administered into the IC. Short-(4 hrs) and long-(72 hrs) term memory were assessed. Results show that NMDA receptors blockade within the IC impairs long-term memory but spares short-term memory. Our results demonstrate that the IC plays a key role in CTP establishment through NMDA receptors activity, particularly the activation of NMDA receptors is related to the consolidation of taste preference memories.

Disclosures: K.G. Medina-Medina: None. **B. Linares-Caballero:** None. **D. Osorio-Gómez:** None. **F. Bermúdez-Rattoni:** None.

Digital Abstract Session

P292. Physiology of Addiction Related Systems

Program #/Poster #: P292.04

Topic: G.02. Reward and Appetitive Learning and Memory

Support: JSPS KAKENHI 18K03182
JSPS KAKENHI 24530917

Title: Effects of reward delays on sign- and goal-tracking behaviors: A preliminary study on changes in response frequency on the time course of reward delay

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Abstract: In instrumental learning situations where an anticipated reward presentation is delayed from a correct behavioral response to acquire a reward, various behaviors that do not directly contribute to appetitive results are observed. Of these behaviors, we determined how frequencies of sign-tracking and goal-tracking behaviors change depending on the reward delay's time course. We measured lever-pressing and nose-poke responses to acquire a pellet in a Skinner box by using seven well-trained male C57BL/6N mice under delay lengths of 0 (no delay), 10, 20, 30, and 40 seconds from pressing the lever to the presentation of a pellet. The frequency of lever-pressing and nose-poke responses at the second, third, and fourth 10-second intervals within the five reward delays was estimated by calculating the difference between the number of these responses observed in the trials with 10 and 20 seconds, 20 and 30 seconds, and 30 and 40 seconds delay lengths. The results showed that nose-poke responses appeared most frequently at the fourth 10-second interval than the former three intervals, while lever-pressing responses were more frequent at the first and second intervals than at the latter two intervals. Data analyses made using Kendall's rank correlation also indicated that the estimated number of lever-pressing responses at the second 10-second interval was negatively correlated with that of the lever-pressing responses at the third (r = -0.68, p = 0.033). However, the former correlated positively with the estimated number of nose-poke responses at the fourth interval (r = 0.78, p = 0.015). This suggests that the animals that pressed the lever more frequently during the earlier period showed the same response less frequently later, followed by a higher increase in nose-poke responses. A transition process from sign tracking to goal tracking when withholding a reward could be affected by the response frequency of a frustrated animal in the earlier period. On the other hand, the total number of correct responses to acquire a pellet, as an index of performance of learning in this task, appeared to be related to neither the frequency of the lever-pressing nor nose-poke responses in the second, third, and fourth 10-second intervals (r < 0.40). A medium but insignificant correlation (r = 0.52, p = 0.099) was identified between the total of correct responses and that of the lever-pressing response in the first 10-second interval, suggesting that learning performance in the whole session may be moderately related to the number of lever-pressing responses in earlier but not in later intervals. This work was supported by JSPS KAKENHI #24530917 and #18K03182.

Disclosures: T. Sato: None. **T. Yamakuni:** None.

Digital Abstract Session

P292. Physiology of Addiction Related Systems

Program #/Poster #: P292.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NSERC RGPIN-2018-06285

Title: Comparing motivation and attention in sign-tracking behaviors

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Abstract: Some animals attribute more importance to the reward cues than the rewards themself. This behavior is called ‘‘sign-tracking’’ and it is observable in several spices as well as in humans. It has been previously found that sign-tracking (ST) behaviors may contribute to the development of addiction and other compulsive behavioral disorders. Recent publications been shown to link sign-tracking to a poor sustained attention, suggesting differences in the cholinergic system. However, sign-tacking behaviors has also been shown from a motivational perspective, in particular in the dopamine system. Given that sign-tracking behavior can have motivational or attentional origins, it would be important to quantify the contribution of each. To answer this question, 21 Long-Evans rats (13 females and 8 males) were first classified as either sign-trackers or goal-trackers according to their result to Pavolvian conditioned approach. This test has classified 6 rats as STs and 7 rats as GTs. They were then further tested by completing the sustained attention task to assess their attentional performance and motivation. To evaluate their performance the relative number of good answers (hits), false alarms, correct rejections and misses were used to measure their attentional performance while the omission rate and reward retrieval time were used to measure their motivation. No significative differences were observed between the animals in regard to their attentional performance. However, there was a significant difference in the rate of omission. Precisely, STs demonstrated less omissions than GTs. In the conditions in which we have tested our rats, our results suggest that sign-tracking behavior has a greater correlation with motivation than it has with attention. Thus, motivation can lead sign-trackers to have behaviors that have little ecological value and make them vulnerable, for example, to addiction disorders.

Disclosures: F. Huppé-Gourgues: None. **K. Mawulom Kindedji:** None.

Digital Abstract Session

P292. Physiology of Addiction Related Systems

Program #/Poster #: P292.06

Topic: G.02. Reward and Appetitive Learning and Memory

Support: CIHR

Title: Chronic administration of D2/3 agonist ropinirole enhances the ability of win-paired cues to drivedevelopment oflong-lasting preference for riskychoice in a rat gambling task

Authors: *L. MORTAZAVI[1], T. HYNES[2], S. KAUR[3], C. A. WINSTANLEY[3];
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Abstract: Dopamine replacement therapies (DRTs) such as ropinirole are common treatments for Parkinson's Disease (PD). While effective in mitigating the motor symptoms of PD, they can induce development of impulse control and gambling disorders in a considerable proportion of patients with chronic use. To investigate the mechanisms by which DRTs precipitate these conditions, we tested the effects of chronic administration of D2/3 receptor agonist ropinirole on animal models performing a rat Gambling Task (rGT). Male Long-Evans rats (N=112) received either saline, 2.5 or 5 mg/kg/day ropinirole via subcutaneously implanted osmotic pumps over 28 days while they acquired the rGT. In this task, animals choose between four options with varying probability and magnitude of winning sucrose pellets or losing time. Half of the animals were trained on a cued version of the task where delivery of rewards was paired with audiovisual cues. We found that only in the animals performing the cued rGT, administration of ropinirole, specifically during the acquisition phase of the task, biased rats towards the high-risk/high-reward options. This risk preference remained and became progressively more pronounced long after termination of drug treatment. Furthermore, consistent with previous research on the rGT, motor impulsivity was dissociable from choice effects in that it only increased transiently but returned to normal levels before the end of the drug delivery period. These findings suggest a critical role for D2/3 activity to specifically modulate the ability of win-paired cues to increase preference for risky choice. Put together with previous work, this effect may be especially powerful and long-lasting when options are being sampled and evaluated but not after a preference has been shaped.

Disclosures: L. Mortazavi: None. **T. Hynes:** None. **C.A. Winstanley:** None. **S. Kaur:** None.

Digital Abstract Session

P292. Physiology of Addiction Related Systems

Program #/Poster #: P292.07

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant RO1 DA033123

Title: Infralimbic cortical control of current versus previous behavioral state

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Abstract: In instrumental learning, extensive training of a response generates habit. The transition from goal-directed to habitual behavior is often considered unidirectional, but this is not necessarily the case; certain manipulations switch a behavior from habit back to action. We identify the infralimbic cortex (IL) as critical to this process: Pharmacological inactivation of IL converted an extensively trained behavior that had renewed as an action back to habit status but

did not affect a behavior that had only been action. We also examined the role of IL in contextual fear extinction and its reacquisition, where IL had parallel effects. In each of two experiments, 32 male Wistar rats were implanted with bilateral cannula to IL and food deprived to 90% free-feeding weight. In Experiment 1a, rats received limited lever-press training in one context, then extensive training of the same response in another context (all reinforced on an RI 30-s schedule) prior to reinforcer devaluation via taste aversion learning. (Paired rats received pairings of the sucrose-pellet reinforcer and injections of lithium chloride (LiCl).) Prior to a test of lever pressing in extinction, half the rats received an infusion of baclofen/muscimol (B/M) to IL and half received saline. Testing in the minimal training context, which prior work suggested would renew goal-direction, revealed a devaluation effect in saline animals, but not B/M animals, as if action had renewed but returned to habit under IL inactivation. In a second experiment, a separate cohort of rats received limited training in a single context (RI 30-s schedule) prior to reinforcer devaluation. Here, both B/M and saline rats exhibited a devaluation effect, suggesting IL inactivation had no effect on an instrumental response that had been only an action. These results suggest that, instead of controlling habit performance (the canonical view), IL inactivation removes a "recency" effect and returns the preceding behavioral state. To further test this idea, the rats in Experiment 1a underwent contextual fear conditioning, extinction, and reacquisition in Experiment 1b. IL inactivation was tested after extinction or reacquisition in different groups. The effect of IL inactivation interacted significantly with test phase: B/M rats tested after extinction showed heightened freezing relative to saline, suggesting a suppression of extinction (a well-known effect), but in rats tested after reacquisition, there was an opposite trend in which inactivated animals showed recovery of extinction performance, as if the reacquired fear memory was removed and behavior was returned to the preceding state of extinction.

Disclosures: M.C. Broomer: None. **M.E. Bouton:** None.

Digital Abstract Session

P292. Physiology of Addiction Related Systems

Program #/Poster #: P292.08

Topic: G.02. Reward and Appetitive Learning and Memory

Support: UH3 NS096833
R01 DA049544

Title: Investigating mechanisms unique to memories associated with methamphetamine and cocaine

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Abstract: Persistent drug-associated memories perpetuate substance use disorders (SUD) by inducing motivation to seek drug. We previously reported that methamphetamine (METH)-associated memories can be disrupted by systemic or intra-basolateral amygdala (BLA) administration of Blebbistatin, which inhibits the actin motor ATPase nonmuscle myosin II (NMII), leading to actin depolymerization. The effect is specific, as it does not interfere with other memories (e.g., fear or cocaine; COC) or have the same effect in dorsal hippocampus (dHPC) or nucleus accumbens (NAc). Therefore, to determine mechanisms underlying this selectivity, we examined the differences between METH and COC. We used RNA-seq to identify potentially unique transcriptional changes in BLA, NAc, and dHPC tissue collected after the final METH, COC, or saline CPP conditioning session. Sequencing revealed relatively large differences between METH and COC-conditioned mice in BLA. One gene selectively upregulated in METH- compared to COC-treated mice was *crhr2.* Corticotrophin releasing factor (CRF) binds to CRF receptor 1 (CRF1; gene: *crhr1*) and CRF receptor 2 (CRF2; gene: *crhr2).* Others have found selective roles for CRF1 in COC-associated memories and sensitization, and CRF2 in METH sensitization, but its role in METH-associated memories was unknown. Therefore, we infused mice in BLA with vehicle (veh) or CRF2 antagonist (Astressin-2B, AS2B) before each CPP conditioning session. AS2B-treated mice did not express a METH CPP when tested 48 hours later, indicating CRF2 is necessary for METH-associated learning. Next, to determine if CRF2 was necessary for memory formation or consolidation, mice were infused with veh or AS2B after the final (METH) conditioning session. AS2B-treated, but not veh-treated, mice expressed a METH CPP; possibly due to an anxiolytic effect of AS2B. To test this, mice were tested in elevated plus maze and open field following similar methods. Results suggest that veh infusions increased some anxiety measures, but not following AS2B infusions. To control for this, mice were habituated by receiving mock infusions prior to each conditioning session. CRF2 inhibition after the final conditioning session was not sufficient to disrupt the memory, indicating that CRF2 must be blocked early during METH-associated memory acquisition to disrupt the memory. Here, we began to assess the differences between two stimulant drug-associated memories, which allows for a better understanding of SUDs, including identifying the mechanism(s) responsible for METH-associated memory's selective vulnerability to NMII inhibition. These may yield avenues to target other pathogenic memories.

Disclosures: M. Hafenbreidel: None. **S.B. Briggs:** None. **S.E. Sillivan:** None. **A.B. Hall:** None. **M. Arza:** None. **A. Tiller:** None. **L. Lin:** None. **S. Khan:** None. **M.D. Cameron:** None. **G. Rumbaugh:** None. **C.A. Miller:** None.

Digital Abstract Session

P292. Physiology of Addiction Related Systems

Program #/Poster #: P292.09

Topic: G.02. Reward and Appetitive Learning and Memory

Support: CIHR Grant PJT-162312

Title: Investigating the behavioural effects of win-associated cues, outcome-associated cues, and randomly occurring cues on risky decision making

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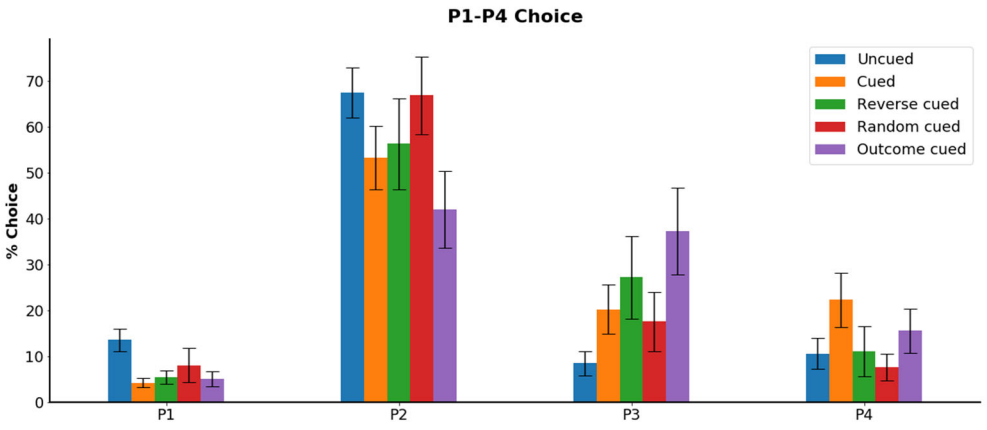
Abstract: Win-associated cues can enhance disadvantageous risky choice in both humans and rodents. Indeed, the impact of reward-paired cues on behaviour is a major component of many theories of addiction. However, the role of specific elements of such cues in producing this behavioural effect has not been thoroughly investigated. Accordingly, the present experiments assessed the effect of four different cue administration paradigms on risky decision making. Three cohorts of 32 to 48 male Long-Evans rats were trained on 5 variants of the rat Gambling Task (rGT), a rodent analog of the human Iowa Gambling Task. Optimal performance on this task is attained by avoiding the two high-risk, high-reward options (P3 and P4) and instead favouring the options associated with lower per-trial gains (P1 and P2). The standard cued version of the task features the addition of reward-paired audiovisual cues that scale in magnitude and complexity with reward size. In each cohort, 8 to 16 rats were trained on either the standard cued or uncued rGT. Sixteen rats in each cohort were trained on one of three variants of the cued task. The first variant reversed the cues, such that the longest and most complex cue was paired with the smallest reward. The second variant delivered cues during every outcome - both wins and losses. The third variant randomly delivered cues with a 50% probability across both winning and losing trials.
As observed in previous experiments, there was a higher proportion of risk-preferring rats on the standard cued task compared to the uncued task. The reverse-cue variant produced a similar choice profile to the standard cued task. Outcome-associated cues increased risky choice to an even greater degree. Conversely, rats trained on the random-cue variant did not exhibit this cue-induced risk preference. These results suggest that audiovisual cues do not need to occur only during rewarded trials in order to drive risky choice. However, the cues do need to be predictive, as random cues did not significantly alter behaviour compared to the uncued task.



Disclosures: **B.A. Hathaway:** None. **K.M. Hrelja:** None. **C.B.W. Harris:** None. **T.J. Hynes:** None. **C.A. Winstanley:** None.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA R01AA024434

Title: Open "bar": An operant paradigm to examine variability in alcohol self-administration in Drosophila melanogaster

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Abstract: Escalation of alcohol self-administration facilitates the transition from alcohol use to compulsive drinking, which is a worldwide biomedical concern. Alcohol research has largely focused on understanding the neural mechanisms underlying excessive or compulsive alcohol intake. Less is understood of the neural substrates underpinning individual differences in alcohol preference and seeking, and how escalation arises in some individuals but not others. The size, heterogeneity and complexity of mammalian reward circuits make investigating variation in alcohol preference extremely difficult. In *Drosophila*, the neural circuits required for encoding valence are well-studied and include identifiable connections, genetic and/or biochemical profiles and characterized temporal changes underlying learning. We developed a 3-day operant paradigm to evaluate self-administration of volatilized pharmacological doses of ethanol in *Drosophila,* and demonstrated population variance in ethanol self-administration. Almost 30% of flies escalated self-administration of a dose of ethanol which increased average velocity. This starkly contrasts with population self-administration patterns observed for known appetitive or aversive odors. We also demonstrate a requirement of mushroom body α'3 circuitry in escalation of ethanol self-administration, and reveal subtle behavioral features associated with manipulating mushroom body α'3 neurons that predict escalation of ethanol self-administration. Our data demonstrate that *Drosophila* express innate preferences for self-administering ethanol and that manipulating learning and memory circuits impacts the trajectory of those expressed preferences. Results allow for a useful experimental approach to study alcohol use and abuse disorders.

Disclosures: **E.R. Glenn:** None. **J.S. Hernandez:** None. **N. Mei:** None. **J. Catalano:** None. **R. Azanchi:** None. **K.R. Kaun:** None.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.02

Topic: G.09. Drugs of Abuse and Addiction

Support: Miami University, Psychology Department
Miami University, College of Arts and Sciences

Title: Sex chromosome complement and gonadal status contributions to ethanol intake, preference, and relapse-like behavior

Authors: *E. A. SNEDDON, L. RASIZER, N. CAVALCO, A. JAYMES, N. OSTLIE, A. K. RADKE;
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Abstract: Background: The diagnosis of alcohol use disorder (AUD) in women is on the rise in the United States. Rodent models show behavioral sex differences in different aspects of ethanol (EtOH) drinking behaviors such as escalation and relapse. The mechanisms associated with increased vulnerability to EtOH in females are still unclear. The four-core genotype (FCG) mouse model allows for the investigation of sex chromosome complement and gonadal status on behavior. We used the FCG mouse model to investigate the influence of gonadal hormones and sex chromosomes on EtOH intake and relapse susceptibility. **Methods:** FCG mice were given access to EtOH in their home cage about 6 hours into the light cycle. Mice had access to two bottles for 24-h. One bottle contained reverse-osmosis (RO) drinking water and the other bottle contained EtOH in RO water (v/v). EtOH concentrations increased from 5%, 10%, 15%, and 20%. Mice were exposed to each concentration of EtOH for a total of 5 drinking sessions. After the last 20% EtOH session, mice underwent a 6-day EtOH deprivation period. After each deprivation period, mice were reintroduced to 20% EtOH for 24-h. This cycle of deprivation and re-exposure was repeated for a total of 5 deprivation sessions. At least two weeks following deprivation, water consumption was measured in one 24-h session and 2.5% sucrose consumption was assessed over five 24-h drinking sessions. **Results:** Mice with female gonads and XX chromosomes consumed greater amounts of EtOH. Mice with XX chromosomes preferred EtOH to a higher degree than mice with XY chromosomes. Mice with XX chromosomes drank more EtOH during deprivation sessions when consumption was expressed as a percent of baseline. Mice with female gonads consumed greater amounts of water when EtOH was present and alone. No differences were observed between groups on sucrose intake. **Conclusions:** Female gonads and XX chromosomes were associated with greater amounts of EtOH intake. Also, mice with XX chromosomes had a higher preference for EtOH and were more prone to relapse. These results indicate individuals with XX chromosomes may have a predisposition to prefer alcohol and have a higher susceptibility to relapse than individuals with XY chromosomes. Water consumption was increased in mice with female gonads during EtOH and control drinking sessions. There was no association between gonadal status or sex chromosome complement on sucrose intake. These findings indicate that EtOH intake,

preference, and relapse-like behavior may be differentially regulated by gonadal status vs. sex chromosome complement and suggest novel, future avenues for investigating the neural mechanisms of female vulnerability to alcohol.

Disclosures: E.A. Sneddon: None. **A.K. Radke:** None. **L. Rasizer:** None. **N. Cavalco:** None. **A. Jaymes:** None. **N. Ostlie:** None.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIAAA grant AA07611

Title: Development of compulsive-like binge quinine-adulterated alcohol drinking in male and female C57BL/6J mice

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Abstract: Alcohol use disorder (AUD) is a chronic disease that is characterized by an inability to control alcohol use despite negative consequences, or compulsive alcohol drinking (American Psychiatric Association, 2013). The bitter compound quinine has been used to model compulsive-like drinking in mice. Mice with previous alcohol exposure will drink quinine-adulterated alcohol (QuA) at the same level as unadulterated alcohol, despite the negative consequence of the taste, whereas mice that are alcohol-naïve will drink significantly less QuA. Here we investigated the establishment of compulsive-like quinine-adulterated front-loading and 2-hour binge alcohol drinking in male and female C57BL/6J mice after 3 weeks of either alcohol or water exposure using a mouse model of binge drinking, Drinking-in-the-Dark (DID). We hypothesized that after 3 weeks of binge-like drinking, mice will display compulsive-like QuA consumption. The mice (n=12/group) were allowed free access to 20% ethanol for 2 hours each day for a total of 26 days. On days 22, 24, and 26, the mice were given QuA (0.5mM quinine in 20% ethanol) during DID in place of the 20% ethanol. Bottles were read and intakes were measured at 30-minutes and 2-hours into DID. On day 26, immediately following DID, blood ethanol concentrations (BECs) were determined with retro-orbital sinus eye bloods. We found that both male and female alcohol history mice binge-drank significantly more QuA than water history mice ($p < 0.0001$), and alcohol history mice drank the same amount of QuA as unadulterated alcohol at baseline (i.e. the day prior to QuA; $p > 0.05$). Alcohol history mice front-loaded significantly more QuA than water history mice suggesting that alcohol history mice were motivated to a greater extent than water history mice to consume the QuA ($p < 0.0001$). Importantly, alcohol history mice front-loaded significantly less QuA than unadulterated alcohol at baseline suggesting that the mice find the QuA solution aversive as they are less willing to drink it prior to post-alcohol absorption effects ($p < 0.0001$). After three weeks

of binge-like drinking, both male and female C57BL/6J mice displayed compulsive binge-like QuA drinking; this pattern did not fluctuate between three QuA exposures. BECs were significantly higher in the compulsive-like alcohol history mice than the non-compulsive water history mice on day 26 ($p < 0.05$). These data provide a robust two-criteria model of compulsive-like alcohol drinking in male and female C57BL/6J mice. This model can be used to advance the mechanistic and neurobiological underpinnings of compulsive-like alcohol drinking and AUD.

Disclosures: M.M. McVey: None. **M.R. Bauer:** None. **S.L. Boehm:** None.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant F32MH115432
NIH Grant R21AA026949
NIH Grant R01AA025368

Title: Relating alcohol consumption behavior to ligand bias at the kappa opioid receptor

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Abstract: The kappa opioid receptor (KOR) is a G protein coupled receptor that regulates pain, motivation, and reward. While activation of the KOR can cause desirable pharmacological effects, including the inhibition of addictive drug reward and analgesia, KOR activation also causes a host of undesirable effects such as sedation and dysphoria. Some of these physiological outcomes have been associated with different signaling pathways at the KOR. For example, previous work has shown that KOR-mediated dysphoria and sedation are dependent on signaling proteins recruited by arrestin. Accordingly, these findings have driven the development of "biased" ligands at the KOR that signal preferentially through arrestin-independent pathways with the aim of designing therapeutics at the KOR that treat pain or drug dependence without side effects. However, the relationship between KOR and drug reward is complex and both time- and drug-exposure specific: in drug naïve animals, the KOR can either inhibit or potentiate the reward of addictive drugs such as alcohol depending on the timing of agonist administration relative to drug exposure. With more drug exposure, and as an individual enters severe drug dependence, the KOR system becomes dysregulated, driving the negative symptoms of withdrawal that in turn promote further drug seeking and relapse. Thus, while the role of KOR in modulating drug seeking makes it an attractive target for therapeutics treating dependence, the complex nature of this regulation means further understanding is needed to properly design and carry out treatment through the KOR. To this end, we investigated whether differentially biased KOR agonists would uniquely modulate alcohol use in mice. For this we relied on KOR agonists

that were deemed to be G-protein-biased (HS666), balanced (U50,488), and β-arrestin 2-biased (nalfurafine), as assessed in *vitro* assays. We then administered these three KOR agonists to male and female wild-type and β-arrestin 2 knockout C57BL/6 mice (n≥8) trained to consume 10% ethanol in a two bottle choice drinking-in-the-dark paradigm. Our results appear to suggest that β-arrestin 2 signaling does not drive KOR-mediated elevation in drinking, and that sedation, as measured by hypolocomotion, and alcohol use are interdependent physiological effects at the KOR. Our results suggest biased signaling at the KOR is not a driving factor behind the pharmacological effects of KOR activation on alcohol intake, in stark contrast to the delta opioid receptor, where alcohol use is tightly correlated with arrestin recruitment.

Disclosures: **A.R. French:** None. **A.M. Gutridge:** None. **Q.H. Royer:** None. **R.M. Van Rijn:** F. Consulting Fees (e.g., advisory boards); Carmot Therapeutics.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01AA024044
NSF Graduate Student Fellowship

Title: Selective inhibition of PDE4B reduces binge-drinking in two C57BL/6 substrains

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Abstract: The cyclic AMP (cAMP)-hydrolyzing phosphodiesterase 4 (PDE4) enzyme family is a critical component of signaling pathways implicated in many neurological conditions, including Alcohol Use Disorder. For example, treatment with the prototypical non-selective PDE4 inhibitor Rolipram lowers voluntary alcohol intake under limited-access drinking procedures. The PDE4 family consists of 4 subtypes (PDE4A-D) and the advent of isozyme-selective inhibitors currently enable dissection of their relative roles in regulating brain and behavior, with fewer off-target effects. To this end, the present study determined the effects of the PDE4B-selective inhibitor A33 on alcohol versus sucrose intake in a murine model of binge-drinking, as well as on alcohol-induced changes in motor behavior. For this, female and male C57BL/6NJ mice (B6NJ; n=7-8/sex) and male C57BL/6J mice (B6J; n=11; female study on-going) were presented concurrently with 10, 20 and 40% alcohol for 2h/day. Using a within-subjects design, mice were pretreated (30 min prior) with 0, 0.03, 0.1, 0.3 or 1.0 mg/kg A33, with the order of dosing randomized across subjects. Three to four days were allowed between dosing to examine for potential carry-over effects. Following characterization of the dose-response function for alcohol intake, the effects of the 1.0 mg/kg A33 dose on the intake of a

20% sucrose solution was determined. The same mice were then examined for the effects of 1.0 mg/kg A33 upon locomotion activity, intoxication on a rotorod and sedation induced respectively by 1.5, 3 and 4 g/kg alcohol. In these latter assays, mice underwent 2 days of testing during which half of the mice received either vehicle or A33 30 min prior to the alcohol injection and testing and then received the opposite pretreatment prior to testing the next day. A33 pretreatment dose-dependently reduced binge-alcohol intake in both substrains, with significant reductions detected at the 0.03 mg/kg dose in male and female B6NJ mice and at the 0.1 mg/kg dose in male B6J mice. Pretreatment with 1.0 mg/kg A33 did not alter sucrose intake in B6J males, but augmented sucrose intake by B6NJ mice. No A33 effect was detected for alcohol-induced locomotor activity, intoxication or sedation in either substrain. These data provide novel evidence that selective inhibition of PDE4B is sufficient to reduce binge alcohol-drinking, without reducing the intake of a non-drug reinforcer or altering sensitivity to alcohol's psychomotor effects. If relevant to humans, such findings pose PDE4B inhibition as an effective pharmacotherapeutic strategy for reducing heavy or problem drinking, with low likelihood of off-target or negative side-effects.

Disclosures: K.K. Szumlinski: None. **C.L. Jimenez Chavez:** None. **C.D. Bryant:** None. **M.A. Munn Chernoff:** None.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.06

Topic: G.09. Drugs of Abuse and Addiction

Support: RO1 AA019793
T32 AA07468

Title: Effect of oxytocin treatment on ethanol intake in group-housed male and female C57BL/6J mice

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Abstract: Alcohol use disorder (AUD) is a prominent public health problem affecting approximately 9 million men and 5 million women in the United States alone. Notably, the relationship between social environment and AUD is complex, in that one's social group may serve to provide support and promote sobriety or to encourage drinking and increase the risk of relapse. Not surprisingly, oxytocin, a neuropeptide system with established roles in various social behaviors, has recently been identified as a potential modulator of alcohol intake, with preclinical studies demonstrating that systemic oxytocin treatment tends to attenuate voluntary consumption. Therefore, we sought to identify the effects of repeated, systemic oxytocin treatment (i.p.) on voluntary ethanol intake in group-housed male and female C57BL/6J mice.

Mice were housed in Herdsman 2 (HM2) cages to enable the tracking of individual water and ethanol intake while allowing for continuous social interaction among cagemates. We found that under baseline conditions, group-housed females consumed significantly more g/kg ethanol than males, an effect largely mediated by females' lower average body weight when compared to males. Following 4 consecutive treatments of 3mg/kg oxytocin, both males and females decreased ethanol intake, but not water intake, relative to vehicle-treated controls. Importantly, though, this effect varied between treatment days. Additionally, the high temporal resolution provided by the HM2 system allowed for the identification of compensatory increases in ethanol intake approximately 12hr after oxytocin was administered, a phenomenon that could present significant issues when considering treatment in patient populations. Therefore, future studies will assess the effects of oxytocin over longer periods of repeated treatment and the potential of additional oxytocin receptor agonists to decrease ethanol consumption.

Disclosures: H. Fulenwider: None. **M. Caruso:** None. **A.E. Ryabinin:** None. **M. Robins:** None.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.07

Topic: G.09. Drugs of Abuse and Addiction

Support: PAPIIT-DGAPA IN201420

Title: Effect of chronic sugar consumption on ethanol preference in adolescent and adult male rats

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Abstract: The exacerbated consumption of sugar and its cognitive processing involves the activation of the brain reward system, in which areas such as the prefrontal cortex (PFC) mediate learning and behavior related to the reinforcement of the consumption of sweet substances. On the other hand, it is known that the late maturation of PFCs during adolescence is related to the early appearance of the intake of addictive substances such as ethanol. However, few studies have analyzed the impact of high sugar consumption and its relationship with the consumption of addictive substances, such as ethanol, particularly when it is present in sugary alcoholic mixtures. Given that alcohol intake usually begins in adolescence through these alcoholic mixtures, the objective of this study was to evaluate, in adolescent (5 weeks at the beginning of the experiments) and/or adult (9 weeks) male rats, the preference for the consumption of sugary ethanol, after prolonged and permanent sugar exposure.Male Wistar rats were housed individually and kept at 23 °C, in a reverse light-dark cycle. All behavioral procedures were performed during the dark phase of the cycle; food pellets were available *ad libitum*. Rats were

randomly assigned to the groups: Naive (N) or Chronic (C), with access to water or sugar, respectively, as the only liquid available for 24 hrs/21 days. Bodyweight, liquid, and food intake were recorded daily. After 21 days, the chronic group was divided into a subgroup with continuous access to sugar (CND) and a subgroup deprived of sugar, with access only to water (CD), between each of 4 preference tests (24 h each) of sugary ethanol (10% ethanol-10% sugar) vs. water.In groups CND and CD, both adolescent and adult rats, the chronic consumption of sugar-induced a suppressive intake effect of the sugary ethanol solution during the 4 preference tests. However, despite always preferring water, only adolescent CD rats increased their consumption of sugar-ethanol mixture from the second preference test. When analyzing the data independently, between rats with high or low sugary ethanol consumption, the effect of sugar deprivation -between preference tests- was related to significantly higher sugary ethanol consumption in adolescent rats compared to adults.Taken together, the results suggest that adolescent rats have a higher susceptibility to sugar deprivation that could be related to late brain maturation as well as decision-making and increased impulsive and risk-seeking behaviors, such as the initiation of use of addictive substances.

Disclosures: **M. Reséndiz:** None. **G. Vera:** None. **A. Rangel:** None. **M.I. Miranda:** None.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.08

Topic: G.09. Drugs of Abuse and Addiction

Support: CSULB CLA
CSUPERB
NIH Grant UL1GM118979
NIH Grant TL4GM118980
NIH Grant RL5GM118978

Title: Dose-dependent differences of ethanol-induced conditioned place preference in juvenile and adolescent male and female sprague-dawley rats

Authors: ***T. M. NELSON**[1], D. Q. PHAM[1], D. FRANCO[1], E. A. BATES[1], T. A. GONZALEZ[1], S. D. INIGUEZ[2], A. R. ZAVALA[1];
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Abstract: Ethanol, the type of alcohol consumed, is the most abused recreational drug in the United States. Ethanol use commonly begins during adolescence, a period of brain development that is susceptible to ethanol's effects, leading to long-term changes in reward pathways. Surprisingly, studies that have used the conditioned place preference (CPP) paradigm, a validated animal model of drug reward, have shown that adolescent rats do not readily demonstrate ethanol-induced CPP. To further characterize ethanol preference in adolescence, we

hypothesized that sex differences would emerge across adolescence and that low doses of ethanol are necessary for adolescent male rats to demonstrate ethanol-induced CPP. Rats underwent a 10-day CPP procedure to assess ethanol preference during early adolescence (postnatal day (PD) 23-32) in experiment 1 and adolescence (PD 31-40) in experiments 2 and 3. On day 1, preference for a two-chamber CPP apparatus was assessed during 15 min (experiment 1 and 2) or 20 min (experiment 3) sessions. On days 2-9, rats were conditioned with saline in their initially preferred chamber or ethanol in their initially non-preferred chamber on alternating days for 15 min. On day 10, the preference for the ethanol-paired chamber was assessed using identical procedures to day 1. In experiments 1 and 2, rats were randomly assigned to receive an injection of ethanol (0.0, 0.5, 1.0, or 2.0 g/kg, intraperitoneally) before being placed in the ethanol-paired chamber. Similarly, in experiment 3, rats were randomly assigned to receive an injection of ethanol (0.0, 0.0156, 0.0313, 0.0625, 0.125, 0.5, or 2.0 g/kg, intraperitoneally) before being placed in the ethanol-paired chamber. In experiment 1, regardless of sex, rats demonstrated ethanol-induced CPP when administered the highest dose of ethanol (2.0 g/kg). In experiment 2, sex differences emerged as female rats continued to prefer a high dose of ethanol (2.0 g/kg). However, male rats exhibited an increased preference for the ethanol-paired chamber only with the lowest ethanol dose (0.5 g/kg). In experiment 3, male rats demonstrated a robust preference for ethanol when administered low ethanol doses (0.0625, 0.125 g/kg). Overall, sex differences did not emerge in preadolescence, as both male and female rats exhibited a strong preference for ethanol. However, during adolescence, ethanol preference shifted in male rats, as only low doses of ethanol resulted in a preference for the ethanol-paired chamber. Establishing preclinical models that allow for the examination of ethanol reward in adolescence is crucial in understanding the neurobiological mechanisms that underlie ethanol abuse in adolescents.

Disclosures: T.M. Nelson: None. **D.Q. Pham:** None. **D. Franco:** None. **E.A. Bates:** None. **T.A. Gonzalez:** None. **S.D. Iniguez:** None. **A.R. Zavala:** None.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.09

Topic: G.09. Drugs of Abuse and Addiction

Support: FRQS

Title: Internal desynchronization and ethanol consumption in female rats

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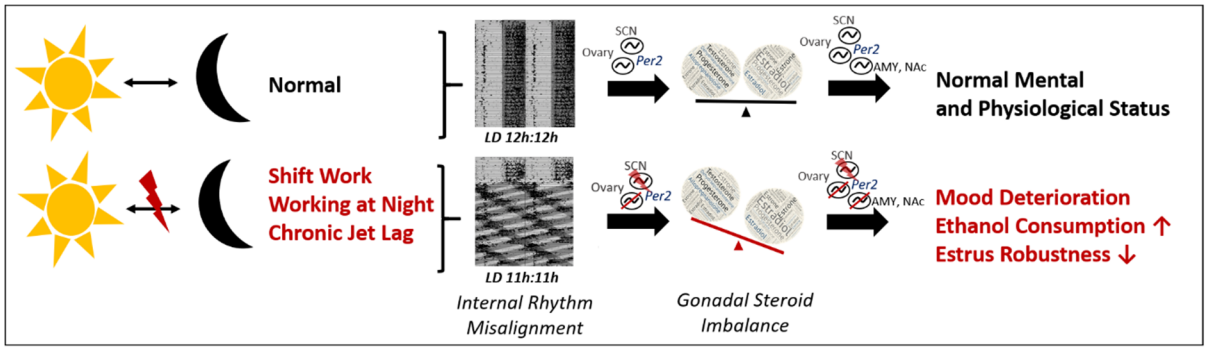
Abstract: To meet the demand for a 24-hour-business service in our modern society, the number of workers in abnormal working time, such as shift work, increased by 29% from 1997 to 2010 (Rydz et al., 2020). Shiftwork is associated with a higher incidence of alcohol abuse, especially

among women. However, there is a fundamental lack of knowledge regarding the underlying mechanism in the female organism, as most studies investigate the adverse effects of chronodisruption focusing on males. Women might be more vulnerable to chronodisruption as a result of endocrine imbalances. An impaired gonadal hormonal rhythm affects the clock gene *Per2* in brain regions related to mood and reward (Perrin et al., 2006). Blunted rhythms in *Per2* expressions were observed in a sub-set of mood-regulating brain areas in animals showing anxiety-like behaviors (Landgraf et al., 2016). Moreover, *Per2* knockout animals display alterations in the glutamatergic system, associated with an increased alcohol intake (Spanagel et al., 2005). We hypothesize that an impaired *Per2* expression in brain areas related to mood and reward might be exacerbated in the presence of dysregulated gonadal steroids, thus contributing to increased susceptibility in behavioral changes related to mood and reward. To address this, we exposed adult female rats to an internal desynchrony inducing 11:11 h light/dark (LD22) schedule. During the experiment, female rats had voluntary access to an intermittent two-bottle alcohol paradigm, followed by an ethanol abstinence period. Animals are tested for ethanol preference, and we evaluated mood and reward behavior. The estrus robustness was assessed via locomotor activity. Our findings indicate that internal desynchronization suspends the regular estrus cycle and augments alcohol preference. Under acute ethanol withdrawal, animals show a higher sucrose preference, whereas prolonged ethanol abstinence increases anxiety-like behavior and reduces natural marble-burying behavior. These data highlight the critical link between dysregulated gonadal steroids and behavioral alterations.



Disclosures: **C. Meyer:** None. **K. Schottner:** None. **S. Amir:** None.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.10

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 AA028218 (JRB)
K01 AA023874 (ANK)

Title: Kappa-opioid receptor stimulation in the nucleus accumbens shell differentially and selectively affects ethanol drinking along a rostro-caudal axis

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Abstract: The kappa-opioid receptor (KOR) and its cognate ligand, dynorphin, are canonically understood to produce negative affective states and, thus, to influence motivated behavior; however, a growing body of evidence demonstrates that the KOR within the nucleus accumbens (NAc) shell exerts opposing effects on affective behaviors, depending on the site of its stimulation. Importantly, no research to date has demonstrated that KOR stimulation in the NAc shell affects ethanol intake. Thus, to determine if KOR stimulation in the NAc shell can impact ethanol drinking, and if this depends on its rostro-caudal location, we trained adult, male Long-Evans rats to drink 20% v/v ethanol under an intermittent access two-bottle choice paradigm for 8-10 weeks and then bilaterally microinjected the selective KOR agonist U-50,488H (0.008, 0.08, 0.8 and 8.0 nmol) vs. saline vehicle (0.3 µl), prior to daily ethanol access, in the rostral, middle, or caudal NAc shell, using a within-subject Latin-square design. Then, to determine if the observed effects were selective for ethanol drinking, we trained a separate group of rats to drink 2.5% w/v sucrose under the same intermittent access model and then microinjected them within-subject prior to daily sucrose access with U-50,488H (0.8 and 8.0 nmol) or saline vehicle (0.3 µl), into either the rostral or caudal NAc shell. We found that, in rats trained to drink ethanol, while KOR stimulation in the middle shell had no effect on ethanol intake, stimulation in the rostral shell decreased ethanol consumption and stimulation in the caudal shell instead increased it. In contrast, in rats trained to drink sucrose, KOR stimulation in neither the rostral nor the caudal shell affected sucrose intake. These results demonstrate that KOR stimulation at distinct rostro-caudal points in the NAc shell induces opposing and selective effects on ethanol drinking. In light of prior research demonstrating that rostral KOR stimulation induces positive affective states while caudal stimulation induces negative affective states, the present findings appear to be consistent with the idea of the “dark side of addiction,” whereby rostral KOR stimulation reduces, and caudal stimulation enhances, ethanol drinking.

Disclosures: B.E. Pirino: None. **A.T. Gargiulo:** None. **G.R. Curtis:** None. **A.N. Karkhanis:** None. **J.R. Barson:** None.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.11

Topic: G.09. Drugs of Abuse and Addiction

Support: AA26117
AA17531
AA26551
AA26455

Title: Sex differences in operant alcohol self-administration in rats following a covid-19 induced abstinence

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Abstract: Although men have a higher incidence of developing alcohol use disorder than women, this gap is narrowing and women may also be more sensitive to the maladaptive CNS effects of excessive alcohol use. Unfortunately, relatively few non-human animal studies have addressed possible sex difference in alcohol drinking-related behaviors. To begin to address this gap in our knowledge, we investigated whether there were any sex-specific outcomes associated with varying durations of alcohol abstinence in Long Evans rats. Male and female rats (8/gp) were trained to complete a 20 lever press response requirement to receive access to a sipper tube containing a 10% alcohol solution for a 20 minute free consumption period. After a baseline period of responding, rats underwent a series of increasing periods of forced abstinence, culminating in a 74 day abstinence necessitated by a Covid-related lab shutdown. Extinction probe trials, during which no amount of lever pressing resulted in delivery of the sipper tube, were conducted during the baseline period and following each abstinence period, to assess seeking behavior. All phases of acquisition, maintenance, and extinction probe responding were performed at the same time of day to reduce external variables. No significant sex differences were noted in the acquisition rate of alcohol self-administration. During a two week baseline, females consumed significantly more alcohol than males but there were no sex differences in extinction responding. A 2- and 10 day forced abstinence period had minimal effects on alcohol intake and the 10-day abstinence transiently reduced extinction responding in both sexes. Interestingly, following the post-Covid abstinence period, male rats showed a transient decrease in alcohol intake and extinction responding, while neither measure was affected in females. Finally, after baseline responding had been re-established in both cohorts, we found that females were more resistant to quinine adulteration of the alcohol solution. Together, these findings reveal the female rats are markedly less sensitive to the effects of prolonged abstinence on appetitive and consummatory alcohol drinking-related measures and may be more prone to exhibit compulsive drinking behaviors.

Disclosures: H.N. Carlson: None. **S.C. Gironda:** None. **S.R. Pitcairn:** None. **M.I. Allen:** None. **J.L. Weiner:** None.

Digital Abstract Session

P293. Alcohol: Intake and Preference

Program #/Poster #: P293.12

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH intramural funding ZIA-AA000218
Peter G. Dodge Foundation (PGDF) funding

NIH/NIAAA Grant R01-AA15971

Title: Long-term but not short-term alcohol binge drinking leads to changes in the gut microbiota and the fecal metabolome in adult male baboons

Authors: *D. PIACENTINO[1], S. GRANT-BEURMANN[2], C. VIZIOLI[3], X. LI[4], V. RUIZ-RODADO[5], M. R. LEE[1], P. V. JOSEPH[3], C. F. MOORE[6], C. M. FRASER[2], E. M. WEERTS[7], L. LEGGIO[1];
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Abstract: Binge drinking is a pattern of drinking that brings the blood alcohol (EtOH) level (BAL) to over 0.08 g/dL in about 2 hours. It's a public health problem, causing 45,000 deaths in the US each year. Research is lacking on the role of the microbiota-gut-brain axis on EtOH binge drinking. We previously showed that EtOH binge-like exposure induces a significant and detrimental decrease in gut microbial α-diversity in Wistar rats. Here, we investigated whether EtOH binge drinking is associated with significant changes in the gut microbiota and/or in fecal metabolites related to gut microbial dysbiosis in adult male baboons of the sp. *Papio Anubis*. We studied three groups: **S**=**S**hort-term EtOH exposure group, self-administering EtOH for 2-3 yrs (*N*=5); **L**=**L**ong-term EtOH exposure group, self-administering EtOH for >10 yrs (*N*=4); **C**=**C**ontrol group, self-administering Tang®, an isocaloric nonalcoholic drink, for 8 yrs (*N*=5). Daily blood draws confirmed that L and S binge drank 7 dd/week, with BALs constantly >0.08 g/dL (range=0.09-0.15 g/dL) after a 2-hour self-administration period. Fecal samples were collected in two conditions: **D**=during 3 days of active **D**rinking (dd 1-3) and **A**=during 3 days of **A**bstinence (dd 4-6). Diet did not vary among animals. Gut microbiota analysis consisted of DNA extraction and 16S rRNA gene sequencing, analysis of microbial species diversity, and comparison of relative taxonomic abundances through Linear Discriminant Analysis Effect Size (LEfSe). Fecal metabolomics relied on UPLC-MS/MS, which identified 564 metabolites. ANOVA and linear mixed-effects models were used for data analysis. The baboons did not differ significantly in age and weight. Microbial α- and β-diversity were significantly decreased in L *vs.* S and C (p's<0.001). At LEfSe, the genera *Lactobacillus* and *Streptococcus* showed high relative abundances in L, the family *Ruminococcaceae* in S, the family *Anaeroplasmataceae* in C (p's<0.01). Microbiota-generated metabolites of aromatic amino acids increased in L *vs.* S and C (p's<0.01). Secondary bile acids produced by the gut microbiota increased in both L and S *vs.* C (p's<0.05). In line with long-term EtOH exposure, mucosal damage markers (N-acetylated amino acids) increased in L *vs.* S and C (p's<0.05). The gut microbiota and the fecal metabolome did not significantly differ under conditions D and A. These novel findings suggest that changes in the gut microbiota and the fecal metabolome occur after long- but not short-term EtOH binge drinking. These changes are not affected by acute forced withdrawal from chronic EtOH exposure. Future research will need to examine if prolonged and sustained abstinence restores the normal gut microbiota.

Disclosures: **D. Piacentino:** None. **S. Grant-Beurmann:** None. **C. Vizioli:** None. **X. Li:** None. **V. Ruiz-Rodado:** None. **M.R. Lee:** None. **P.V. Joseph:** None. **C.M. Fraser:** None. **E.M. Weerts:** None. **L. Leggio:** None.

Digital Abstract Session

P294. Alcohol: Cognitive and Behavioral Effects

Program #/Poster #: P294.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA R01AA024434

Title: Neuromolecular and behavioral changes associated with alcohol deprivation in Drosophila

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Abstract: Alcohol use disorder (AUD) is a chronically relapsing disorder, characterized by loss of control in limiting intake, and sometimes involving intermittent periods of abstinence and relapse. Little is understood about how alcohol deprivation during periods of abstinence alters the molecular landscape, and consequently behavior. Using a new *Drosophila melanogaster* alcohol deprivation model, we identify how alcohol deprivation alters spontaneous behavior, uncover the associated neural structures, and determine correlated changes in gene expression in the brain. Using high content behavioral tracking approaches, we found that repeated alcohol experiences followed by a period of alcohol deprivation induce behavioral features associated with a negative affect state, including changes in social behavior. We mapped the behavioral features to activation of specific regions in the brain and found that the mushroom body and surrounding neuropil were involved in the social behavioral shifts associated with alcohol use as well as alcohol deprivation. Using RNAseq, we found brain-wide alterations in gene expression, implicating genes associated with sensory responses, and neural plasticity. Immunity genes became upregulated following daily repeated alcohol exposures, and persisted with one or two days of alcohol deprivation, suggesting a persistent change in molecular function. Our study provides the framework for understanding how alcohol deprivation alters the molecular landscape in neural circuits, thus initiating behaviors that can prompt relapse into alcohol use and abuse.

Disclosures: **K.S. McCullar:** None. **N. D'Silva:** None. **K. Kaun:** None. **A. Conard:** None. **E. Larschan:** None. **R. Azanchi:** None. **T. Blackwater:** None. **U. Heberlein:** None.

Digital Abstract Session

P294. Alcohol: Cognitive and Behavioral Effects

Program #/Poster #: P294.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIAAA grant AA07611

Title: A single alcohol pre-exposure alters dorsolateral striatal AMPA receptor dependent binge and quinine-adulterated alcohol drinking in C57BL/6J mice.

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Abstract: Compulsive alcohol drinking is a defining characteristic of alcohol use disorder and the dorsolateral striatum (DLS) is implicated in regulating this inflexible behavior. Synaptic restructuring occurs following long-term use of alcohol leading to hyperactivation of ionotropic glutamate receptors. Glutamatergic afferents bind post-synaptic AMPA-gated GABAergic medium spiny neurons in the DLS, a brain region involved in the development of behavioral compulsivity. AMPA receptors (AMPARs) have been implicated in both goal-directed (dorsomedial striatal dependent) and DLS dependent inflexible behaviors with DLS inhibition altering inflexible behavior including habit and compulsion. The AMPAR antagonist, NBQX, has been shown to reduce alcohol consumption when administered systemically in mice (Bauer, Garcy, & Boehm, 2020) or into the DLS of rats (Corbit, Nie, & Janak, 2014). However, much remains unknown about the role of AMPARs in the control of binge-like alcohol drinking or compulsive alcohol drinking in mice. The purpose of this experiment was to antagonize DLS AMPARs during compulsive-like quinine-adulterated alcohol (QuA; 0.5 mM) drinking and non-compulsive binge-like alcohol drinking using Drinking-in-the-Dark (DID). C57BL/6J mice were given a total of 21 days of either a binge alcohol history, to establish a compulsive-like phenotype, or a water history to serve as alcohol naïve controls, prior to infusion. On days 22 and 24 mice were given a bilateral infusion of one of three concentrations of NBQX (0, 0.3, or 1 μg; n = 7-11/group) into the DLS, immediately prior to DID. We found that DLS NBQX reduced 2-hour alcohol drinking in male water history mice ($p < 0.01$) and reduced 2-hour QuA drinking when QuA was the second solution presented (i.e. following a single alcohol pre-exposure) in male water history mice only ($p < 0.05$; n = 3-6). We also found that NBQX trended toward reducing 20-minute front-loading of alcohol in female alcohol history mice only ($p = 0.07$). Importantly, locomotor activity was not disrupted by NBQX at any dose (p's > 0.05). Together, these data suggest that DLS infusion of NBQX reduces alcohol drinking in male water history mice, QuA drinking in male water history mice but only after an initial alcohol pre-exposure, and alcohol front-loading in female alcohol history mice only. These results demonstrate the importance of DLS AMPA receptors for prevention of binge-like alcohol drinking in naïve male mice, the relevance of a single alcohol pre-exposure on DLS mechanisms, namely susceptibility to AMPAR antagonism, and demonstrates that DLS mechanisms of binge-drinking differ by sex. This work was supported by NIH/NIAAA grant AA07611.

Disclosures: **M.R. Bauer:** None. **M.M. McVey:** None. **S.L. Boehm:** None.

Digital Abstract Session

P294. Alcohol: Cognitive and Behavioral Effects

Program #/Poster #: P294.03

Topic: G.09. Drugs of Abuse and Addiction

Support: Research supported by a Programme grant from the MRC (MR/NO2530X/1) to BJE and DB
LMP is supported by a Leverhulme Trust Early Career fellowship (ECF-2018-713)

Title: The vulnerability to develop compulsive alcohol drinking as a coping strategy is mediated by the kappa opioid system

Authors: *L. MARTI-PRATS, C. GIULIANO, A. BELIN-RAUSCENT, M. FOUYSSAC, C. VELAZQUEZ-SANCHEZ, X. ZHANG, B. J. EVERITT, D. BELIN;
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Abstract: Most individuals drink alcohol recreationally and enjoy its positive and anxiolytic effects. However, a subset of vulnerable individuals progressively lose control over intake and develop the compulsive alcohol seeking and drinking that is a key feature of alcohol use disorder (AUD). While the psychological and neural basis of this vulnerability are complex, it is well established that the medication of an internal distress as a coping strategy is an important factor in AUD, as indicated by the recent exacerbation of problem drinking associated with social isolation in response to the COVID-19 pandemic lockdowns. However, the underlying neural and behavioural mechanisms are not well understood. Here, we developed a novel procedure that enables the identification of inter-individual differences in drinking alcohol to cope with distress, as measured in a Schedule-Induced Polydipsia procedure (SIP). We causally tested the hypothesis that this individual tendency to drink alcohol as a coping mechanism predicts an increased vulnerability to develop compulsive, quinine-resistant, alcohol drinking that is mediated by the dynorphin/kappa opioid receptor (KOR) system, long associated with negative emotional states. Two cohorts of Sprague-Dawley rats were exposed to SIP, a procedure in which intermittent food delivery triggers a state of internal distress that some rats learn to cope with by developing adjunctive anxiolytic behaviours, such as excessive intake of freely available water. We showed that while some rats learned to cope with distress triggered by the SIP procedure by drinking water, as evidenced by a profound decrease in anxiety as measured in an elevated plus maze task, others only acquired this adjunctive, anxiolytic response when alcohol, but not water, was available. The subpopulation that acquired alcohol adjunctive drinking did not differ from those adjunctively drinking water in terms of alcohol exposure, but they persisted in drinking alcohol when adulterated with quinine. Hence, only those rats that drank alcohol to cope with distress went on to drink alcohol compulsively. Furthermore, acute administration of the KOR antagonist norbinaltorphimine, decreased alcohol intake selectively in this vulnerable

subpopulation, suggesting an important role of the dynorphin/KOR system in the use of alcohol to self-medicate distress. These results suggest that acquisition of alcohol drinking under negative reinforcement, associated with an alteration of the dynorphin/KOR system, enhances the later likelihood of developing compulsive drinking in individuals that had been unable to learn to cope with negative states by alternative means.

Disclosures: L. Marti-Prats: None. **C. Giuliano:** None. **A. Belin-Rauscent:** None. **M. Fouyssac:** None. **C. Velazquez-Sanchez:** None. **X. Zhang:** None. **B.J. Everitt:** None. **D. Belin:** None.

Digital Abstract Session

P294. Alcohol: Cognitive and Behavioral Effects

Program #/Poster #: P294.04

Topic: G.09. Drugs of Abuse and Addiction

Support: CAPES/CNPQ
NIH Grant P60AA011605

Title: Adolescent ethanol exposure alters neuronal firing patterns during conditioned approach in orbitofrontal cortex and nucleus accumbens of female rats

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Abstract: Adolescence is a critical period of brain development. Exposure to drugs of abuse, including alcohol, during the adolescent period can lead to impairments in cortical and limbic brain regions that are still developing. While many adverse and persistent consequences of adolescent intermittent ethanol (AIE) exposure have been described in male rodents, behavioral and neuronal changes promoted by AIE exposure in females are less described. The present study examined the effect of AIE exposure on neuronal activity in the orbitofrontal cortex (OFC) and nucleus accumbens (NAc) of female rats during Pavlovian conditioned approach (PCA). Sprague-Dawley rats received AIE (5g/kg i.g. ethanol, 2-day-on/2-days-off) or water control (CON) throughout adolescence (P25-54). At approximately P70, rats began PCA training. During the PCA sessions, a 30-second conditioned stimulus (CS), presented as a cue light and lever extension, predicted delivery of a 20% sucrose reward. Electrode arrays were implanted in the OFC and NAc allowing single unit neuronal activity recordings during the PCA sessions and also during a reward omission session. Based on a major expression of a sign tracking (ST) phenotype in both groups and to better focus on the effects of AIE, we conducted analysis only in ST animals (AIE: n=14 rats; CON: n=8 rats). AIE-exposed rats exhibited less reward receptacle interaction, represented by a more negative elevation score (ES) in the baseline

session ($P \leq 0.05$), although this difference disappeared during the reward omission session. OFC neurons in AIE rats displayed greater excitation at CS onset and CS offset during the baseline session (P's≤ 0.05) and at CS onset during the omission session ($P \leq 0.05$). These data describe a stronger neural response to cues in the OFC of rats after AIE exposure compared to control rats. AIE exposure also promoted a decrease in NAc neuronal firing rates during the receptacle entry after CS onset ($P < 0.01$). The firing rate to CS onset in a subset of neurons in the OFC and NAc predicted subsequent conditioned approach in a trial-by-trial analysis. The proportion of these correlated neurons was higher in the NAc of AIE-exposed rats than CON in the baseline session ($P < 0.01$), and the difference disappeared in the omission session. In contrast, correlated neurons in the OFC did not differ between AIE and CON rats in the OFC on baseline day, but was smaller in AIE rats on omission day ($P \leq 0.05$). In summary, these results describe persistent AIE-promoted changes in fronto-limbic processing of conditioned cues and approach, indicating that adolescent alcohol exposure can produce lasting effects on the neurocircuitry of reward-motivated behavior.

Disclosures: **J.A. Pochapski:** None. **A. Gómez-A:** None. **S.J. Stringfield:** None. **H. Jaggers:** None. **C. Dannenhoffer:** None. **C.A. Boettiger:** None. **C. Da Cunha:** None. **D.L. Robinson:** None.

Digital Abstract Session

P294. Alcohol: Cognitive and Behavioral Effects

Program #/Poster #: P294.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA P50 AA026117
NIAAA T32 AA007565

Title: Abstinence Driven Changes in Functional Brain Network Activity when Viewing Alcohol Cue Imagery

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Abstract: Alcohol is a leading risk factor for substantial health loss and global disease burden, but cessation of alcohol consumption is highly associated with stress and alcohol cravings, which drive risk of relapse. An increased understanding of the physiology underlying alcohol abstinence will help to illuminate these and other underlying risk factors and contribute to increases in global health. To examine the biological effects of abstinence, this study recruited daily drinkers without symptoms of an alcohol use disorder (AUD), who drink frequently enough that abstinence was a novel or unusual state. These daily drinkers followed their typical consumption pattern and abstained for four sequential days, respectively, in randomized order. Functional MRI (fMRI) data was collected on the fourth day of each experimental period.

During scanning, participants viewed a series of neutral images, followed by a series of alcohol related images. Functional connectivity of the Default Mode Network (DMN) and the Salience Network (SN) was compared across the imagery tasks and the drinking states. Significantly stronger connections were found within both networks during the alcohol viewing task, with no significant difference observed between the neutral and alcohol tasks in a control network. Furthermore, in the SN, this strength difference was only observed following abstinence (no significant difference across tasks following normal drinking), and the increase in connectivity strength during alcohol cue viewing was significantly stronger during abstinence compared to normal drinking in the DMN. These findings suggest an increase in self-referential thinking and selective attention during alcohol cue viewing driven by alcohol abstinence and the personal experiences associated with it: the neutral cues seem personally irrelevant and are filtered for inattention rather than detection, while the alcohol cues are personally significant to the daily drinkers and become highly salient, even after only a brief period of abstinence.

Disclosures: H. Peterson: None. **P.J. Laurienti:** None.

Digital Abstract Session

P294. Alcohol: Cognitive and Behavioral Effects

Program #/Poster #: P294.06

Topic: G.09. Drugs of Abuse and Addiction

Support: CIHR Grant 156070

Title: Approach-avoidance conflict resolution: Sex differences and relationship with voluntary ethanol drinking and compulsive seeking

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Abstract: Alcohol use disorder (AUD) presents a significant health and financial burden for society. Women, although diagnosed with AUD at a lower rate, are susceptible to faster progression to problematic drinking than men. Additionally, women suffer more severe negative health consequences as a result of AUD. Despite this, female subjects are underrepresented in preclinical AUD research. One major component of AUD, compulsive alcohol seeking, may be instigated by an aberrant approach-avoidance conflict resolution, or the inability to avoid a stimulus that simultaneously signals rewarding and aversive outcomes. We had previously shown that repeated cocaine exposure biases male rats towards approach of conflict cues, but little is known about how conflict resolution relates to ethanol drinking in females. The current study used 28 Long-Evans rats of each sex to examine sex differences in approach-avoidance conflict behavior and determined the ability of conflict-induced approach/avoidance tendencies to predict future drinking. First, rats underwent a y-maze conditioning paradigm in which they learned to associate distinct visuo-tactile bar cues with sucrose (appetitive), footshock (aversive), or no outcome (neutral). On the conflict test, the rats were presented with an arm lined with both

appetitive and aversive cues, and an arm containing neutral cues, with the time spent in the conflict vs. neutral arms used to assess approach/avoidance tendencies in the face of conflict. Subsequently, animals were trained to self-administer 20% v/v ethanol on a FR3 15-min schedule. Rats were then challenged with a progressive ratio test, extinction, and mild footshock delivery during ethanol consumption. The following sex differences were observed: 1) males demonstrated greater approach tendencies than females on the conflict test, 2) females, however, displayed higher levels of drinking and reached higher breakpoints in the progressive ratio test, and 3) females lever pressed at higher rates and consumed more ethanol in the face of footshock administration. Approach-avoidance tendencies were not related to ethanol consumption, but higher conflict-induced approach tendencies were associated with increased resistance to footshock in both males and females. This work highlights novel sex differences in approach-avoidance conflict behavior while replicating known sex differences in ethanol drinking. Most significantly, this work identifies approach tendencies in the face of motivational conflict as a potential predictor of compulsive drinking.

Disclosures: T.A. McNamara: None. **R. Ito:** None.

Digital Abstract Session

P294. Alcohol: Cognitive and Behavioral Effects

Program #/Poster #: P294.07

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institutes of Health (2U54GM104942-03) to MLR
Department of Veteran Affairs Career Development Award (1lK2BX002505) to MLR

Title: Investigating the sex-specific effects of socialization on voluntary ethanol consumption using vapor self-administration in rats

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Abstract: Introduction: Social interactions are crucial in adolescent development. Adolescence is characterized as a transitional developmental period between childhood and adulthood that is associated with increased freedom and new experiences that are often peer-influenced. During adolescence, there is a higher prevalence of alcohol consumption, which is in part due to the heightened social facilitating and rewarding effects of alcohol. Previous work shows that excess alcohol consumption during adolescence increases the risk of developing an alcohol use disorder (AUD) later in life. However, the contributions of social interaction and sexual dimorphism in alcohol consumption are not fully understood. Currently, there are several rat models used to study the characteristics of ethanol (EtOH) use and the emergence of AUD. However, many require the addition of a sweetener to coerce EtOH consumption, which has been shown to

confound results in adolescent rats. Here we use a novel self-administration vapor system to investigate the sexual dimorphic nature of socially facilitated EtOH consumption without added sweeteners.
Methods: Adolescent (PND30) and adult (PND70) male and female Sprague-Dawley rats used nosepoke initiated self-administration vapor chambers administering 20mg/L of vaporized EtOH or air (control) in response to each nose poke. Animals underwent intermittent EtOH vapor access every other day for a total of 40 sessions. All animals underwent 10 sessions with their cagemate (social) followed by 10 sessions in isolation, 10 day forced abstinence, 10 isolation sessions, and 10 social sessions.
Results: We observed that while female rats consumed more EtOH than age-matched males, male rats increase EtOH preference over sessions regardless of age. All rats consumed more EtOH during the first social session than the subsequent isolated sessions. There was an increase in EtOH consumption in adult females during the second social session compared to the previous isolated sessions that was not observed in males.
Conclusion: These data reveal that female and male rats are vulnerable to socially facilitated EtOH consumption regardless of age. This is consistent with human data showing that alcohol consumption increases among adolescents and young adults in social settings. However, only male rats demonstrate escalation across sessions indicating that they may be more vulnerable to the development of EtOH dependence. These data demonstrate that the self-administration vapor system is an effective alternative to other methods of voluntary EtOH administration for investigating factors that contribute to alcohol use and escalation during adolescence.

Disclosures: C.D. Walker: None. **H.G. Sexton:** None. **M. Risher:** None.

Digital Abstract Session

P294. Alcohol: Cognitive and Behavioral Effects

Program #/Poster #: P294.08

Topic: G.09. Drugs of Abuse and Addiction

Support: Western University of Health Sciences, College of Pharmacy, Department of Pharmaceutical Sciences

Title: The development of depression-like behavior and alcohol self-administration following exposure to repeated variable stress in female mice: Interaction between age and endogenous dynorphin

Authors: *S. AHMEDYAR, K. LUTFY;
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Abstract: Prolonged activation of endogenous dynorphin-kappa opioid receptor (DYN-KOR) system via chronic stress has been implicated in the development of neuropsychiatric disorders as well as in alcoholism. Prevalence of depression is higher in women and individuals aged 18-35 (SAMHSA, 2017). In a preliminary study, we evaluated the impact of age and the role of

DYN-KOR system in the development of negative affective states that occur following repeated variable stress (RVS) exposure in female mice. RVS paradigm was developed to mimic the variable stress events associated with post-traumatic stress disorder. We also assessed the effect of depression-like behavior on alcohol consumption in an age-related manner. Naïve female preprodynorphin (ppDYN) null mice and their littermates/controls underwent a 10-day RVS exposure followed by alcohol self-administration using (6%, v/v) alcohol (ethyl alcohol) in the home cage. Younger mice lacking DYN (n=4) span 1.4-2.5 months, older mice lacking DYN (n=2) between 11-12 months, and age-matched wild-type (WT) littermates/controls (n=4-5 per age group) were used. The 10-day RVS model consists of forced swim test (FST) at temperatures (15°C, 32°C), physical restraint, and elevated plus maze. Experiments were repeated twice with mice from each genotype and age group, except for old mice due to low availability of KO mice. Experimenter was blind to the mouse genotype. All data were analyzed by repeated measures three-way ANOVA (mean ± S.E.M.) followed by Tukey's *post-hoc* test with significance set at $p<0.05$. Our results revealed an interaction between endogenous DYN and age regarding the development of depression-like behavior and initial alcohol self-administration. Young DYN null mice exposed to RVS exhibited greater depression-like behavior as evident by an increase in immobile time in the FST compared to young WT mice and old DYN null mice. Correspondingly, there was a decrease in swim and escape time. Initial alcohol intake (EtOH g/days/mouse kg) was higher in young mice lacking DYN compared to WT. These differences were not observed in old mice of the two genotypes. Together, these results suggest that there is an influence of age in the development of negative affective states following exposure to RVS and endogenous DYN may serve as a protective role in the brain against stress-induced depression-like behavior in young female mice. Moreover, the neuropeptide attenuates initial alcohol consumption in young but not old female mice. In conclusion, endogenous DYN may serve as an internal brake to reduce the development of depression-like behaviors and the subsequent increase in alcohol self-administration in young female mice.

Disclosures: S. Ahmedyar: None. **K. Lutfy:** None.

Digital Abstract Session

P295. Neural, Molecular and Genetic Mechanisms of Alcohol

Program #/Poster #: P295.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA RO3 AA026758-01A1

Title: High baseline lateral habenula firing correlates with low motivation to seek ethanol in an intermittent access to ethanol paradigm

Authors: *S. TANDON;
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Abstract: Many adults consume alcohol regularly; however, only some vulnerable individuals develop alcohol use disorder (AUD). This variability in the risk for alcohol abuse is multifactorial and includes differences in behavioral traits. The lateral habenula (LHb) is known to mediate aversive state-related behavioral responses. Interestingly, in both humans and rodents, depression-like symptoms are associated with high LHb activity. Moreover, there is a high co-morbidity between major depressive disorder and AUD in humans. Hence, we wanted to determine whether individual variation in LHb activity and behavioral state in ethanol-naïve rats affect their home-cage ethanol drinking patterns. Thus, in this study, we determined the correlation between individual variability in baseline LHb neural activity, the negative-affective state-related ultrasonic vocalizations (USVs; 22-28 kHz), and the extent of ethanol use over time. To do so, we surgically implanted a unilateral 16-wire electrode array in the LHb of adult male Long Evans rats (n=11). Rats were placed in sound-insulated chambers for two hours, where they were free to explore while we simultaneously recorded neuronal signals from their LHb and their ultrasonic vocalizations. Next, these rats underwent an intermittent access to ethanol (IAE) paradigm, where they received 20% ethanol for 24 hours on alternate days and ad-libitum water in their home cages for four weeks. The change in ethanol intake over time differed between rats, with some rats escalating their ethanol intake in the four weeks, while other rats showed no meaningful change in ethanol intake over time as compared to the first session. We found a significant negative correlation between average LHb firing rates and changes in ethanol intake in the first week of IAE, such that rats with higher baseline LHb activity did not escalate their ethanol intake with more sessions. We also found a weak positive correlation between the number of 22-28 kHz USVs and the average baseline LHb firing rates of these rats. These results indicate that higher baseline LHb neuronal activity in individual rats is associated with decreased motivation to seek ethanol during the early stages of ethanol consumption. Currently, we are determining the correlation between the number of 22-28 kHz vocalizations and the positive affective state vocalizations (50-55 kHz) emitted by adult male Long Evans rats rats (n=12) prior to their ethanol sessions each week in the IAE paradigm and the increase in ethanol intake over time.

Disclosures:

Digital Abstract Session

P295. Neural, Molecular and Genetic Mechanisms of Alcohol

Program #/Poster #: P295.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R00AA025384

Title: Intermittent access to alcohol drives increased colocalization of glutamate and GABA markers in ventral pallidum

Authors: *R. GAO, G. BADGER, M. CARPIO, A. SOOD, C. A. CAYTON, J. M. RICHARD; Univ. of Minnesota, Minneapolis, MN

Abstract: Compulsive use is a major characteristic of alcohol use disorder. Aversion-resistant drinking is a model of compulsive alcohol use, in which animals continue to consume alcohol despite its adulteration with bitter quinine. Neuroadaptations in corticostriatal inputs to nucleus accumbens are important for aversion-resistant drinking, but it remains unclear how these changes impact downstream mediators of aversion-related behaviors. Ventral pallidum (VP) is a major output of the nucleus accumbens known for its role in reward and addiction. Recent work has ascribed reward-related functions largely to VP GABA neurons, whereas VP glutamate neurons mediate aversion-related behaviors. Despite this, whether or how VP is involved in aversion-resistant alcohol drinking remains unknown. In this study, we examined how intermittent access to alcohol (IAA) alters VP neurons and their activity during aversion-resistant drinking. Here, Wistar rats (M:F = 17:19) were allowed to voluntarily drink 15% alcohol with or without adulteration of 0.03 g/l quinine in operant chambers after different durations of IAA experience (3, 8 and >14 weeks). After a final alcohol plus quinine drinking test, brains were quickly extracted and flash frozen. We applied fluorescence *in situ* hybridization (RNAscope) on VP slices, labeling vesicular glutamate transporter 2 (*Slc17a6* [*Vglut2*]), Glutamate Decarboxylase 1 (*Gad1*), and c-fos (*Fos*) RNA to evaluate cell-type-specific activity. Longer IAA increased alcohol consumption, specifically in male rats, but all exposure groups were resistant to quinine adulteration. As predicted, in rats with 8 and >14 weeks of IAA, a lower proportion of *Vglut2*-positive neurons were *Fos*-positive. Interestingly, we found that rats with > 14 weeks of IAA also had a higher proportion of *Vglut2*/*Gad1* co-labeled neurons in VP. Female rats also had a higher proportion of *Vglut2*/*Gad1* co-labeled neurons, but this sex difference did not depend on the length of alcohol exposure. Altogether, these results suggest that intermittent alcohol experience both 1) blunts recruitment of (aversion sensitive) glutamate neurons in VP, and 2) increases co-expression of glutamate/GABA neurons markers, potentially altering downstream co-release. Both effects could contribute to aversion-resistant drinking. Additionally, sex differences in *Vglut2*/*Gad1* co-expression may impact both baseline aversion processing and the development of aversion-resistant drinking. Alterations in cell-type specific activity or co-release in VP are promising targets for further efforts to investigate the mechanisms underlying compulsive alcohol use.

Disclosures: R. Gao: None. **G. Badger:** None. **M. Carpio:** None. **A. Sood:** None. **C.A. Cayton:** None. **J.M. Richard:** None.

Digital Abstract Session

P295. Neural, Molecular and Genetic Mechanisms of Alcohol

Program #/Poster #: P295.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5R01AA024044-04
NSF GRFP Fellowship
BBRF NARSAD Young Investigator Award

Title: Binge drinking induces age-dependent protein changes in the mouse BNST

Authors: *S. L. SCUDDER, C. L. JIMENEZ CHAVEZ, T. N. KELLEY, K. K. SZUMLINSKI; Psychological & Brain Sci., Univ. California-Santa Barbara, Santa Barbara, CA

Abstract: Patterns of alcohol consumption differ across age groups and age of drinking onset can contribute to adult drinking behavior and likelihood of developing an alcohol use disorder. Binge drinking in particular is highly prevalent amongst adolescents and serves as a risk factor for substance abuse later in life. In rodent models, behavioral manifestations of withdrawal symptoms are more pronounced in adult drinkers relative to adolescents, despite increased consumption in younger animals. Multiple weeks of daily binge ethanol drinking produces robust changes in neuronal activity and protein expression in brain areas such as the nucleus accumbens and the bed nucleus of the stria terminalis (BNST). While the latter region is thought to be essential for controlling an animal's propensity to binge drink, little is known about the age dependence of alcohol-evoked changes in protein expression and neuronal physiology therein. To address this, adolescent (P28) and adult (P56) C57BL/6J mice of both sexes were allowed to voluntarily binge drink during daily 2-hour drinking-in-the-dark sessions for two weeks. Mice were subjected to behavioral evaluations of negative affect and tissue was collected from the anterior dorsal BNST for Western blotting. As in previous studies, adolescent mice consumed more alcohol during sessions on average and showed reduced measures of negative affect at an early withdrawal timepoint. In the BNST, levels of calcium/calmodulin-dependent kinase II alpha (CaMKIIalpha) and extracellular signal-regulated kinase (ERK) were more elevated after drinking in adolescents compared to adults. This engagement of BNST proteins involved in excitatory synaptic scaffolding and plasticity could contribute to adolescent resilience to withdrawal symptoms. Future studies will explore age-dependent differences in BNST plasticity electrophysiologically and examine changes in this region after prolonged withdrawal.

Disclosures: S.L. Scudder: None. **C.L. Jimenez Chavez:** None. **T.N. Kelley:** None. **K.K. Szumlinski:** None.

Digital Abstract Session

P295. Neural, Molecular and Genetic Mechanisms of Alcohol

Program #/Poster #: P295.04

Topic: G.09. Drugs of Abuse and Addiction

Title: Lrrk2, a Parkinson's-associated protein, regulates striatal D1 receptor function and alcohol drinking in mice

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Abstract: The transition from hedonic to compulsive drinking is a hallmark of alcohol use disorders. Alcohol exerts acute and chronic actions on striatal circuitry and function, which are thought to promote compulsive-like alcohol drinking despite negative consequences. Leucine-rich repeat kinase 2 (Lrrk2) is a protein with high expression in the striatum which has been shown to regulate dopamine receptor function. Mutations in the Lrrk2 gene are the most common cause of genetic form of Parkinson disease and have been shown to impair D1 receptor internalization and to enhance D1R signaling. In this study, we test the hypothesis that Lrrk2 in striatal neurons regulates dopamine receptor signaling to affect alcohol reinforcement and compulsive-like drinking. We found that acute alcohol administration or alcohol drinking decrease LRRK2 phosphorylation and its kinase activity. The alcohol induced inhibition of LRRK2 kinase activity was seen preferentially in the dorsomedial striatum (DMS), a region involved in mediating goal directed actions. We generated cell-specific knockout mice and evaluated whether a lack of Lrrk2 gene in different neuron types in the striatum would affect alcohol consumption in mice. Mice with global deletion of Lrrk2 or cell-specific deletion of Lrrk2 in D2-expressing neurons in the striatum showed no difference in alcohol drinking compared with littermate controls, however, deletion of Lrrk2 in D1R-positive neurons (D1-Lrrk2 KO) produced an increase in alcohol consumption relative to littermate control mice. When trained to self-administer alcohol in an operant self-administration task, D1-Lrrk2 KO mice also showed higher responding and intake. In addition, these mice also showed higher breakpoints and drinking was more resistance to shock punishment and taste adulteration. These results indicated higher motivation to consume alcohol and more compulsive-like alcohol drinking in mice lacking Lrrk2 in D1 MSN. We further explore possible mechanisms underlying these observations and found that D1 receptors function is enhanced in D1-Lrrk2 KO mice. D1-Lrrk2 KO mice show an upward shift in the dose response to the locomotor effects of D1-like agonist. Moreover, electrophysiological recordings from D1R positive neurons in the DMS of D1-Lrrk2 KO mice showed an increase in the excitability of these cells in response to D1-like receptor agonist compared to D1R neurons of littermate wild type mice. These findings suggest that loss of Lrrk2 in direct-pathway striatal neurons causes an upregulation of D1R response, both at the cellular and behavioral level, which is conducive to excessive drinking and compulsive-like alcohol use.

Disclosures: D. da Silva e Silva: None. **A. Matsui:** None. **A. Mamais:** None. **E. Murray:** None. **D. Ron:** None. **M.R. Cookson:** None. **V.A. Alvarez:** None.

Digital Abstract Session

P295. Neural, Molecular and Genetic Mechanisms of Alcohol

Program #/Poster #: P295.05

Topic: G.09. Drugs of Abuse and Addiction

Support: Dutch NWO-Vidi Grant

Title: The role of the nucleus accumbens shell in alcohol use despite negative consequences in rats

Authors: *A. J. MCDONALD, I. ALONSO LOZARES, D. SCHETTERS, Y. VAN MOURIK, T. J. DE VRIES, N. J. MARCHANT;
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Abstract: Alcohol use is widespread across most societies. While most people can control their alcohol use, a vulnerable sub-population may develop alcohol use disorder (AUD), characterized by continued use of alcohol despite negative consequences. We used a rodent model of alcohol use despite negative consequences, to identify neurobiological differences that may underlie addiction-like behavioral vulnerability. We and others have previously shown that this approach reliably identifies two sub-populations of rats. One group substantially decreases alcohol use in the face of punishment (punishment-sensitivity, controlled use) and another group continues alcohol use despite negative consequences (punishment-resistance or insensitivity, addictive-like behavior). In this study we aim to identify the role of the nucleus accumbens shell (NAcSh) in this divergent response to punishment of alcohol use. We trained Long-Evans outbred rats (n=60, m/f) to self-administer alcohol, and then introduced punishment of alcohol self-administration with response-contingent foot-shock. We found a significant proportion of rats persisted with alcohol self-administration in the face of mild punishment. Interestingly, we found that more female rats developed punishment-resistant alcohol use compared to male rats. In one group of rats (n=24) we used immunohistochemical detection of the neuronal marker of activity c-Fos to identify brain activity associated with punishment-resistant alcohol use. We found that lower c-Fos expression in NAcSh was associated with punishment-resistant alcohol use, compared to punishment-sensitive rats, and rats tested without punishment. To test for a causal role of NAcSh activity in punishment-resistant alcohol use, in another group of rats (n=36) we used chemogenetic inhibition (hM4Di) of NAcSh throughout different phases of the experiment. We found that chemogenetic inhibition of NAcSh had no effect on either unpunished alcohol self-administration or motivation for alcohol in a progressive ratio test. During punishment however, NAcSh inhibition increased alcohol self-administration in the punishment-resistant rats. In an unpunished probe test, alcohol-seeking was induced in all rats regardless of punishment-sensitivity. This work identifies an important role of NacSh in the expression of behavioral control in response to alcohol use despite negative consequences. Understanding the contribution of NAcSh, and associated neural circuits, in the suppression of addictive-like behavior will provide us with a greater understanding of the neurobiological underpinnings of AUD, and may lead to future treatment developments.

Disclosures: A.J. McDonald: None. **I. Alonso Lozares:** None. **D. Schetters:** None. **Y. van Mourik:** None. **T.J. de Vries:** None. **N.J. Marchant:** None.

Digital Abstract Session

P295. Neural, Molecular and Genetic Mechanisms of Alcohol

Program #/Poster #: P295.06

Topic: G.09. Drugs of Abuse and Addiction

Support: AA7565

AA26117
AA17531
AA26551
AA26455

Title: Comparative effects of selective chemogenetic inhibition of projections from basolateral amygdala to nucleus accumbens core and the ventral subiculum to nucleus accumbens shell on an operant alcohol self-administration task

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Abstract: Emerging evidence suggests inputs from the basolateral amygdala and ventral subiculum to the nucleus accumbens play a prominent role in a wide array of motivated behaviors, including those associated with drugs of abuse. However, there remains a paucity of research evaluating the involvement of these circuits in alcohol-drinking related behaviors. Thus, the present experiments aimed to characterize the comparative contributions of these projections on motivation for alcohol in an operant self-administration task designed to methodologically dissociate discrete phases of appetitive and consummatory behavior. Male Long-Evans rats (N=16) were trained to complete a 20 lever press response requirement to receive access to a sipper tube filled with a 10% ethanol solution for free consumption. Additionally, extinction probe trials during which no amount of lever pressing resulted in delivery of the sipper tube were included in the experimental design to assess responding independent of immediate reinforcement. Following training, animals received surgery for viral injection of inhibitory (Gi) DREADDs into either the BLA or vSub and bilateral guide cannula implantation into the NAcC or NAcSh, respectively. Following recovery and baseline behavior monitoring, counterbalanced injections of clozapine n-oxide (30, 100, and 300 nM) or vehicle (ACSF) were delivered once weekly 5 minutes prior to behavioral testing, such that each animal received each dose once. Histological confirmation of viral expression and cannula placement is ongoing. We report that temporary inactivation of either the BLA-NAcC or vSub-NAcSh reduced extinction probe responding with minimal effects on intake, suggesting that both circuits play an important role in appetitive, but not consummatory, alcohol-drinking related behaviors. Furthermore, inhibition of BLA-NAcC, but not vSub-NAcSh, produced a significant alteration in extinction responding for sucrose. Together, these studies have further elucidated novel circuits that might be targeted by pharmacological treatment for alcohol seeking.

Disclosures: H.N. Carlson: None. **J. Weiner:** None.

Digital Abstract Session

P295. Neural, Molecular and Genetic Mechanisms of Alcohol

Program #/Poster #: P295.07

Topic: G.09. Drugs of Abuse and Addiction

Support: AA26117
AA17531
AA26551
AA26455

Title: Chronic intermittent ethanol dependent hyperexcitability of the basolateral amygdala to ventral subiculum projection and intrinsic activity of the ventral subiculum

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Abstract: The hippocampus has been implicated in the pathophysiology of alcohol use disorder (AUD) in both human and animal studies. While perturbations in hippocampal function are known to contribute to the cognitive deficits associated with AUD, there is a growing appreciation that this brain region, and particularly the ventral domain, also plays a role in the negative affective symptoms of this disease, as well as those of comorbid conditions like generalized anxiety disorder and post-traumatic stress disorder. The ventral hippocampus (vHC) receives strong excitatory input from the basolateral amygdala (BLA), a central hub of emotional processing that is also known to play an integral role in AUD and comorbid affective conditions. Rodent studies have shown that the BLA-vHC projection bidirectionally modulates anxiety-like behaviors and both brain regions exhibit hyperexcitability in animal models of AUD and anxiety/stressor disorders. However, no studies to date have examined the effects of chronic alcohol on the BLA-vHC circuit. In the present study, we used ex vivo electrophysiology and optogenetics to examine the effects of a well-established rodent model of AUD, chronic intermittent ethanol exposure (CIE), on BLA-vHC synaptic transmission as well as putative intrinsic vHC synaptic activity, in male Long-Evan rats. We discovered that the BLA primarily innervates pyramidal neurons in the subicular region of the vHC (vSub) and that withdrawal from CIE significantly increases excitatory and inhibitory neurotransmission in the BLA-vHC circuit. Notably, CIE led to an overall increase in excitatory/inhibitory ratios along with an increase in the AMPA/NMDA ratio, and no change in pair-pulse ratios, consistent with a postsynaptic increase in excitability. CIE treatment also led to an increase in intrinsic network excitability as evidenced by an increase in sEPSC frequency and the duration of elevated spontaneous excitatory neurotransmission. Overall, our findings suggest that a hyperexcitable state in BLA-vSub specific inputs, as well as intrinsic inputs to vSub pyramidal neurons, may contribute to the negative affective behaviors associated with CIE.

Disclosures: E.C. Bach: None. **S. Ewin:** None. **H. Carlson:** None. **J.L. Weiner:** None. **A. Baldassaro:** None.

Digital Abstract Session

P295. Neural, Molecular and Genetic Mechanisms of Alcohol

Program #/Poster #: P295.08

Topic: G.09. Drugs of Abuse and Addiction

Support: Department of Veterans Affairs Merit Research Award I01BX002661
NIH Grant AA028175-01

Title: Clock genes in the shell region of nucleus accumbens regulate binge drinking in mice

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Abstract: INTRODUCTION: Binge drinking is a deadly pattern of alcohol abuse and poses a serious threat to the health and economy of society. Clinical and preclinical studies suggest that misalignment in circadian timing due to genetic variation in clock genes is strongly associated with alcohol abuse, however, the neuroanatomical basis for such a relationship is unknown. The shell region of nucleus accumbens (NAcSh) is well-known for its role in binge drinking. Hence, we ask if clock genes in the NAcSh regulate binge drinking? **METHODS:** To address this question, two experiments were performed on C57BL/6J mice exposed to extensively used, 4-day drinking-in-the-dark (DID) paradigm. The first experiment examined the effects of binge drinking on the expression of circadian clock genes (Clock, Per1, and Per2) in the NAcSh and suprachiasmatic nucleus (SCN). Mice were euthanized on Day 4 after four hours of alcohol consumption and brains were processed for RT-PCR. The second experiment determined the effects of antisense-induced knockdown of circadian clock genes in the NAcSh, using bilateral guide cannula above the NAcSh, on binge drinking. On Day 4, one hour prior to the onset of alcohol exposure, mice were bilaterally infused with either a mixture of Clock, Per1 and Per2 antisense oligodeoxynucleotides (AS-ODNs; Antisense group) or nonsense/random ODNs (R-ODNs; Control group) into the NAcSh. Blood alcohol concentration was measured to confirm binge drinking. Separate groups of mice, infused with AS or R-ODNs were used to examine sucrose (10% w/v) and water consumption using the DID paradigm. Microinfusion sites were histologically verified using cresyl violet staining. **RESULTS:** As compared to sucrose, binge alcohol consumption increased the expression of circadian genes in the NAcSh but not in the SCN. Knockdown of clock genes in the NAcSh caused a significant reduction in the amount of alcohol consumed during four hours of binge drinking as compared to the control treatment. No differences were found in sucrose and water consumption. **CONCLUSIONS:** Our results suggest that the circadian misalignment of clock genes in the NAcSh plays a crucial role in binge drinking.

Disclosures: R. Sharma: None. **M. Parikh:** None. **V. Mishra:** None. **P. Sahota:** None. **M. Thakkar:** None.

Digital Abstract Session

P295. Neural, Molecular and Genetic Mechanisms of Alcohol

Program #/Poster #: P295.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH(AA028175-01)
Department of veterans Affairs Merit Research Award(I01BX002661)

Title: Cholinergic interneurons in the shell region of neucleus accumbens promotes binge drinking in mice

Authors: *M. PARIKH, R. SHARMA, V. MISHRA, P. SAHOTA, M. THAKKAR; Research, Neurol., Truman VA Med. Center/University of Missouri, Columbia, MO

Abstract: Background: Binge drinking is the most common and deadly pattern of alcohol consumption. However, the underlying neuronal mechanism is not clearly understood. Since cholinergic interneurons, present in the nucleus accumbens (NAc), are crucial for reward and addiction, we hypothesized that chemogenetic activation of CIN in the NAc will increase binge drinking.**Methods:** To address this question, we used C57BL/6J transgenic mice expressing Cre-recombinase in the cholinergic neurons. Mice were bilaterally infused either with excitatory DREADD (Gq, AAV/hSyn-DIO-hM3Dq-mCherry; 500nL/side) or non-specific virus (Ns, AAV/hSyn-DIO-mCherry; 500nL/side; Control) in NAcSh and left them undisturbed for 21 days to allow it to express. Next, mice were subjected to binge alcohol consumption using the extensively used, 4-day drinking-in-the-dark (DID) paradigm. On Day 4, two hours prior to the onset of alcohol exposure, mice either received clozapine-N-oxide (CNO; 5 mg/Kg; Ns-CNO and Gq-CNO; for CIN activation) or saline (Ns-Saline and Gq-Saline; Controls). Blood alcohol concentration was measured to confirm binge drinking. Separate groups of mice, infused with Gq or Ns, were used to examine sucrose (10% w/v) using the DID paradigm. Microinfusion sites were histologically verified using choline acetyltransference (ChAT) immunofluorescence.**Results:** As compared to the Controls, mice in the Gq-CNO group displayed a significant increase in the amount of alcohol consumed during four hours of binge drinking on day 4 of the DID paradigm. No differences were found in sucrose consumption.**Conclusions:** Our results suggest that binge drinking is regulated by CIN neurons in the NAcSh.

Disclosures: M. Parikh: None. **R. Sharma:** None. **V. Mishra:** None. **P. Sahota:** None. **M. Thakkar:** None.

Digital Abstract Session

P295. Neural, Molecular and Genetic Mechanisms of Alcohol

Program #/Poster #: P295.10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA020919
NIH Grant DA035958

Title: Cd5 knockout mice exhibit reduced sensitivity to the sedative effects of alcohol and alcohol consumption

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Abstract: Cluster of differentiation 5 (CD5), a transmembrane protein expressed in both T and B lymphocytes, modifies inhibitory synaptogenesis during development and displays altered expression profiles following chronic ethanol (EtOH) use. Specifically, the number of CD5+ B cells is reduced during EtOH withdrawal while the number of T cells is increased. Given recent research suggesting that some of EtOH effects are accounted for by neuroimmune interactions, we assessed the effects of EtOH on neuronal activity in the ventral tegmental area (VTA), dopamine release in the nucleus accumbens (NAc), and behavior in CD5 knockout (KO) mice vs wild-type (WT) controls. There was no significant difference in baseline firing rate or EtOH inhibition of VTA GABA neurons or evoked DA release in the NAc of CD5 KO vs WT mice. However, CD5 KO mice were characterized by markedly reduced DA overflow (i.e., frequency response) compared to WT mice. CD5 KO mice were characterized by lower baseline locomotor activity in the open-field paradigm, with reduced sensitivity to EtOH sedation (0.5 - 2.0 g/kg) and loss of righting reflex (4.0 g/kg) compared to WT mice. In a 24-hr access two-bottle choice drinking paradigm, CD5 KO mice exhibited markedly less consumption of EtOH than WT mice (9.4 vs 19.1 g/kg/day). These results suggest that CD5 KO mice are less sensitive to some of the effects of EtOH and that peripheral neuroimmune mechanisms involving CD5 may be a factor in EtOH consumption and sedation.

Disclosures: B.M. Williams: None. **A.J. Payne:** None. **J.D. Obray:** None. **C.J. Chapman:** None. **C.A. Small:** None. **J.T. Yorgason:** None. **S.K. Weber:** None. **S.C. Steffensen:** None. **J.N. Brundage:** None.

Digital Abstract Session

P295. Neural, Molecular and Genetic Mechanisms of Alcohol

Program #/Poster #: P295.11

Topic: G.09. Drugs of Abuse and Addiction

Title: Mechanisms underlying alcohol augmentation of COVID-19 pathologies

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Abstract: The coronavirus disease 2019 (COVID-19) pandemic is a worldwide crisis caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Many COVID-19 patients present with fever in the early phase, with some progressing to a hyperinflammatory phase.

Ethanol (EtOH) can increase intestinal permeability to gut-derived microbial endotoxins, leading to systemic inflammation. We hypothesized that exposure to EtOH can thereby augment SARS-CoV-2-induced inflammation. Unfortunately, medical records of COVID-19 patients are sparse for drinking history. Therefore, this study utilized an *in silico* bioinformatic approach to examine the relationship between EtOH consumption and COVID-19 pathologies. Molecules associated with EtOH exposure were identified by analysis with QIAGEN Knowledge Base (QKB). Molecules associated with COVID-19 were identified from published literature on COVID-19, QIAGEN Coronavirus Network Explorer (QCNE), and analysis of the RNA-Sequencing data of autopsied lungs of COVID-19 patients retrieved from the Gene Expression Omnibus (GEO) database to identify genes whose expression was perturbed by COVID-19. QIAGEN Ingenuity Pathway Analysis (IPA) was used to analyze molecules associated with both EtOH and COVID-19. Our analysis suggests that EtOH exposure may augment the effects of SARS-CoV-2 infection on hepatic fibrosis signaling pathway, cellular metabolism and homeostasis, inflammation, and neuroinflammation, and that EtOH may enhance the activity of key meditators including cytokines, such as interleukin (IL)1β, IL6, interferon (IFN)γ, and tumor necrosis factor (TNF) and transcription factors, such as hypoxia inducible factor (HIF)1α, JUN, Nuclear factor (NF)κB, and signal transducers and activators of transcription (STAT) while inhibiting the activity anti-inflammatory mediators, such as glucocorticoid receptor (GR) and peroxisome proliferator-activated receptor (PPAR)/retinoid X receptor (RXR). Furthermore, five of these inflammatory mediators, IL1β, IL6, TNF, JUN, STAT, were mapped to ten out of fourteen pathways, including high mobility group protein (HMGB)1, IL1, and IL-6 signaling pathways, predicted to associate with SARS-CoV-2 proteins. This augmentation of symptoms by alcohol can be more fully explored when more complete medical records are available from a large cohort.

Disclosures: H. Zhou: None. **W. Huang:** None. **A. Montero:** None. **D. Goldman:** None. **S.L. Chang:** None. **C. Hodgkinson:** None.

Digital Abstract Session

P296. Behavioral and Neural Mechanisms of Cannabinoids

Program #/Poster #: P296.01

Topic: G.09. Drugs of Abuse and Addiction

Support: Kakenhi Grant-in-Aid 17F17388
JSPS Fellowship P17388

Title: Rodent 3D motion capture system uncovers distinct behavioral effects of cannabinoids in freely moving mice

Authors: *B. M. IGNATOWSKA-JANKOWSKA[1], A. GURKAN-OZER[1], A. KUCK[1], M. NIPHAKIS[2], D. OGASAWARA[2], B. CRAVATT[2], M. UUSISAARI[1];
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Abstract: One of behavioral effects of cannabinoid CB_1 receptor activation is inhibition of locomotor activity. However, effects of endogenous cannabinoids on behavior have not been extensively studied. Here we aimed to assess whether enhancing endocannabinoid signaling produces effects similar to exogenous cannabinoid agonists.To elevate signaling of endocannabinoids 2-Arachidonoylglycerol and Anandamide we used selective inhibitors (MJN110 and PF3845, respectively) of Monoacylglycerol lipase and Fatty Acid Amide Hydrolase, enzymes responsible for their degradation. High-speed, high-resolution 3D motion capture system (Qualisys) was used to track movement (3D trajectories and velocity of markers) during voluntary locomotor tasks: open field exploration, vertical and horizontal climbing, beam walking, aerial righting reflex.The results revealed distinct behavioral phenotypes induced by different cannabinoids. Low doses of synthetic cannabinoid agonist CP55,940 (0.03, 0.1, 0.3 mg/kg) produced significant bidirectional, task-dependent effects: a decrease of activity and widened stance in the open field, but increased activity in the vertical climbing task (n=10). MJN110 (1.25, 2.5 mg/kg) significantly increased the activity both in the open field and climbing task (n=12). PF3845 significantly decreased locomotor activity (10, 30 mg/kg) in both tasks without affecting stance width (n=12). Moreover, MJN110 (1.25 mg/kg, n=12) increased escape behavior in the open field. In our models, CB1 receptor antagonist AM251 (3 mg/kg) induced significant decrease in activity in all tasks, that was more pronounced than inhibition caused by PF3845 and occluded stimulatory effects induced by MJN110.The results suggest that selective elevation of 2-AG and AEA signaling results in distinct, bidirectional effects on behavior that are different from exogenous cannabinoid agonists. Furthermore, the work highlights the strength of 3D motion capture as precise and sensitive tool to evaluate wide range of behaviors in rodents.

Disclosures: B.M. Ignatowska-Jankowska: None. **A. Gurkan-Ozer:** None. **A. Kuck:** None. **M. Niphakis:** None. **D. Ogasawara:** None. **B. Cravatt:** None. **M. Uusisaari:** None.

Digital Abstract Session

P296. Behavioral and Neural Mechanisms of Cannabinoids

Program #/Poster #: P296.02

Topic: G.09. Drugs of Abuse and Addiction

Support: Marshall Scholarship Awarded to M.L.M.
CIHR Project Grant Awarded to C.A.W.

Title: The effects of acute and chronic cannabis sativa and indica consumption on decision-making strategies used by rodents gambling: a cross-over design

Authors: *M. R. LYSENKO-MARTIN[1], C. P. HUTTON[2], H. BRODIE[1], C. A. WINSTANLEY[1];
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Abstract: Cannabis is being legalized globally at increasing rates, making it pressing to understand how acute or chronic exposure to cannabis perturbs decision-making and leads to addiction. The impact of cannabis exposure on decision-making can be quantified using a validated rodent assay of cost-benefit decision-making, the rat Gambling Task, in which exposure to cue-paired probabilistic rewards triggers neurobiological adaptations leading to a pro-addiction phenotype. Long-Evans male rats (N=32) were orally dosed with *Cannabis sativa*, *Cannabis indica*, or a vehicle-control solution daily for 25 days, and regularly performed the cued rat Gambling Task. Animals then underwent a washout period of 40 days with no drug or gambling, after which a treatment cross-over was performed where animals previously given vehicle were chronically dosed with *sativa*, and those previously given *sativa* were given vehicle, and their decision-making strategies were again evaluated on the free choice portions of the gambling task. This cross-over was performed because it is important to distinguish whether decision-making strategies were selected due to the influence of the drug on choice or the influence of the drug on learning. A linear mixed-effects model was used to evaluate rodent risk-profiles. Acutely, rodents exposed to *sativa* exhibited biased decision-making in favour of riskier choices, compared to the optimal response strategies used by *indica*-exposed and control rats. Over chronic use, *sativa*-exposed rats caught up to controls and began to exceed the optimal decision-making strategies compared to all other groups. When the groups were switched after a washout period, choice preference remained unchanged in all groups, but rodents receiving *sativa* took longer to make a choice and completed fewer trials. These results suggest that regular consumption of *sativa* may delay cued reward learning by initially biasing users towards high-risk/high-reward decisions, while *indica* does not impair optimal decision-making on a gambling task. All animals in all conditions end up learning the most optimal choice preference, but the time they take to do so, and the impulsiveness with which they perform, are negatively affected by *Cannabis sativa.*

Disclosures: M.R. Lysenko-Martin: None. **C.P. Hutton:** None. **H. Brodie:** None. **C.A. Winstanley:** None.

Digital Abstract Session

P296. Behavioral and Neural Mechanisms of Cannabinoids

Program #/Poster #: P296.03

Topic: G.09. Drugs of Abuse and Addiction

Support: College of Medicine, University of Saskatchewan

Title: Exposure to high-THC cannabis smoke during gestation alters development and anxiety of Sprague Dawley rat offspring

Authors: T. SANDINI[1], A. ROEBUCK[2], M. HERDZIK[1], D. MCELROY[1], T. ONOFRYCHUK[1], S. ORVOLD[1], A. ZAGZOOG[1], Q. GREBA[1], R. LAPRAIRIE[1], *J. G. HOWLAND[1];
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Abstract: Rationale and Objective: Cannabis use among pregnant women is a growing public health concern. With recent legalization of cannabis in many jurisdictions, there is an urgent need to understand the impact of its use during pregnancy on fetal neurodevelopment and behavioural outcomes in exposed offspring. Currently, there is limited data regarding the effects of exposure to the smoke of combusted cannabis flowers in preclinical rodent studies. To address this limitation, we tested the effects of repeatedly exposing female Sprague Dawley (SD) rats during pregnancy on behaviour of the male and female offspring. **Methods**: Rats were exposed to the smoke of combusted cannabis flowers in two experiments using a commercially available system from La Jolla Alcohol Research, Inc. In experiment one, we performed an acute dose response experiment to assess plasma levels of Δ9-tetrahydrocannabinol [THC] and its metabolites following exposure to the smoke generated by combusting 60, 200, or 600 mg of a commercially available strain of cannabis flower (Aphria 'Mohawk'; 19.51% THC, <0.07% cannabidiol [CBD]). In a second experiment, pregnant SD rats were exposed to 200mg of cannabis smoke (n = 12) or a room air control (n = 10) daily from gestational day (GD) 6 to 20. We used this model to determine the effects of prenatal cannabis smoke exposure on maternal reproductive parameters and long-term behavioral alterations of male and female adulthood offspring. **Results:** Plasma THC levels were approximately 1 ng/mL and 10 ng/mL in rats exposed to 200 or 600 mg of cannabis, respectively. 11-OH-THC and 11-COOH-THC were also significantly increased in a dose-dependent manner. Exposure of pregnant rats to the smoke generated by combusting 200 mg of cannabis flower from GD6 to GD20 significantly decreased body temperature in the cannabis exposed dams immediately following each session. However, maternal weight gain, food intake, gestational length, litter number, and litter weight were not altered. There was a significant increase in the male-to-female ratio in the cannabis-exposed litters. In young adulthood, both male and female cannabis smoke-exposed offspring spent less time exploring the inner zone of an open field. Analysis of sociability and cognition in the offspring are in progress. **Conclusions**: These results demonstrate that repeated exposure to high-THC containing cannabis smoke during gestation alters sex ratios of the offspring and anxiety-like behaviors in the offspring.

Disclosures: T. Sandini: None. **A. Roebuck:** None. **M. Herdzik:** None. **D. McElroy:** None. **T. Onofrychuk:** None. **S. Orvold:** None. **A. Zagzoog:** None. **Q. Greba:** None. **R. Laprairie:** None. **J.G. Howland:** None.

Digital Abstract Session

P296. Behavioral and Neural Mechanisms of Cannabinoids

Program #/Poster #: P296.04

Topic: G.09. Drugs of Abuse and Addiction

Support: Canadian Insitute of Health Research (CIHR)
Saskatchewan Health Research Foundation (SHRF)
University of Saskatchewan College of Medicine
Natural Sciences and Engineering Research Council (NSERC)

Title: Dissociable changes in spike and wave discharges following exposure to injected cannabinoids and smoked cannabis in Genetic Absence Epilepsy Rats from Strasbourg

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Abstract: In the last decade there has been significant interest in the use of cannabinoids for treatment of many epilepsies including absence epilepsy (AE). Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model many aspects of human AE including the presence of spike-and-wave discharges (SWDs) on electroencephalogram (EEG) and behavioural comorbidities, such as elevated anxiety. Compared to Non-Epileptic Control (NEC) rats we have recently identified regional alterations in the endocannabinoid system in GAERS and shown that treatment with a type 1 cannabinoid receptor positive allosteric modulator reduces SWDs. However, the effects of cannabis plant-based phytocannabinoids have not been tested in GAERS. Therefore, we investigated how SWDs in GAERS may be altered by the 2 most common phytocannabinoids, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), and exposure to smoke from 2 different strains of cannabis. Animals were implanted with bipolar electrodes in somatosensory cortex and EEGs recorded for 2 hours. Injected THC (1-10 mg/kg, *i.p.*) dose-dependently increased SWDs to over 200% of baseline. In contrast, CBD (30-100 mg/kg, *i.p.*) produced an ~50% reduction in SWDs. Exposure to smoke from a commercially available strain of high-THC cannabis (Mohawk, Aphria) increased SWDs in GAERS. This effect may be strain/dose-dependent as a low-THC/high-CBD strain of cannabis (Treasure Island, Aphria) did not significantly increase or decrease seizures. Lastly, pre-treatment with a CB1R antagonist (SR141716A) did not prevent the high-THC cannabis smoke from increasing SWDs suggesting that the THC-mediated increase is not CB1R-dependent. Together these experiments provide initial evidence that acute phytocannabinoid administration exerts biphasic modulation of SWDs and may differentially impact patients with AE.

Disclosures: A.J. Roebuck: None. **Q. Greba:** None. **T.J. Onofrychuk:** None. **D.L. McElroy:** None. **T.M. Sandini:** None. **A. Zagzoog:** None. **J. Simone:** None. **S. Cain:** None. **T.P. Snutch:** None. **R. Laprairie:** None. **J.G. Howland:** None.

Digital Abstract Session

P296. Behavioral and Neural Mechanisms of Cannabinoids

Program #/Poster #: P296.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA0500116

NIH Grant DA044118

Title: Impact of adolescent THC exposure on reward-related behavior in adulthood

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Abstract: Adolescent brains are particularly vulnerable to the adverse effects of cannabis exposure, which include increased risk of exhibiting neuropsychiatric disorder and neurocognitive dysfunction. Preclinical studies have established that repeated endocannabinoid receptor stimulation during adolescence can induce long-term cognitive and behavioral dysregulation in adult animals. Notably, these studies indicated that adolescent cannabis exposure can induce long-lasting alterations in reward consumption and reward-motivated behavior. We probed the specific long-term effects of adolescent tetrahydrocannabinol (THC) exposure on hedonic, motivational, and cognitive components of reward-seeking behavior in rats. Long-Evans male rats (n=9-10 / group) were exposed to THC (5 mg/kg) or vehicle for 14 days starting at either PD 30 (adolescent) or PD 90 (adults). Behavioral testing was then conducted after a 30 days washout period so that rats exposed to THC or vehicle during adolescence were adult at time of tests. In a first set of experiments, we found that adolescent-THC exposure (vs. age-matched vehicle exposure) leads to decreased consumption of palatable food reward (sweetened condensed milk; SCM) but does not significantly alter more selective measures of hedonic or motivated feeding. In contrast, rats exposed to THC as adults engaged in more bouts of SCM consumption, suggesting a heightened motivational state. This interpretation was confirmed during subsequent operant progressive ratio testing - adult THC exposure resulted in a persistent increase in willingness to exert effort for food reward. A second set of experiments revealed that rats' ability to select actions in a flexible, goal-directed manner (based on action-outcome representation) was not altered by THC exposure. We also conducted a test of impulsivity, in which rats were reinforced for withholding their reward-seeking behavior for intervals of at least 30 seconds (differential reinforcement of low rates 30 s). Relative to vehicle controls, we found that the adult-THC group displayed excessive premature reward seeking, interfering with their ability to earn reward. In contrast, the adolescent-THC group displayed more efficient reward seeking, in that they were better at waiting than vehicle controls.Overall, our findings confirm previous studies that had shown that repeated endocannabinoid receptor stimulation during adolescence is sufficient to induce long lasting changes in reward consumption in adult rats. Our study shows distinct effect of repeated THC exposure on motivational influence of reward seeking depending on whether it took place during adolescence or adulthood.

Disclosures: B. Halbout: None. **C. Hutson:** None. **L. Hua:** None. **J. Montesinos:** None. **S.V. Mahler:** None. **S.B. Ostlund:** None.

Digital Abstract Session

P296. Behavioral and Neural Mechanisms of Cannabinoids

Program #/Poster #: P296.06

Topic: G.09. Drugs of Abuse and Addiction

Support: R21DA046154
Johns Hopkins University Dalio Fund in Decision Making and the Neuroscience of Motivated Behaviors
R01DA042211
Tobacco Related-Disease Research Program grant numbers TRDRP; T31IP1832

Title: Appetitive, antinociceptive, and hypothermic effects of vaped and injected D9-tetrahydrocannabinol (THC) in rats: exposure and dose-effect comparisons by strain and sex

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Abstract: Rationale: Advances in drug vapor exposure systems have enabled evaluation of α-9-tetrahydrocannabinol (THC) vapor effects in laboratory animals. The purpose of this study was to 1) establish a range of parameters of THC vapor exposure in rats sufficient to produce a behavioral dose-effect curve in a battery of tasks sensitive to THC; and 2) to investigate sex differences in the effects of THC vapor exposure and THC injection (intraperitoneal, IP) on these behaviors in two strains of outbred rats. **Methods:** Male and female Sprague Dawley and Wistar rats (N=22, 5-6/sex per group) received THC via passive vapor exposure (200 mg/ml; 5 conditions) and IP injection (1-20 mg/kg) in a within subject design. The effects of vaped and injected THC on progressive ratio responding for food pellets. Nociception with a tail withdrawal assay and measurements of body temperature were also assessed during a 5-hr test period for evaluation of time course of effects. Plasma THC concentrations were assessed after THC vapor and 10 mg/kg IP THC. **Results**: THC vapor produced dose related increases and decreases in motivation to obtain food under the progressive ratio schedule. 1-20 mg/kg IP THC reduced breakpoints. Vaped and injected THC produced exposure and dose-dependent antinociception and hypothermia. Sex and strain differences in THC effects were also observed. Plasma THC concentrations were higher after 10 mg/kg IP THC (152 ng/mL) compared to the highest vapor exposure condition tested (38 ng/mL), but magnitude of behavioral effects were comparable. **Conclusions**: THC vapor exposure produced reliable, dose orderly effects on food-maintained behavior, nociception, and body temperature that are comparable to effects of IP THC, although there were differences in the time course of behavioral outcomes.

Disclosures: C.F. Moore: None. **C. Davis:** None. **E. Weerts:** None. **M. Taffe:** None.

Digital Abstract Session

P296. Behavioral and Neural Mechanisms of Cannabinoids

Program #/Poster #: P296.07

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIDA grant to JL CC
Leon Levy grant to RTDR

Title: Cannabinoid signals modulate the amygdalostriatal circuit for learning proactive threat-coping.

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Abstract: In humans, proactive threat-coping enhances the individual perception of control over a threatening environment (agency). This can result in the suppression of Pavlovian freezing responses. We used the signaled active avoidance test (SigAA) in rats to model proactive actions that suppress freezing. The cannabinoid-1 receptor (CB1r) and endocannabinoids (eCBs) are known for facilitating plasticity in neuronal circuits for defensive responses, reward seeking, and negative reinforcement. We hypothesize that SigAA responses are facilitated by cannabinoid-dependent plasticity in the nucleus accumbens (NAcc), stimulated by glutamatergic projections from the basal amygdala (BA). Using pharmacological, chemogenetic and optogenetic inhibition, we show that glutamatergic projections from BA to NAcc subregions underlie the acquisition of SigAA responses. We found a correlation between the success to acquire SigAA responses and eCB levels in the brain, which could be a biomarker for proactive responses. SigAA responses were modulated pharmacologically by systemic manipulation of CB1R, or locally by altering CB1R signaling in NAcc. Nevertheless, preliminary results indicate that the systemic CB1r-agonist administration failed to rescue SigAA responses during optogenetic inhibition (BA-NAcc). Ongoing experiments seek to assess molecular, cellular and network mechanisms using different approaches: Cre-dependent chemogenetics combined with pharmacological manipulations of CB1r in NAcc, Cre-dependent fiber photometry to examine endocannabinoid dynamics *in vivo* in NAcc, quantification of the levels of CB1r and eCB in the brain and serum of animals, using mass spectrometry, correlating to their performance to learn

SigAA responses. These data suggest that the novel use of endocannabinoid-based drugs combined with proactive learning strategies might have a lasting effect in modulating defensive responses. An adapted approach in humans could help to reduce anxiety disorder symptoms.

Disclosures: **R. Triana-Del Rio:** None. **E. Andrade:** None. **V. Yaragudri:** None. **L. Branigan:** None. **C. Farb:** None. **M. Hou:** None. **W. Piper:** None. **J. Oyarzun:** None. **C. Cunha:** None. **Y. Li:** None. **P. Shrestha:** None. **C.M. Alberini:** None. **C. Constantinople:** None. **E. Klann:** None. **R. Sears:** None. **C.K. Cain:** None. **J.E. LeDoux:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.01

Topic: G.09. Drugs of Abuse and Addiction

Support: This research was supported by NIDA-IRP

Title: Social reward prevents extended access cocaine self-administration and incubation of cocaine craving in male and female rats

Authors: ***M. VENNIRO**, Y. SHAHAM;
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Abstract: Background: We recently developed a community-reinforcement model using operant social reward to promote abstinence in rats. We found that social choice-induced abstinence prevents methamphetamine and heroin self-administration and decreases incubation of craving. Here, we determined the effect of social reward on intermittent- and continuous-access cocaine self-administration, and incubation of cocaine craving. We also determined parameters to reverse social preference. Methods: In experiment 1, we trained male and female rats for social self-administration (6 days) and then for cocaine self-administration, initially continuously (2-h/day; 4 days), and then either continuously (12-h/day) or intermittently (5-min ON, 25-min OFF × 24) for 8 days. We assessed relapse to cocaine seeking after 1 and 15 days. Between tests, the rats underwent either forced or social choice-induced abstinence. In experiment 2, after establishing stable preference for social interaction, we manipulated the delay for both rewards or social reward alone, or the fixed-ratio schedule requirements for social reward. Results: The rats self-administered more cocaine during continuous access, but the inter-infusion-interval was higher during intermittent access. Social reward prevented incubation of cocaine craving independent of access conditions and sex. The rats' social preference decreased by increasing the delay for both rewards or for social reward alone, or by increasing the fixed-ratio requirement for social reward. Conclusions: The protective effect of operant social reward generalizes to cocaine self-administration and incubation of cocaine craving. Delaying the social reward or increasing the effort to obtain it increases cocaine choice. This human-relevant choice procedure can be used to identify brain mechanisms of individual differences in addiction vulnerability and testing novel medications. This research was supported by NIDA-IRP

Disclosures: **M. Venniro:** None. **Y. Shaham:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.02

Topic: G.09. Drugs of Abuse and Addiction

Title: Effects of chronic stress on cocaine seeking behavior in male and females rats

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Abstract: Post-Traumatic Stress Disorder (PTSD) and Substance Use Disorder (SUD) are generally studied independently in preclinical studies, although they can occur concurrently in patients. PTSD patients exhibit higher rates of SUD. Exposure to stressful or traumatic events can lead to the development of PTSD, and stress has been associated with a higher probability of relapse. Two important brain structures involved in the development of PTSD and SUD are the basolateral amygdala (BLA) and the nucleus accumbens (NAc), the latter being part of the reward circuitry. It has been shown that activation of BLA-NAc projecting neurons increases self-stimulation in optogenetic experiments, but it is not known how stress may alter this projection. Previous studies have shown that modified single prolonged stress reduces cocaine self-administration in rats. Moreover, rats exposed to single prolonged stress had a reduced cue-induced reinstatement of cocaine-seeking behavior, without any effects on acquisition and extinction of cocaine self-administration. Early life stress also facilitates the development of cocaine addiction, but it did not alter the reinstatement of cocaine-seeking behavior. All these studies used different models of PTSD in rodents and a short-access cocaine self-administration paradigm. However, the effects on extended-access cocaine self-administration have not been reported. Our objective is to determine the effects of chronic stress on cocaine-seeking behavior in male and female rats. We hypothesized that unescapable footshock before cocaine self-administration will increase cocaine-seeking behavior in both sexes. To test this hypothesis, we used unescapable footshock for a period of 5 days at an intensity of 0.50mA, presented randomly. After development of chronic stress, animals underwent 6-hour sessions of extended-access cocaine self-administration for 10 days, followed by a 30 day forced abstinence period. Subsequently, we performed cue-primed and drug memory retrieval tests to address cocaine-seeking behavior. Preliminary data show that unescapable footshock before cocaine addiction leads to increased cocaine-primed memory retrieval after withdrawal in male and female rats. Interestingly, female rats show increased cue-primed memory retrieval in the stress group compared with the control group. This was not observed in male rats. These results suggest that chronic stress before cocaine exposure increases the susceptibility to cocaine after forced

abstinence in male and female rats. Although cue-induced cocaine-seeking appears to work differently in male versus female rats, these results need to be confirmed.

Disclosures: **R.J. Morales Silva:** None. **G. Marrero Cristobal:** None. **U. Gelpi Domínguez:** None. **G. Rodriguez Torres:** None. **J.K. Alvarado Torres:** None. **M.T. Sepulveda-Orengo:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.03

Topic: G.09. Drugs of Abuse and Addiction

Support: Medical research Council: MR/NO2530X/1

Title: Biobehavioural basis of the flexible inflexibility that characterises maladaptive drug-seeking at relapse

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Abstract: While motivationally relevant drug-paired conditioned stimuli (CSs) trigger activation in the nucleus accumbens in recreational drug users, in chronic users or individuals with a diagnosed addiction they trigger the activity of, and dopamine release in, the dorsal (or dorsolateral) striatum (DLS), the activation of which is the best predictor of long-term relapse.The psychological and behavioural significance of the functional engagement of the DLS in drug addictive behavior has not yet been elucidated. We have long suggested that it reflects the development of habitual control over drug-seeking behaviour to emphasize the inflexible engagement in drug foraging activities by addicted individuals in order to acquire and use the drug. In the real world, the distinction between motivational control of the initiation of drug-seeking and its later performance is especially important in that drug seeking is also motivated by response-produced drug-associated CSs - conditioned reinforcers - that invigorate instrumental seeking responses and bridge delays to the procurement, and eventually use, of the drug, hence playing a key role in relapse during abstinence.The neural basis of cue-controlled cocaine seeking, operationalised as second-order schedules of reinforcement (SOR), is well established. However, the influence of a long history of cocaine seeking under the control of the conditioned reinforcing properties of drug-paired cues on the tendency to relapse has not been investigated. Here, in a series of longitudinal studies combining functional neuroanatomy and causal manipulations of the dorsal striatum in behaving rats we showed that a long (several weeks) history of cocaine seeking under a SOR, at a time when it depends upon the DLS habit system, results in aberrant drug seeking at relapse after abstinence. We further showed that this exacerbated tendency to relapse depends on the dorsomedial striatum-dependent goal-directed

system that is transiently engaged by the inability of an individual to perform drug seeking habits during abstinence and independently of withdrawal from, or unavailability of, cocaine.These data suggest that a prolonged history of cocaine seeking under the conditioned reinforcing effects of cocaine-paired cues results in the recruitment of neural mechanisms that establish maladaptive drug seeking habits, or incentive habits. Incentive habits underlie the DLS-dopamine dependent inflexible initiation of drug seeking behaviour However, when they cannot be performed, or expressed, they perpetuate vigorous drug seeking behaviour via the transient engagement of the pDMS goal-directed system in a drug-independent manner.

Disclosures: D.J. Belin: None. **M. Fouyssac:** None. **Y. Pena Oliver:** None. **M. Puaud:** None. **C. Giuliano:** None. **B.J. Everitt:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.04

Topic: G.09. Drugs of Abuse and Addiction

Support: R00DA042895
T32DA007234

Title: Hierarchical cue control of cocaine seeking in the face of cost

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Abstract: Drug addiction is characterized by intermittent, persistent drug seeking despite rising costs. Drug-associated cues are a powerful trigger of this behavior, capable of inciting relapse in recovering addicts. We set out to model three key aspects of human drug use in rats: the intermittent, binge-and-stop nature of drug intake, the motivational conflict of drug seeking in the face of escalating negative costs, and the ability of different types of drug cues to modulate seeking and spur relapse. Critically, we found that the ability of proximal cues to trigger relapse was gated by the presence of a global cue signaling drug-availability within the animal's environment, suggesting that hierarchical cue interactions exert an important modulating influence on drug-seeking motivation. Dopamine release within the nucleus accumbens core (NAc) has been implicated in cue-induced relapse of drug seeking. It is less clear, however, if dopamine signaling may encode hierarchical drug-related learning states where drug cues interact to guide seeking. To address this, we measured changes in dopamine receptor activity within the NAc with fiber photometry, using the genetically encoded dopamine sensor dLight. Our data suggests that the dopamine preferentially encodes drug availability over delivery with continued use and is modulated by escalating cost. Together, these results demonstrate hierarchical cue control of drug seeking despite cost, and point to a role for NAc core dopamine in this process.

Disclosures: V.L. Collins: None. **K.N. Bornhoft:** None. **B.T. Saunders:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA IRP / NIH

Title: Behavioral and neurobiological substrates underlying discriminative stimulus-control of cocaine seeking in rats

Authors: *O. R. DRAKE, M. B. BRENNER, V. A. LENNON, S. J. WEBER, L. E. KOMER, R. MADANGOPAL, L. A. RAMSEY, B. J. TUNSTALL, J. M. BOSSERT, Y. SHAHAM, B. T. HOPE;
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Abstract: Persistent susceptibility to cue-induced relapse is a cardinal feature of addiction. Discriminative stimuli (DSs) signal drug availability (DS+) or unavailability (DS-) and control drug seeking prior to relapse. We previously used a trial-based procedure to train rats to self-administer cocaine under DS-control and demonstrated that DS-controlled cocaine-seeking persists up to 300 abstinence days. Here we investigate the behavioral and neurobiological substrates underlying persistent DS-controlled cocaine seeking. We first investigated individual contributions of DS+ and DS- to cocaine seeking in our task by measuring non-reinforced lever-presses during 4 trial types 21 days after cocaine self-administration: no DSs, DS+ only, DS- only, both DSs. Rats' responding was low during 'no DSs' and 'DS- only' trials, intermediate when 'both DSs' were presented, and maximal during 'DS+ only' trials. Next, we used GABAA+GABAB receptor agonists (muscimol+baclofen, M+B) to examine the role of prelimbic (PL) and infralimbic (IL) subregions of medial prefrontal cortex (mPFC) in DS-controlled cocaine seeking and taking. M+B microinjections into IL, but not PL, reduced DS-controlled cocaine seeking on abstinence day 21. M+B injection into either area had no effect on DS-controlled cocaine self-administration. Finally, we used ex vivo whole-cell recordings and found that M+B suppressed electrically evoked excitatory postsynaptic current magnitude and decreased spontaneous EPSC frequency in mPFC neurons. Our data indicate that DS+ and DS- independently control the expression and suppression of DS-controlled drug seeking in our task. Further, DS-controlled drug seeking during abstinence is mediated in part by neural activity in IL but not PL.

Disclosures: O.R. Drake: None. **M.B. Brenner:** None. **V.A. Lennon:** None. **S.J. Weber:** None. **L.E. Komer:** None. **R. Madangopal:** None. **L.A. Ramsey:** None. **B.J. Tunstall:** None. **J.M. Bossert:** None. **Y. Shaham:** None. **B.T. Hope:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.06

Topic: G.09. Drugs of Abuse and Addiction

Title: Acquisition rates of cocaine self-administration are affected by estrous stage and naive terminal state

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Abstract: Cocaine Use Disorder (CUD) is a debilitating mental illness that affects over 1 million people in the United States annually. Despite a recent rise in CUD cases, including a tripling in overdose deaths since 2012, there remains no FDA approved pharmaceutical to treat CUD. This is partially due to the fact that the majority of cocaine research has focused on male behavior and neurobiology. However, recent clinical and preclinical work indicates distinct sex differences in cocaine abuse vulnerability. Thus, it is likely that many compounds are failing in clinical trials due to a lack of knowledge regarding how biological sex impacts CUD development. Though males consistently exhibit greater cocaine consumption, females show significant increases in other aspects of CUD-like behavior including motivation for cocaine, escalation of intake, and acquisition of cocaine taking. In rodents, it has been repeatedly shown that females in the estrus phase of the estrous cycle exhibit the highest levels of CUD-like behaviors suggesting that ovarian hormones may play a critical role in modulating this vulnerability. Further, despite the integral role of the mesolimbic dopamine system in CUD, there has been little work done to directly asses interactions between the estrous cycle and the mesolimbic dopamine system in the context of CUD. Here, adult Sprague-Dawley rats trained to self-administer cocaine show that female rats in the estrus stage of the estrus cycle exhibit significantly greater acquisition behaviors, notably time to acquisition, as well as increased cocaine potency at the dopamine transporter (DAT). When collapsed by cycle there appears to be no difference in DAT function between males and females, however, females in estrus show significantly greater DAT sensitivity to cocaine compared to males and females in non-estrus. This is significant as many studies rule out sex and the estrous cycle as potential influences on behavior and neurobiology based on a lack of difference between males and females not separated by cycle stage. This work demonstrates that by not staging intact females, it is possible to miss cycle specific effects that may not be initially evident by comparing just males and females. Further, we show that while males appear to show no “preference” for acquisition dose, females exhibit significantly greater acquisition behavior at lower doses. This effects does not appear to be due to alterations is cocaine potency at the DAT which are mostly unchanged across sex and dose. Together, this data suggests that sex differences significantly complicate acquisition and that greater emphasis on this aspect of drug taking is required in CUD research.

Disclosures: M.I. Mauterer: None. **S.R. Jones:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.07

Topic: G.09. Drugs of Abuse and Addiction

Support: DA031900
Drexel University Dean's Fellowship on Excellence in Collaborative Research

Title: Impact of sleep on incubation of cocaine craving and dopamine terminal adaptations following abstinence

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Abstract: Finding effective and tolerable treatments for cocaine addiction has been extremely challenging due to the high rates of relapse. Clinical and animal studies have shown a progressive intensification of cocaine seeking and craving during abstinence, increasing the likelihood of relapse. In addition, sleep disruptions are commonly observed during recovery from chronic cocaine use and manifest as abnormal sleep architecture which is posited to promote incubation of cocaine craving. While the neural mechanisms underlying the association between sleep disruption and incubation of cocaine craving are unclear, accumulating evidence suggests that alterations in mesolimbic dopamine (DA) neurotransmission may contribute to these processes. To determine to what degree cocaine craving is associated with sleep disruptions and whether this involves DA terminal adaptations, adult female and male rats underwent intermittent access to cocaine self-administration followed by a period of imposed abstinence. Incubation of cocaine craving was assessed by cue-induced drug seeking test on abstinence day 1 and 7. To examine sleep disruptions, sleep/wake activity was recorded before cocaine exposure and throughout the abstinence period. Changes in DA release and uptake in the nucleus accumbens were then recorded using fast scan cyclic voltammetry on day 8 of abstinence following the seeking test. Intermittent access to cocaine followed by abstinence engendered a significant intensification of cocaine seeking, decreases in REM sleep, and increases in DA release and uptake compared to cocaine naïve rats. Based on these findings, we then tested whether restoring sleep during abstinence prevents incubation of cocaine craving via normalization of DA terminal function. Sleep restoration was achieved by exposing the rats to a continuous movement of a rotating bar near the floor of a custom-designed chamber during their normal active period (dark phase) to accumulate sleep pressure and enhance sleep quality during their normal inactive period (light phase). Restoring sleep attenuated incubation of cocaine craving and reversed DA terminal adaptations. These results suggest that sleep disruptions might not only be a symptom of cocaine withdrawal, but also an important factor for promoting incubation of cocaine craving.

Disclosures: I.P. Alonso: None. **E.A. Zihal:** None. **R.A. España:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH T34GM118272

Title: Cocaine preference over sucrose in a rat model of voluntary abstinence is associated with increased cocaine relapse and vOFC activation

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Abstract: Cocaine use disorder (CUD) is characterized by high rates of relapse after long periods of abstinence. Contingency management (CM) is an effective behavioral treatment for CUD, offering non-drug rewards to maintain abstinence. However, relapse typically occurs once CM is discontinued. Thus, choice-based "voluntary abstinence" is a translationally relevant rodent model of abstinence that has not yet been used following cocaine self-administration. We hypothesized that the combination of voluntary abstinence (VA) and ceftriaxone would reduce relapse to cocaine-seeking once VA was stopped. Ceftriaxone is a beta-lactam antibiotic reliably found to attenuate cocaine-seeking after instrumental extinction and forced abstinence. Here, we trained 25 male Sprague-Dawley rats to self-administer sucrose pellets (5 d, 2 hr/d). Rats next self-administered intravenous cocaine (12 d, 2 hr/d). Discrete and contextual sucrose- and cocaine-associated cues were used. Rats then underwent either VA or forced abstinence for 14 days. During VA, rats experienced 20 trials/d during which they chose between sucrose and drug levers to receive the associated reinforcer. Half of the rats received ceftriaxone (200 mg/kg IP) during the last 7 days of VA. Rats assigned to forced abstinence were handled daily but did not go back to the operant environment during abstinence. On day 15, a 2 hr relapse test was conducted; only the drug-paired and inactive lever were available, with cocaine-associated cues. Rats were perfused immediately after the relapse test for the quantification of Fos protein expression. The majority of rats preferred the sucrose lever during VA; however, 33-44% of vehicle and ceftriaxone-treated rats continued to prefer cocaine to sucrose. Relapse to cocaine-seeking was equivalent between groups. However, the amount of cocaine intake during VA positively correlated with the number of cocaine lever presses during the relapse test. Relapse-induced Fos expression was greater in the vOFC in the VA group relative to forced abstinence, indicating that this brain region may be engaged by choice-based abstinence. Both of these effects were present only in vehicle-treated rats, indicating that ceftriaxone treatment alters the relationship between relapse and both cocaine-choice and vOFC activity, possibly via increasing VTA activity during relapse. In conclusion, these results are the first to indicate that cocaine-experience rats show a range of preference for sucrose over cocaine during VA, and this choice was not influenced by the anti-relapse medication ceftriaxone.

Disclosures: G. Rojas: None. **Y. Padovan- Hernandez:** None. **L.A. Knackstedt:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA CEBRA R21DA046227

Title: Cell Subtype Transcriptional Role of Nab2 in Cocaine Action

Authors: *E. CHOI, R. CHANDRA, M. MCGLINCY, A. CHOW, M. LOBO;
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Abstract: Drug abuse is a debilitating chronic disease which is a leading cause of disability around the world. Exposure to psychostimulants, such as cocaine, is associated with molecular and functional imbalance in the two medium spiny neuron subtypes (MSNs) in the NAc, the dopamine receptor 1 and 2 enriched MSNs (D1-MSNs and D2-MSNs). We have previously reported the bidirectional alterations of a transcription factor, early growth response 3 (Egr3), that is up-regulated in D1-MSNs and down regulated in D2-MSNs with repeated exposure to cocaine. Interestingly, we observed that the NGFI-A binding protein 2 (Nab2), corepressor of Egr3, is also bidirectionally altered in D1-MSNs and D2-MSNs, but in the opposite direction to the Egr3 expression. The role of Nab2 in cocaine induced behavior, however, remains largely elusive. In this study, we developed a CRISPR targeted transcription activator and inhibitor tools that can be targeted to precise loci in selective neuron subtypes. In cell culture, our constructs, which code for catalytically dead dCas9 fused with VP64 for transcription activation, or KRAB for transcription inhibition, were transfected into Neuro2A cells along with sgRNA targeting for early growth response 3 (Egr3) and NGFIA-binding protein 2 (Nab2). Egr3 mRNA level was significantly down regulated in cells transfected with the sgRNA targeting Egr3. Consistent with this, Nab2 mRNA level was significantly down regulated in cells with Nab2 targeting sgRNA. These constructs were packaged into AAVs for *in vivo* delivery into the brain. In mice that received the CRISPR I AAV targeted for Nab2, we observed a significant KD of Nab2 protein expression. We then assessed locomotor behavior during exposure to cocaine, when Nab2 transcription was inhibited in D2-MSNs using the CRISPR I AAV, we found reduced locomotor behaviors in this group. Together, our study demonstrates a distinct role of Nab2 in D1 and D2 MSNs. Our future studies, in which we plan to perform cocaine self-administration in these mice with CRISPR mediated Nab2 overexpression and knockdown in D1 and D2 MSNs, and subsequent RNA sequencing from NAc tissues of these mice after the self-administration will further test our hypothesis and shed light into the molecular mechanisms occurring in D1 and D2 MSN NAc cell subtypes after cocaine operant self administration.

Disclosures: E. Choi: None. **R. Chandra:** None. **M. McGlincy:** None. **A. Chow:** None. **M. Lobo:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.11

Topic: G.09. Drugs of Abuse and Addiction

Support: R00 DA041462

Title: Behavioral effects of Metformin on Cue-Induced Cocaine Reinstatement

Authors: *L. WILLARD[1], A. CHAN[1], S. MULLOY[2], A. SCIACCOTTA[3], N. IBRAHIM[3], S. SPENCER[1];
[1]Pharmacol., [2]Neurosci., [3]Univ. of Minnesota, Minneapolis, MN

Abstract: Behavioral effects of Metformin on Cue-Induced Cocaine Reinstatement in RatsAuthors Lexi Willard, Amy Chan, Sarah Mulloy, Allegra Sciaccotta, Noor Ibrahim, and Sadé SpencerUniversity of MinnesotaDepartment of Pharmacology **Disclosures**Lexi Willard: None. Amy Chan: None. Sarah Mulloy: None. Allegra Sciaccotta: None. Noor Ibrahim: None. Sadé Spencer: None.
Abstract Currently there are no FDA approved pharmacological agents to alleviate cocaine use disorder, and cocaine use fatalities have tripled since 2011. Adenosine monophosphate-activated protein kinase (AMPK) is a heterotrimeric enzyme vital for regulating cellular oxidative stress through sensing ATP:AMP levels. Cocaine increases oxidative stress through manipulation of dopaminergic transmission. Following cocaine self-administration and extinction the amount of phosphorylated (active) AMPK (pAMPK) in the nucleus accumbens core (NAc) is decreased. Metformin, an FDA approved type 2 diabetes drug, is found to increase pAMPK levels and shows efficient blood-brain barrier permeability, unlike other pAMPK activators. We hypothesized that intracranial microinjections of metformin into the NAc would curb active lever presses in a cue-induced reinstatement paradigm by returning AMPK function back to a baseline level. In this study we utilize metformin to reduce cue-induced cocaine seeking in male and female rats after cocaine self-administration and extinction. Women have been seen to escalate towards cocaine addiction faster and have an increased risk of relapse compared to men. Intracranial microinjections of metformin (125 micrograms/hemisphere) into the NAc reduced cue-induced reinstatement of cocaine seeking in both male and cycling female rats. We employed a sucrose self-administration paradigm to check if intracranial metformin's effect was due to a general attenuation of appetitive behavior and found that metformin did not decrease sucrose seeking in males but did in females. We performed locomotor assay analysis to control for effects on activity and found that intracranial microinjections of metformin did not decrease locomotor activity. Further exploration of metformin's effects towards curbing cocaine reinstatement could prove beneficial towards treating cocaine use disorder and provide more information on AMPK signaling.

Disclosures: L. Willard: None. **A. Chan:** None. **S. Mulloy:** None. **A. Sciaccotta:** None. **N. Ibrahim:** None. **S. Spencer:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.12

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA031734

Title: CRF_2 receptor knockdown in the nucleus accumbens attenuates cocaine anticipation in rats

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Abstract: Cocaine-dependent individuals experience intense drug cravings in anticipation of imminent drug availability. Increased arousal in states of drug-expectation may heighten the propensity to seek and consume drug. The present studies examine accumbens-specific actions of the stress peptide corticotropin releasing factor (CRF) as a candidate mechanism for maladaptive arousal evoked by drug-predictive stimuli. We show that CRF is released in the nucleus accumbens (NAc) immediately preceding drug-reward opportunity (i.e. cocaine availability), and this coincides with a rise in extracellular dopamine (DA). Interestingly, we find that infusion of CRF into the NAc stimulates DA release in a CRF-R_2-dependent manner in cocaine-experienced rats, whereas CRF-R_2 activation was ineffective in naïve animals. To clarify the role of NAcC-CRF-R_2 on instrumental behavior directed towards cocaine procurement, rats were first trained to self-administer cocaine under a chained schedule of reinforcement (FI-FR) in order to dissociate *anticipatory* ('drug-seeking') from *consummatory* ('drug-taking') behavior. Completion of FI-5 was followed by 5 min of continuously reinforced (FR1) responding for cocaine. On consecutive days, rats received bilateral infusions of siRNA designed to target either CRHR2, or a scrambled control, into the NAc. Knockdown of CRF-R_2 selectively attenuated cocaine-seeking responding during the FI link of the chain schedule without affecting subsequent cocaine intake. Behavior returned to baseline 36-hours after the last injection. These data suggest the CRF-R_2 may be specifically involved in generating arousal during cocaine expectation, whereas the unconditioned aspects of drug-reward are differentially controlled. Because qPCR analysis of the NAc revealed no differences in CRF-R2 transcript levels between cocaine-experienced and drug-naïve rats; ongoing studies examine potential contributions of CRF binding protein (CRF-BP) - a known modulator of CRF-R_2 activity - to cocaine-dependent behavioral outcomes.

Disclosures: M.Z. Leonard: None. **H. Covington III:** None. **S.L. Fulton:** None. **I.S. Maze:** None. **K.A. Miczek:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.13

Topic: G.09. Drugs of Abuse and Addiction

Title: Exposure to a ketogenic diet blocks the formation of a conditioned place preference for cocaine in young adult Sprague-Dawley rats

Authors: *L. A. MARTINEZ, M. HUSTON;
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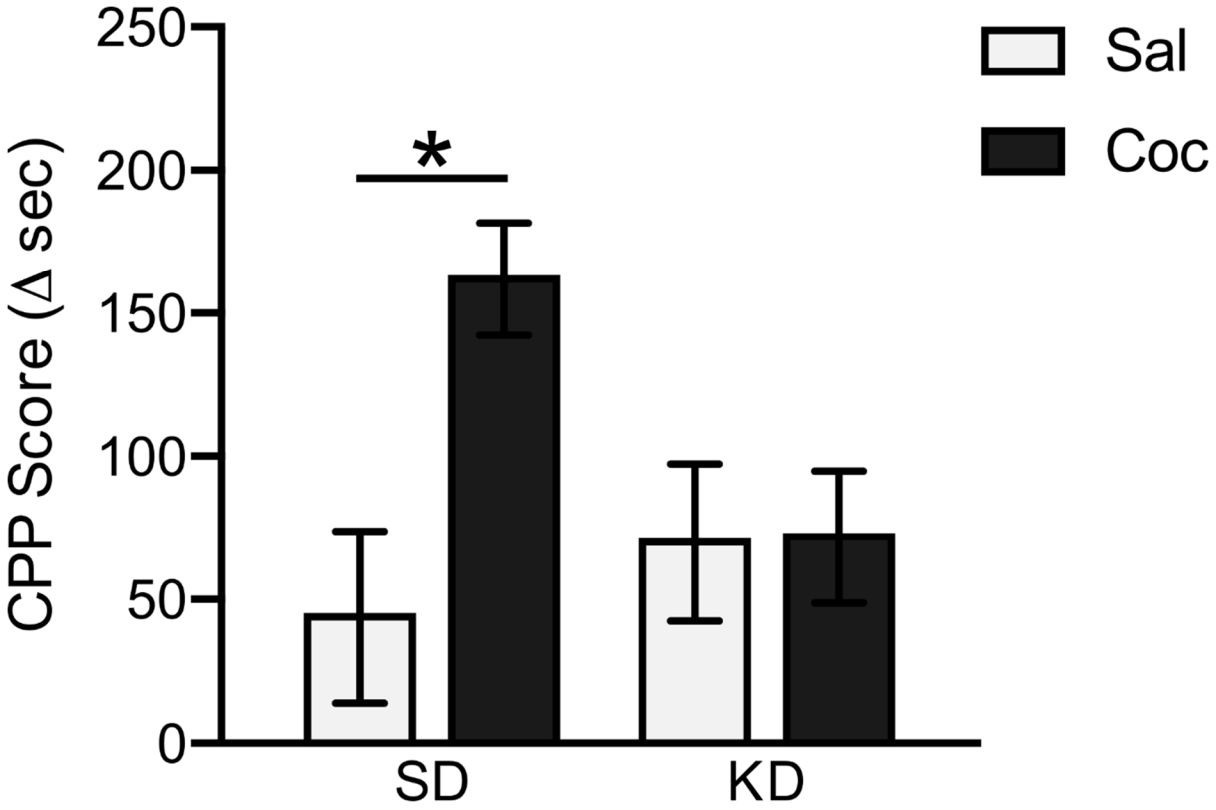
Abstract: Ketogenic diets (KD) are high fat, low carbohydrate and adequate protein formulations that have traditionally been used as a treatment for epilepsy; however, there is growing evidence that these diets have broader therapeutic potential for disorders featuring disruptions to brain structure/function, including drug addiction. Previously we have found that administration of a KD to young adult male and female Sprague-Dawley rats blocks the development of locomotor sensitization and decreases stereotyped behaviors exhibited in response to repeated cocaine injections. These effects led us to speculate that the rewarding effects of cocaine are also disrupted by KD diet exposure. To determine if this is indeed the case, male and female Sprague-Dawley rats were placed on a strict 6.6:1 (fat:[carbohydrates+protein], by weight) KD or standard diet (SD) at 5 weeks of age and then were maintained on those diets for 3 weeks prior to behavioral testing. Rats then underwent conditioned place preference (CPP) testing using a biased design in a two-chamber apparatus. Briefly, rats underwent an initial 15-min pretest with free access to both chambers, followed by a series of 30-min daily conditioning sessions with either cocaine (Coc; 15 mg/kg) or saline (Sal) in the initially non-preferred chamber and saline in the initially preferred chamber on alternating days. Rats underwent a final 15-min posttest to determine the effects of conditioning on their preference for the initially non-preferred chamber. Rats maintained on a KD failed to develop a CPP for 15 mg/kg cocaine, whereas SD rats did form a robust CPP for this dose. These results suggest that administration of a KD diminishes the rewarding properties of cocaine, providing further evidence in support of the therapeutic potential of these diets in drug addiction.



Disclosures: **L.A. Martinez:** None. **M. Huston:** None.

Digital Abstract Session

P297. Cocaine: Reinforcement, Seeking, and Reinstatement

Program #/Poster #: P297.14

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA044308

Title: Microbiome-derived metabolites regulate cocaine-seeking and transcriptional homeostasis in the rat nucleus accumbens

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Abstract: Psychostimulant addiction represents a public health crisis leading to tremendous morbidity. Despite this, there are no FDA-approved medications for treatment of psychostimulant use disorders. Burgeoning research has detailed the important connections between the resident population of bacteria in the gut, the gut microbiome, and the

pathophysiology of neuropsychiatric disease. Robust literature implicates bacterially-derived Short-Chain Fatty Acid (SCFA) metabolites in altering transcriptional profiles and chromatin structure in the brain. Recent work from our group demonstrated that acute depletion of the gut microbiome results in enhanced reward and altered gene expression in a mouse cocaine place preference model, and that repletion of SCFAs reverses these effects. However, the role of gut microbiome and its metabolites in modulating cocaine-seeking behavior after prolonged abstinence is unknown. Given that prevention of relapse following abstinence is the most clinically challenging issue in treating patients with substance use disorders, studies examining the effects of microbiome manipulations in relapse relevant models are critical for the development of translational strategies in this space. For these studies, depletion of gut bacteria and their metabolites was induced in Sprague-Dawley rats via addition of antibiotics in their drinking water and compared to untreated controls. To identify specific mechanistic contributions of SCFA, the metabolites were replenished via addition to drinking water in animals with depleted gut microbiomes. Rats were trained to self-administer cocaine and subjected to either within-session threshold testing to evaluate motivation for cocaine at a range of doses or 21 days of abstinence followed by a cue-induced cocaine-seeking task to model relapse behavior. Nucleus accumbens was isolated and tissue processed for RNA-sequencing analysis. Animals lacking a complex gut microbiome show significantly increased cocaine-seeking behaviors and altered expression of synaptic plasticity genes. In the absence of a normal microbiome, repletion of bacterially-derived SCFA metabolites reverses behavioral and molecular changes associated with microbiome depletion. Molecular analyses reveal marked changes in activity-dependent gene expression in antibiotic-treated rats compared to controls. These findings suggest that gut bacteria via their metabolites may serve as homeostatic regulators of gene expression in the brain, and suggest the microbiome has potential as a translational research target.

Disclosures: K.R. Meckel: None. **R.S. Hofford:** None. **A. Godino:** None. **E.G. Peck:** None. **E.S. Calipari:** None. **D.D. Kiraly:** None.

Digital Abstract Session

P298. Cocaine: Cognitive and Behavioral Effects

Program #/Poster #: P298.01

Topic: G.09. Drugs of Abuse and Addiction

Support: CIHR Project grant to CAW (PJT-162312)
UBC Psychiatry salary support to TJH

Title: Chemogenetic stimulation of VTA dopamine neurons causes cumulative deficits in decision making and enhanced addiction vulnerability

Authors: *T. HYNES, K. HRELJA, C. CHERNOFF, L. CALDERHEAD, S. KAUR, C. WINSTANLEY;
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Abstract: Addiction is a complex affliction, characterized not only by the use of drugs, but also by deficitsin cost/benefit decision making. A critical biological factor in the development of addiction isaberrant dopamine (DA) neurotransmission. Biological sex is also an important consideration.Investigating such phenomena is difficult in humans, because of the profound influence thesocio-political construct of gender has on behaviour. We therefore sought to longitudinally probethe complex intersection of addiction, decision making, sex, and DA neurotransmission bycombining our cued rat gambling task (crGT; a rodent assay of cost/benefit decision making) withoperant intravenous cocaine self-administration and chemogenetic manipulations of DAneurons. We infected the VTA DA neurons of female and male rats with an excitatory designerreceptor, before training them in our crGT. Prior to daily crGT training sessions, we administeredclozapine-N-oxide to activate the receptor and increase the excitability of VTA DA neurons duringtask performance. Once rats achieved stable crGT performance, we allowed them to self-administer intravenous cocaine. We also quantified the psychomotor reactivity to cocaine in agroup of otherwise cocaine-naïve rats. Chemogenetic stimulation of VTA DA neurons causeddeficits in decision making and increased the propensity of both sexes to self-administer cocaine.Moreover, this manipulation resulted in a phenotype which was hyperactive at baseline and thatexhibited a heightened psychomotor response to cocaine. These findings suggest that VTA DAneurons are a critical node in decision making and that repeated stimulation of VTA DA neuronscan pre-sensitize the DA system, rendering female and male rats more vulnerable to addiction-like behaviour.

Disclosures: T. Hynes: None. **K. Hrelja:** None. **C. Chernoff:** None. **L. Calderhead:** None. **S. Kaur:** None. **C. Winstanley:** None.

Digital Abstract Session

P298. Cocaine: Cognitive and Behavioral Effects

Program #/Poster #: P298.02

Topic: G.09. Drugs of Abuse and Addiction

Support: R01-DA-042057

Title: Chronic cocaine administration induces sex-dependent increases in locomotor activity and behavioral sensitization

Authors: *A. TROMBLEY;
Wayne State Univ..

Abstract: Chronic cocaine administration induces sex-dependent increases in locomotor activity and behavioral sensitizationTrombley A.T[1,2], Gheidi A.[1], Sadik N.[1], Matchynski, J.[1,2], Karavidha K.[1], Kallakuri S.[1], Brummelte, S.[2,3], Perrine S.A.[1,21]Department of Psychiatry and Behavioral Neurosciences, [2]Translational Neuroscience Program, [3]Department of Psychology, Wayne State University, Detroit, MI.Binge-pattern cocaine use is an on-going public health concern with recent increased prevalence for both women and men. Repeated cocaine exposure has been

shown to induce locomotor sensitization in both female and male rats. To investigate the biological basis of potential sex differences in locomotor sensitization, we measured the effects of cocaine on locomotor activity and behavioral sensitization in both female and male rats as well as characterized the estrous phase of female rats. Adult Wistar rats (N=32, n=16/sex, or n=8/group) received either cocaine (15 mg/kg via intraperitoneal injection 3 times daily, one hour apart) or saline (1 ml/kg via same schedule) for 14 days. Locomotor activity was measured at baseline and on days 1 and 14 to be used as an index of behavioral sensitization. Estrous phase tracking via vaginal lavage (or sham procedure in males) was conducted to characterize estrous phase and determine the impact of estrous on female's behavior. Preliminary results indicate that both male and female rats showed increased locomotor activity acutely after cocaine (day 1) and after chronic cocaine (day 14) administration. However, this effect was significantly heightened in females. Estrous data are currently being analyzed and will be presented. These results further validate that sex differences in cocaine-induced behaviors have a biological underpinning and emphasize the need to better understand sex-differences in models of drug abuse.

Disclosures:

Digital Abstract Session

P298. Cocaine: Cognitive and Behavioral Effects

Program #/Poster #: P298.03

Topic: G.09. Drugs of Abuse and Addiction

Support: Elise Triano Biology Research Fund
WCU CSM Undergraduate Research Award

Title: Behavioral changes induced by cocaine antagonist

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Abstract: Drug overdose is an increasingly large problem in the United States. In 2018, there were 67,367 reported drug overdose deaths in the United States. Of these drugs, opioids, including those with synthetic narcotics were the leading drugs to cause overdoses with a total number of deaths occurring at 46,802. Cocaine follows behind opioids at a total number of deaths by overdose of 14,666. While, opioid overdoses can be combated pharmacologically there is no safe and effective pharmacological agent to combat cocaine overdose. Our lab is exploring the ability of cocaine antagonists to be used in a similar fashion to interventions used in opioid misuse. We are seeking to understand how cocaine induced changes to locomotion and anxiety might be ameliorated by examining the effects of a potential cocaine antagonist (PCA1) on mouse behavior in the open field and elevated plus behavioral tasks. To address hormone mediated differences in behavioral response to cocaine both male and female mice were tested. Estrus stage of female mice was tracked using non-invasive vaginal lavage and automated analysis of behavior was performed using ezTrack. In this exploratory work, our results show

that that PCA1 alone can induce behavioral changes in control mice. Future work will examine the behavioral changes of PCA1 in combination with cocaine.

Disclosures: E.S. Sweet: None. **C.A. Gallagher:** None. **S.R. Martonick:** None.

Digital Abstract Session

P298. Cocaine: Cognitive and Behavioral Effects

Program #/Poster #: P298.04

Topic: G.09. Drugs of Abuse and Addiction

Support: CNPq, grant number 432061/2018-5 (Da Cunha, PI) 02/2019 – 01/2022
CNPq, grant number 306855/2017-8 (Da Cunha, PI) 03/2018 – 02/2021
CNPq-MCT, protocol 465346/2014-60 (Lent, R., PI; Da Cunha,C, Co-I) 01/2017 - 12/2020
FINEP, grant number 01.19.0153-00 (D'Oliveira, A.S.C.M. PI, Da Cunha,C. Co-PI) 01/2020-12/2021
CAPES-PROBRAL-DAAD grant, protocol 88881.198683/2018-01 PIs: Da Cunha C.(Brazil) Scwarting RW(Germany) 2019-2020

Title: Diazepam attenuates the effects of cocaine on locomotion, 50 kHz ultrasonic vocalizations and phasic dopamine release in the nucleus accumbens of rats

Authors: *W. N. SANCHEZ LUNA[1,2], J. POCHAPSKI[2], L. JESSEN[2], M. ELLENBERGER[3], R. SCHWARTING[3], D. L. ROBINSON[4], R. ANDREATINI[2], C. DA CUNHA[2,1];
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Abstract: Currently, there is no effective drug to treat cocaine use disorders, which affect millions of people worldwide. Benzodiazepines are potential therapeutic candidates since microdialysis and voltammetry studies have shown that they can decrease dopamine release in the nucleus accumbens of rodents. In addition, we have recently shown that diazepam blocks the increase in dopamine release and 50 kHz ultrasonic vocalizations induced by amphetamine in rats. Here we tested whether the i.p. administration of 2.5 mg/kg diazepam in adult male Wistar rats could block the effects of 20 mg/kg cocaine (i.p.) on electrically evoked dopamine release in the nucleus accumbens measured by fast-scan cyclic voltammetry, 50-kHz USVs, and locomotor activity. Cocaine injection increased evoked dopamine release in up to 3-fold by within 5 min and was significantly higher than baseline for at least 90 min. The injection of diazepam 15 minutes later attenuated the cocaine effect by nearly 50% and this attenuation was maintained for at least 30 min. Stimulant drugs, natural rewards and reward predictive cues are known to evoke 50 kHz ultrasonic vocalizations (USVs) in adult rats. In the present study, cocaine increased by 12-fold the number of 50 kHz USVs of the flat, step, trill, and mixed kinds. This effect was

maximum 5 min after cocaine injection and, although it decreased exponentially with time, it lasted at least 40 min. Diazepam significantly blocked this effect for the entire duration of the session. The distance travelled by control rats during a 40-min session of exploration in an open field was maximum in the first 5 min and decayed exponentially until the end of the session. Cocaine-treated rats travelled significantly longer distances as compared to the control group, while diazepam significantly attenuated cocaine-induced locomotion by up to 50%. These results suggest that the neurochemical, affective, and stimulant effects of cocaine can be mitigated by diazepam.

Disclosures: **W.N. Sanchez Luna:** None. **J. Pochapski:** None. **L. Jessen:** None. **M. Ellenberger:** None. **R. Schwarting:** None. **D.L. Robinson:** None. **R. Andreatini:** None. **C. da Cunha:** None.

Digital Abstract Session

P298. Cocaine: Cognitive and Behavioral Effects

Program #/Poster #: P298.05

Topic: G.09. Drugs of Abuse and Addiction

Support: CONACYT-FOSISS project No. 0201493

Title: Neurite orientation dispersion and density imaging in cocaine use disorder

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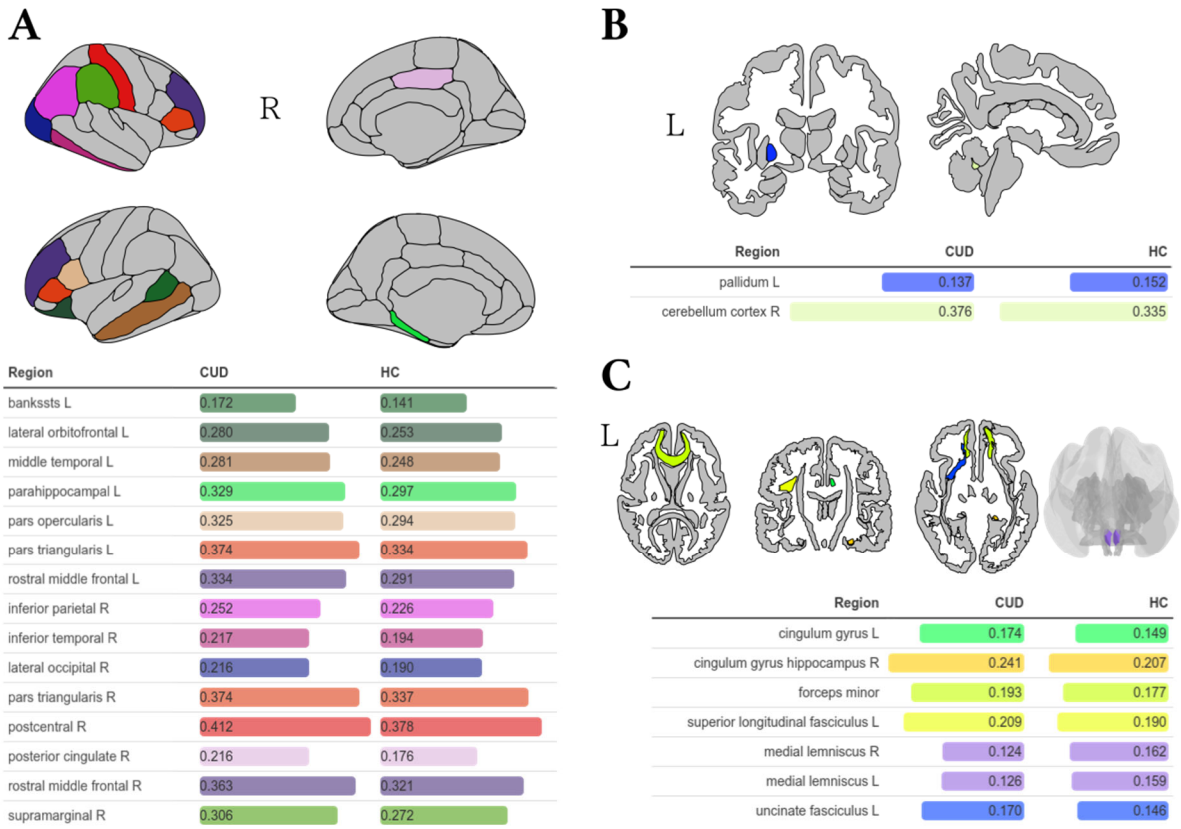
[1]Univ. Nacional Autónoma de México, Querétaro, Mexico; [2]Symbiosis Intl. Univ., Maharashtra, India

Abstract: Cocaine use disorder (CUD) is characterized by a compulsive search for cocaine that chronically produces cognitive deficits, including lack of inhibition and decision-making. Several studies have shown that cocaine users exhibit brain volume and diffusion-based white-matter alterations in a wide variety of brain regions. However, the non-specificity of standard volumetric and diffusion-tensor methods to detect structural micropathology may lead to wrong conclusions. To better understand microstructural pathology in CUD, we analyzed 60 CUD participants (3 female) and 43 non-CUD controls (HC; 2 female) retrospectively from our cross-sectional Mexican SUD neuroimaging dataset (SUDMEX-CONN), using multi-shell diffusion-weighted imaging and the neurite orientation dispersion and density imaging (NODDI) analysis which aims to more accurately model microstructural pathology. We used Viso values of NODDI that employ a three-compartment model in white (WM) and gray-matter (GM). These values were correlated with clinical measures, including psychiatric severity status, impulsive behavior and pattern of cocaine and tobacco use in the CUD group. As hypothesized, we found higher whole-brain microstructural pathology in WM and GM in CUD patients than controls. ROI analysis revealed higher Viso-NODDI values in superior longitudinal fasciculus, cingulum, hippocampus cingulum, forceps minor and Uncinate fasciculus, as well as in frontal and parieto-

temporal GM structures. We also found correlations between significant ROIs and impulsivity, onset age of cocaine use and weekly dosage with Viso-NODDI. However, we did not find correlations with psychopathology measures. Overall, microstructural pathology seems to be present in CUD both in gray and white matter, however their clinical relevance remains questionable.



Region	CUD	HC
bankssts L	0.172	0.141
lateral orbitofrontal L	0.280	0.253
middle temporal L	0.281	0.248
parahippocampal L	0.329	0.297
pars opercularis L	0.325	0.294
pars triangularis L	0.374	0.334
rostral middle frontal L	0.334	0.291
inferior parietal R	0.252	0.226
inferior temporal R	0.217	0.194
lateral occipital R	0.216	0.190
pars triangularis R	0.374	0.337
postcentral R	0.412	0.378
posterior cingulate R	0.216	0.176
rostral middle frontal R	0.363	0.321
supramarginal R	0.306	0.272

Region	CUD	HC
pallidum L	0.137	0.152
cerebellum cortex R	0.376	0.335

Region	CUD	HC
cingulum gyrus L	0.174	0.149
cingulum gyrus hippocampus R	0.241	0.207
forceps minor	0.193	0.177
superior longitudinal fasciculus L	0.209	0.190
medial lemniscus R	0.124	0.162
medial lemniscus L	0.126	0.159
uncinate fasciculus L	0.170	0.146

Disclosures: J. Rasgado Toledo: None. **A. Shah:** None. **M. Ingalhalikar:** None. **E.A. Garza-Villarreal:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.01

Topic: G.09. Drugs of Abuse and Addiction

Support: DA25267
DA48353

Title: In Vitro and In Vivo Pharmacological Comparison of Mu-Opioid Receptor Activity of the Kratom (Mitragyna speciosa) Alkaloid Mitragynine and its Metabolite 7-Hydroxymitragynine

Authors: *S. OBENG[1], F. LEÓN[2], L. F. RESTREPO[1], M. E. REEVES[1], L. R. GAMEZ-JIMENEZ[1], A. PATEL[1], H. P. NICHOLAS[1], T. BRAUN[1], J. D. FORTNER[2], M. L.

CROWLEY[2], V. L. C. PALLARES[1], M. MOTTINELLI[2], C. LOPERA-LONDOÑO[2], C. R. MCCURDY[2], L. R. MCMAHON[1], T. HIRANITA[1];
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Abstract: *In Vitro* and *In Vivo* Pharmacological Comparison of Mu-Opioid Receptor Activity of the Kratom (*Mitragyna speciosa*) Alkaloid Mitragynine and its Metabolite 7-HydroxymitragynineSamuel Obeng[1,2], Francisco León[2], Luis F. Restrepo[1], Morgan E. Reeves[1], Lea R. Gamez-Jimenez[1], Avi Patel[1], Nicholas P. Ho[1], Tobias Braun[1], John D. Fortner[2], Morgan L. Crowley[2], Victoria L.C. Pallares[1], Marco Mottinelli[2], Carolina Lopera-Londoño[2], Christopher R. McCurdy[2], Lance R. McMahon[1], and Takato Hiranita[1]Departments of Pharmacodynamics[1] and Medicinal Chemistry[2], College of Pharmacy, University of FloridaThe μ-opioid receptor (MOR) pharmacology of mitragynine and its 7-hydroxy metabolite have not been fully established. For both we assessed binding affinity and efficacy *in vitro*, as well as discriminative stimulus and antinociceptive effects in rats. Both mitragynine (MG) and 7-hydroxymitragynine (7-MG) had higher binding affinities at human MOR (K_i values 77.9 and 709 nM, respectively) than at the human κ- (KOR) or δ-opioid receptors (DOR). MG was identified as a MOR antagonist while 7-MG was identified as a MOR partial agonist (E_{max}=41%) using the [^{35}S]GTPγS functional assay. In Sprague Dawley rats trained to discriminate 3.2 mg/kg morphine from vehicle (i.p.), MG produced a maximum of 72% morphine-lever responding. Whereas morphine produced a maximum of 65% MG-lever responding in a separate group of rats trained to discriminate 32 mg/kg MG from vehicle (i.p.), other MOR agonists (i.p.) produced high percentages of morphine-lever and MG-lever responding (respectively): 7-MG (100% and 98%), fentanyl (100% and 80%), buprenorphine (100% and 79%), and nalbuphine (99% and 98%). MG (up to 56 mg/kg, i.p.) did not produce significant antinociception, whereas 7-MG (17.8 mg/kg, i.p.) produced robust antinociception in the hotplate assay with the temperature set at 52 °C. Naltrexone (0.032 mg/kg, i.p.) antagonized the discriminative stimulus, rate-decreasing, and antinociceptive effects of morphine and 7-MG. Conversly, naltrexone (0.032 mg/kg, i.p.) antagonized the discriminative stimulus effects of MG, but not its rate-decreasing effects. Pretreatment with MG (5.6 mg/kg, i.p.) potentiated the discriminative-stimulus effects of morphine but antagonized the antinociceptive effects of morphine. Striking differences in MOR efficacy *in vitro* are consistent with 7-MG having greater MOR efficacy than MG *in vivo*. MG exerts both MOR antagonist and agonist activity depending on the MOR efficacy required.Supported by DA25267 and DA48353.

Disclosures: S. Obeng: None. **F. León:** None. **L.F. Restrepo:** None. **M.E. Reeves:** None. **L.R. Gamez-Jimenez:** None. **A. Patel:** None. **H.P. Nicholas:** None. **T. Braun:** None. **J.D. Fortner:** None. **M.L. Crowley:** None. **V.L.C. Pallares:** None. **M. Mottinelli:** None. **C. Lopera-Londoño:** None. **C.R. McCurdy:** None. **L.R. McMahon:** None. **T. Hiranita:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.02

Topic: G.09. Drugs of Abuse and Addiction

Support: IRP/NIDA/NIH

Title: Effect of chronic delivery of the mixed MOR/NOP agonists BU08028 and AT-201 on relapse to heroin seeking and taking in a rat model of opioid maintenance

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Abstract: Background: Maintenance treatment with opioid agonists (buprenorphine, methadone) decreases opioid use and opioid relapse. Using a modified ABA context-induced-reinstatement procedure, we recently found that chronic delivery of either buprenorphine or the novel mu opioid receptor (MOR) partial agonist TRV130 decreases relapse to oxycodone seeking and taking in rats. Here, we tested the effects of the mixed MOR/nociceptin opioid receptor (NOP) partial agonist AT-201 and the buprenorphine analog BU08028 in our model. Methods: We trained male and female rats to self-administer heroin (6-h/d, 14-d) in context A; infusions were paired with a discrete tone-light cue. We then implanted Alzet osmotic pumps containing BU08028 (0, 0.03, or 0.1 mg/kg/d) or AT-201 (0, 3.8, or 12 mg/kg/d) and performed three relapse-related tests: (1) extinction responding reinforced by heroin-associated discrete cues in non-drug context B, (2) reinstatement of heroin seeking induced by re-exposure to context A, and (3) reacquisition of heroin self-administration in context A. Results: Chronic BU08028 delivery had no effect on extinction responding in context B or context-induced reinstatement of heroin seeking in context A, but *increased* reacquisition of heroin self-administration in context A. The experiment with AT-201 is ongoing and we will present the results at the meeting. Conclusions: In our rat model of opioid maintenance, chronic BU08028 delivery had no effect on extinction responding and context-induced reinstatement, but unexpectedly, increased reacquisition of heroin self-administration. To the degree that our results from the model translate to humans, they suggest that mixed MOR/NOP agonists would not be an effective opioid maintenance treatment.

Disclosures: J.M. Bossert: None. **L. Altidor:** None. **H. Korah:** None. **I. Fredriksson:** None. **A. Shekara:** None. **S. Husbands:** None. **N.T. Zaveri:** None. **A. Sulima:** None. **K. Rice:** None. **Y. Shaham:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.03

Topic: G.09. Drugs of Abuse and Addiction

Title: Role of ventral subiculum neuronal ensembles in incubation of oxycodone craving after electric barrier-induced voluntary abstinence

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Abstract: Background: We recently developed a rat model of incubation of oxycodone craving after electric barrier-induced voluntary abstinence and showed a time-dependent increase in drug seeking on abstinence days 15 and 30 compared to day 1. Here, we studied the role of ventral subiculum (vSub) neuronal ensembles in this new form of incubation using the activity marker Fos, muscimol+baclofen (GABA agonists) inactivation, and the Daun02 chemogenetic inactivation procedure. **Methods:** We trained either Sprague-Dawley or Fos-lacZ transgenic male and female rats to self-administer oxycodone (0.1 mg/kg/infusion, 6-h/d) for 14 days. We then introduced an electric barrier of increasing intensity (0.1 to 0.4 mA) near the drug-paired lever that caused cessation of oxycodone self-administration. We tested Sprague-Dawley rats for relapse to oxycodone seeking in the absence of shock and drug on abstinence day 15 and extracted the brains for Fos-immunohistochemistry, or tested the rats after vSub injections of vehicle or muscimol+baclofen on abstinence days 1 and 15. We tested the Fos-lacZ transgenic rats for relapse to oxycodone seeking on abstinence day 18 after selective inactivation of relapse test-activated Fos neurons in vSub on abstinence day 15 using the Daun02 inactivation procedure. **Results:** Relapse after electric barrier-induced abstinence was associated with increased Fos expression in vSub, and both local inactivation of vSub and selective inactivation of vSub Fos-expressing neuronal ensembles decreased "incubated" oxycodone seeking. **Conclusions:** Results demonstrate a role of vSub neuronal ensembles in incubation of opioid craving after cessation of drug taking due to adverse consequences of drug seeking. Supported by NIDA-IRP.

Disclosures: I. Fredriksson: None. **A. Shekara:** None. **S. Applebey:** None. **A. Minier-Toribio:** None. **B.T. Hope:** None. **J.M. Bossert:** None. **Y. Shaham:** None. **C. Cifani:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.04

Topic: G.09. Drugs of Abuse and Addiction

Title: Female rats exhibit increased oxycodone demand and withdrawal-associated anxiety in a rat model of oxycodone-alcohol polysubstance use

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Abstract: From 2007-2017, co-use of oxycodone and alcohol accounted for 14.3-15.4% of all opioid overdose deaths involving prescription opioids (Tori et al., 2020). The compounding effects of alcohol and oxycodone co-use on respiratory depression increases the likelihood for

fatal overdose, highlighting a need for investigations of oxycodone-alcohol polysubstance use (PSU). The present study investigates the effects of alcohol-oxycodone PSU on intake, behavioral economic demand, withdrawal symptoms, and relapse to oxycodone seeking in male and female rats. Forty Sprague-Dawley rats (half male) were permitted daily access to oxycodone only (OXY, n=18), oxycodone+alcohol (OXY+ALC, n=18), or alcohol only (ALC, n=4) groups. OXY and OXY+ALC rats self-administered intravenous oxycodone (0.1 mg/kg/infusion) for 3 hr/day followed by 6 hr access to water only (OXY rats) or both water and 20% (v/v) ALC (OXY+ALC rats). ALC rats self-administered sucrose pellets followed by 6 hours access to ALC and water in the home cage. After 6 days of self-administration on a fixed-ratio (FR) FR-1 schedule, followed by 6 days on an FR-3 schedule of reinforcement, oxycodone demand was assessed by increasing FR requirements across sessions. FR requirements increased every two days on a quarter logarithmic scale (FR-6, FR-10, etc.) until zero reinforcers were earned on both days. Rats re-established oxycodone self-administration on an FR-3 schedule of reinforcement for 2-3 days before extinction training and cue-induced reinstatement testing. Rats were assessed for anxiety-like behavior once and somatic signs of withdrawal at two different time points throughout the experiment, 24 hr after the last drug intake. In both males and females, oxycodone intake was reduced in the PSU group, however, female ALC+OXY rats exhibited greater anxiety-like behavior in withdrawal and increased demand for oxycodone. These results indicate that oxycodone-alcohol PSU can lead to greater withdrawal symptoms and increased motivation to seek oxycodone in females. Such results confirm the importance of considering PSU and sex differences in rodent models of substance use disorder.

Disclosures: C. Wilkinson: None. **G. Rojas:** None. **L.A. Knackstedt:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.05

Topic: G.09. Drugs of Abuse and Addiction

Support: Miami University Department of Psychology

Title: The Role of VTA Afferents in Opioid Withdrawal-Induced Aversion

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Abstract: The ventral tegmental area (VTA) is a dopaminergic region that plays a central role in reward-related behaviors and is implicated in drug addiction. Withdrawal from opioids is characterized by modulations of VTA activity and profound expressions of aversion, which may lead to relapse. However, our understanding of the role the VTA plays in this behavior is complicated by the fact that it receives projections from multiple brain regions, often with different neurotransmitter profiles. These issues preclude a deeper understanding of the neurobiological mechanisms of opioid addiction, which is necessary to develop better treatments.

In order to better understand VTA circuitry as it relates to opioid withdrawal-induced aversion, we chose two regions that send GABAergic projections to the VTA and that have been shown to inhibit dopamine release in the presence of aversive stimuli. The rostromedial tegmental nucleus (RMTg) and the nucleus accumbens (NAc) shell were chemogenetically inhibited during morphine withdrawal to assess their contribution to the development of conditioned place aversion (CPA).
C57BL/6J mice received pENN.AAVrg.hSyn.HI.eGFP-Cre.WPRE.SV40 bilaterally in the VTA to retrogradely infect afferent neurons. pAAV-hSyn-DIO-hM4D(Gi)-mCherry was bilaterally injected into the RMTg or NAc medial shell. Controls received pAAV-hSyn-DIO-eGFP. Mice were subjected to CPA by receiving a cocktail of clozapine-n-oxide (CNO, 1.0 mg/kg) and morphine (10.0 mg/kg), followed by naloxone (2.5 mg/kg) and confined to one side of a conditioning box. The opposite side of the conditioning box was paired with CNO and saline. Data were presented as the time spent on the withdrawal-paired side of the box before and after conditioning. Morphine-induced locomotion and somatic withdrawal experiments served as positive controls.
Inhibition of the RMTg's projection to the VTA did not affect withdrawal-induced CPA, but control experiments verified that the circuit was successfully targeted. It is possible that the RMTg does not play a central role in aversion to morphine withdrawal, but further experimentation is needed. Inhibition of the NAc's projection to the VTA is ongoing, but we hypothesize that inhibiting it will reduce the severity of CPA to morphine withdrawal. Given the seriousness of aversion during the withdrawal phase and its role in relapse, addressing this behavioral phenomenon is critical to gain a better understanding of the neural mechanisms underlying opioid addiction.

Disclosures: S. Monroe: None. **A.K. Radke:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.06

Topic: G.09. Drugs of Abuse and Addiction

Title: The 5α-reductase inhibitor finasteride reduces opioid self-administration.

Authors: *G. D. BOSSE[1], R. CADEDDU[1], G. FLORIS[1], R. FARERO[2], E. VITAGO[1], S. J. LEE[2], T. ZHANG[1], N. GAIKWAD[3], K. A. KEEFE[4], P. E. M. PHILLIPS[2], M. BORTOLATO[1], R. T. PETERSON[1];
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Abstract: Opioid use disorder (OUD) has become a leading cause of death in the US, yet current therapeutic strategies remain highly inadequate. To identify novel potential treatments for OUD, we screened a targeted selection of over 100 drugs, using a recently developed opioid self-

administration assay in zebrafish. This paradigm showed that finasteride, a steroidogenesis inhibitor approved for the treatment of benign prostatic hyperplasia and androgenetic alopecia, reduced self-administration of multiple opioids without affecting locomotion or feeding behavior. These findings were confirmed in rats; furthermore, finasteride did not interfere with the antinociceptive effect of opioids in rat models of neuropathic pain. Steroidomic analyses of the brains of fish treated with finasteride revealed a significant increase in dehydroepiandrosterone sulfate (DHEAS). Treatment with precursors of DHEAS reduced opioid self-administration in zebrafish, in a fashion akin to the effects of finasteride. Our results highlight the importance of steroidogenic pathways as a rich source of therapeutic targets for OUD and point to the potential of finasteride as a new option for this disorder.

Disclosures: **G.D. Bosse:** None. **R. Cadeddu:** None. **G. Floris:** None. **R. Farero:** None. **E. Vitago:** None. **S.J. Lee:** None. **T. Zhang:** None. **N. Gaikwad:** None. **K.A. Keefe:** None. **M. Bortolato:** None. **P.E.M. Phillips:** None. **R.T. Peterson:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.07

Topic: G.09. Drugs of Abuse and Addiction

Support: DA47855
DA48353

Title: In Vitro Pharmacological Characterization of Novel Fentanyl Analogs

Authors: *V. L. C. PALLARES[1], S. OBENG[1], M. MOTTINELLI[2], C. R. MCCURDY[2], L. R. MCMAHON[1], T. HIRANITA[1];
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Abstract: Fentanyl and its analogs are used as heroin adulterants, constituents of counterfeit prescription pills, and in isolation as abused drugs. Fentanyl and its analogs appear to have contributed to the rapidly increasing opioid overdose cases in the United States. Thus, there is the clear need to understand the pharmacology of novel fentanyl analogs. Here, the *in vitro* pharmacological effects of fentanyl were compared to those of six analogs of fentanyl [acryl fentanyl, β-hydroxythio fentanyl, cyclohexyl fentanyl, 4-fluoroisobutyrfentanyl (FIBF), furanyl fentanyl, and tetrahydrofuranyl fentanyl]. Using cell membranes expressing human opioid receptors, the affinity and efficacy were determined by radioligand receptor binding and [^{35}S]GTPγS functional assays, respectively. The affinities of fentanyl at mu- (MOR, [^{3}H]DAMGO), kappa- (KOR, [^{3}H]U69,593), and delta-opioid (DOR, [^{3}H]DADLE) receptors were 7.96, 202, and 539 nM, respectively. Affinities of furanyl and acryl fentanyl at MOR were 3.8- and 1.6-fold higher than that of fentanyl, respectively. Affinity of furanyl fentanyl at KOR was 1.6-fold higher than that of fentanyl. Affinities of furanyl, acryl, and β-hydroxythio fentanyl at DOR were 8.5-, 3.0-, 1.5-fold higher than that of fentanyl. Among the six analogs of fentanyl

tested, affinities of cyclohexyl fentanyl were the lowest at all three opioid receptor subtypes (Ki values greater than or equal to 1,030 nM). The E_{max} values of fentanyl at mu- (%DAMGO), kappa- (%U69,593), and delta-opioid (%SNC80) receptors were 107%, 54.5%, and 41.0%, respectively. All of the fentanyl analogs except furanyl (69.8%) and cyclohexyl (13.3%) fentanyl were full agonists at MOR (87.0% to 105%). Naltrexone (18.4 nM; 10 × Ki value at MOR) produced 13- and 7.5-fold rightward shifts in the concentration-effect functions of fentanyl and acryl fentanyl, respectively. At KOR, E_{max} values ranged from 3.86% (tetrahydrofuranyl fentanyl) to 47.1% (β-hydroxythio fentanyl), whereas at DOR, E_{max} values ranged from 8.67% (cyclohexyl fentanyl) to 52.4% (acryl fentanyl). With the exception of cyclohexyl fentanyl, these results suggest that the relatively high affinities and efficacies of the fentanyl analogs at MOR could contribute to opioid overdoses. Further, the similar affinity and efficacy profiles of fentanyl and acryl fentanyl among the three opioid receptor subtypes indicates that replacing the ethyl group with a vinyl group does not result in significant changes in binding affinity and efficacy; however, replacing the ethyl group with a cyclohexyl group results in a significant reduction in binding affinity and efficacy at MOR.

Disclosures: **V.L.C. Pallares:** None. **S. Obeng:** None. **M. Mottinelli:** None. **C.R. McCurdy:** None. **L.R. McMahon:** None. **T. Hiranita:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH DA25267
NIH DA48353

Title: Consequences of the Pharmacological Inhibition of Cytochrome P4503A on the Effects of the Mitragyna Speciosa (Kratom) Alkaloid Mitragynine in Rats

Authors: ***T. HIRANITA**, F. LEON, S. OBENG, L. R. GAMEZ JIMENEZ, L. F. RESTREPO, A. PATEL, N. P. HO, S. H. ZANDY, C. HARKINS, M. A. ALVAREZ, A. THADISETTI, E. J. KERN, C. R. MCCURDY, L. R. MCMAHON;
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Abstract: Mitragynine (MG) is an abundant alkaloid in the botanical *Mitragyna speciosa* (kratom). MG is converted into 7-hydroxymitragynine (7-OH-MG) by the cytochrome P450 3A isoform (CYP3A). Both MG and 7-OH-MG are μ-opioid receptor (MOR) ligands, and it appears that MG has lower efficacy (i.e., intrinsic activity) than 7-OH-MG. The extent to which *in vivo* conversion to and pharmacological actions of 7-OH-MG contribute to the effects of the parent compound MG has not been established. We hypothesized that inhibiting the conversion of MG into 7-OH-MG would increase the potency of MG *in vivo*. Ketoconazole (KTC) is a CYP3A inhibitor; we demonstrated it does not bind MOR *in vitro* up to 10μM. In separate groups of rats

trained to discriminate either MG (32 mg/kg, i.p.) or morphine (3.2 mg/kg, i.p.) from vehicle, KTC (56 mg/kg, p.o.) produced 4.2- and 4.7-fold leftward shifts, respectively, in the dose-effect functions of MG to increase drug-appropriate responding. However, KTC did not significantly modify the potency of MG to decrease rates of operant responding for food. Moreover, MG did not produce antinociceptive effects in a hotplate assay at 52°C even in the presence of KTC. The effects of KTC to increase the potency of MG to produce discriminative stimulus effects were pharmacologically selective inasmuch as KTC did not significantly increase the potency of morphine to produce discriminative stimulus, rate-decreasing, and antinociceptive effects. KTC also produced a 9.7-fold increase in the potency of 7-OH-MG to produce morphine-like discriminative stimulus effects, and a 4.1-fold increase in the potency of 7-OH-MG to produce antinociceptive effects. However, KTC did not modify the potency of 7-OH-MG to decrease operant response rates. Collectively, these results demonstrate that inhibition of CYP3A increases the potency of not only MG but also 7-OH-MG, suggesting that CYP3A metabolizes both MG and 7-OH-MG. Given that CYP3A mediates the metabolism of many clinically used medicines, and/or is inhibited or activated by various drugs, caution is warranted regarding CYP3A mediated drug-drug interactions in kratom users.

Disclosures: **T. Hiranita:** None. **F. Leon:** None. **S. Obeng:** None. **L.R. Gamez Jimenez:** None. **L.F. Restrepo:** None. **A. Patel:** None. **N.P. Ho:** None. **S.H. Zandy:** None. **C. Harkins:** None. **M.A. Alvarez:** None. **A. Thadisetti:** None. **E.J. Kern:** None. **C.R. McCurdy:** None. **L.R. McMahon:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant P50 DA046373
T32DA007288

Title: Plasticity changes in prelimbic neurons projecting to nucleus accumbens after heroin self administration, abstinence and relapse in Drd1- and Drd2-Cre rats

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Abstract: The cellular mechanisms underlying dysregulation of neurons in the prelimbic (PL) cortex that project to the nucleus accumbens (NAc) in relapse to drug-seeking after heroin self-administration (SA) are unclear. We investigated changes in intrinsic and synaptic plasticity in PL-NAc core neurons in wildtype (WT) and in Drd1-Cre+ and Drd2-Cre+ rats after heroin SA followed by abstinence with or without relapse. Sixty-six male and female Long Evans wildtype (WT), Drd1-Cre+ (D1), and Drd2-Cre+ (D2) rats were implanted with a jugular catheter and

their nucleus accumbens core (NAc core) infused with retrogradely transported AAVrg-hSyn-eGFP (WT) or AAVrg-hSyn-DIO-eGFP (Cre+) to isolate PL-NAc projecting neurons. After recovery, rats received yoked saline or were trained to self-administer heroin on a FR1 schedule for 14 days Following 7d homcage abstinence, a subset of rats underwent a cued relapse test (CRT) prior to whole-cell patch clamp recordings of PL->NAc neurons in oxygenated aCSF containing 100 μM picrotoxin. Current-clamp was employed to examine intrinsic cellular activity and voltage-clamp was used to characterize synaptic plasticity, including baseline sEPSCs and AMPA to NMDA ratios. Overall AP firing in PL->NAc neurons was significantly higher in WT heroin-abstinent rats (17.0±2.8) than in saline-treated rats (11.55±0.6; p=0.04) with no change in rheobase. Moreover, PL->NAc neurons in heroin-abstinent WT rats had greater sEPSC amplitudes (15.2±1.2pA vs 11.6±1.2pA; p=0.049) and sEPSC frequencies (2.9±0.2Hz vs 1.7±0.3Hz; p<0.01) than WT saline rats. AMPA/NMDA were significantly higher in the heroin group (1.6±0.2) than in the saline group (0.9±0.1; p<0.01). Similarly, $Drd1^+$ PL-NAc neurons in heroin-abstinent rats exhibited greater overall AP firing (19.6±0.7) than in saline $Drd1^+$ neurons (13.8±4.2; p=0.02). Moreover, in heroin-abstinent $Drd1^+$ rats, both amplitude and frequency of sEPSCs in PL->NAc neurons were significantly greater than in saline-treated rats. In contrast, AP firing in PL->NAc neurons did not differ, but sEPSC frequency was increased, in heroin-treated vs. saline-treated $Drd2^+$ rats. Following CRT, $Drd1^+$ PL->NAc neurons exhibited significantly less AP firing and decreased sEPSC amplitude and event frequency whereas cells in $Drd2^+$ rats remained unaffected. This study demonstrates heroin-induced neuroadaptations in PL->NAc neurons following 7 days of abstinence in WT rats are due to increases in intrinsic neuronal excitability and synaptic plasticity. Furthermore, these changes appear to be expressed predominantly by $Drd1^+$, but not $Drd2^+$, PL->NAc neurons after abstinence and reversed by cued relapse.

Disclosures: R.D. Cole: None. **J.D. Otis:** None. **J.F. McGinty:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA025646

Title: Differential immediate-early gene expression during context-induced and context-independent heroin-seeking behaviors in rats

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Abstract: Opioid abuse and overdose are major public health concerns, and a key impediment to the treatment of opioid addiction is drug relapse precipitated by exposure to drug-associated

environmental stimuli. The present study aimed to map neural substrates involved in goal-directed and habitual aspects of heroin-seeking behavior following abstinence in an instrumental model of drug relapse. To this end, male Sprague-Dawley rats were trained to lever press for heroin infusions in a distinct environment (i.e., Paired context) during at least 10 daily sessions. Next, the rats were exposed to an alternate environment with no access to drug or operant levers (i.e., Unpaired context) during 14 daily sessions. After forced abstinence, rats received a non-reinforced test session in the Paired or the Unpaired context, each with or without lever access. Brain tissue was collected immediately after the test session, and standard immunohistochemistry protocols were used to assess changes in the expression of c-Fos and zif268 immediate-early genes (IEGs), markers of neuronal activation and plasticity, in 42 brain regions. Our results indicated that heroin-seeking behavior was more robust in the Paired context than in the Unpaired context. Different groups of cortical and subcortical brain regions exhibited significant changes in IEG expression in response to (a) exposure to the Paired context vs Unpaired context with lever access, (b) lever access vs no-lever access independent of context, or (c) exposure to the Paired context depending on lever access. Furthermore, network activation analysis revealed that Paired context exposure with lever access resulted in the coupling, whereas Unpaired context exposure with lever access resulted in the uncoupling, of brain networks critical for goal-directed and habitual drug-seeking behaviors. These findings expand upon existing literature and may provide the rationale for future studies investigating the causal contributions of novel brain regions and novel circuits to heroin relapse.

Disclosures: **J.L. Ritchie:** None. **J.L. Walters:** None. **A.N. Lacey:** None. **J.M. Galliou:** None. **S.E. Swatzell:** None. **T.A. Brown:** None. **J.N. Roland-McGowan:** None. **R.A. Fuchs:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.11

Topic: G.09. Drugs of Abuse and Addiction

Support: DA031900
ommonwealth Universal Research Enhancement Program (PA Dept. Health)

Title: Oxycodone Exposure Modulates Dopamine Transmission in the Nucleus Accumbens

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Abstract: Opioid use disorder is a pervasive public health issue, particularly, prescription opioids have been integral in the progression of the opioid crisis over the past two decades. A major barrier in successful treatment of opioid use disorder is the difficulty for substance users to maintain long periods of drug abstinence, owing to high relapse rates. Therefore, it is paramount to further our understanding of the neural processes underlying the risk for relapse. Interestingly,

humans exhibit an increase in drug craving over time, termed incubation of drug craving. However, the neurobiological mechanisms underlying this incubation phenomenon remains unclear. Dopamine neurotransmission in the mesolimbic pathway has been established in substance use disorder, but remains largely unexplored with incubation of opioid craving. To examine to what extent incubation of opioid craving is associated with alterations in dopamine neurotransmission, female and male rats self-administered oxycodone under an intermittent access schedule of reinforcement. Following self-administration rats underwent a 14-day, forced abstinence period. Cue-induced seeking tests were conducted at the beginning and end of abstinence to assess incubation of oxycodone craving. Eighteen hours after the second seeking test, we conducted fast scan cyclic voltammetry recordings in a slice preparation. Preliminary results suggest individual differences in both oxycodone intake and propensity to incubate oxycodone craving, which was similar in females and males. Further, we found that dopamine neurotransmission in the nucleus accumbens was disrupted following oxycodone exposure but did not appear to differ between rats that displayed incubation of oxycodone craving and those that did not. These observations suggest that exposure to oxycodone may differentially influence future drug craving, but it remains unclear whether changes in dopamine neurotransmission underlie these differences.

Disclosures: **K. Samson:** None. **R. España:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.12

Topic: G.09. Drugs of Abuse and Addiction

Title: Clear volume analysis with machine learning (CVA-ML): an open-source rat-optimized pipeline to identify neuronal ensembles underlying context-induced reinstatement of oxycodone seeking

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Abstract: **Abstract:** The neuronal activity marker Fos is widely used to identify neuronal ensembles of reward seeking and relapse. However, previous studies require immunostained brain sections for targeted ensemble identification of specific regions. Recent advances in tissue clearing and immunostaining allow for unbiased characterization of neural activation patterns across intact mouse brains. The lack of rat-specific analysis pipelines has limited the use of this approach in rats. Here, we optimized the iDISCO+ immunolabelling procedure and developed an open-source analysis pipeline called CVA-ML (Clear Volume Analysis with Machine Learning)

to identify brain-wide ensembles of context-induced reinstatement of oxycodone seeking.
Methods: We trained male Sprague-Dawley rats to self-administer oxycodone (6-h/d, 14-d) in context A and then extinguished their lever pressing in context B. We then tested for reinstatement of oxycodone seeking in both contexts A and B. We perfused and extracted the rats' brains 90 min after reinstatement tests and used our rat-optimized iDISCO+ pipeline to label Fos-positive nuclei. We imaged cleared brains using light-sheet fluorescence microscopy (LSFM) and used CVA-ML to map reinstatement-associated ensembles across the whole rat brain.
Results: We observed reliable context-induced reinstatement of oxycodone seeking after extinction of operant responding in a non-drug context. We labelled brains for Fos, imaged them using LSFM, and used CVA-ML to analyze Fos-expression patterns in these 3D datasets.
Conclusions: We present an open-source CVA-ML pipeline for whole brain activity mapping in rats. We demonstrate the application of this pipeline to identify the functional connectome underlying context-induced reinstatement of oxycodone seeking.
This work was supported by NIDA/NIH

Disclosures: **A. Shekara:** None. **J. Nguyen:** None. **I. Fredriksson:** None. **Y. Shaham:** None. **J.M. Bossert:** None. **M.B. Brenner:** None. **C. Mejias-Aponte:** None. **B.T. Hope:** None. **R. Chen:** None. **S.A. Golden:** None. **R. Madangopal:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.13

Topic: G.09. Drugs of Abuse and Addiction

Support: P30DA0333034
F32DA047026
1FI2GM128603
Intramural Research Program of NIDA

Title: Lack of effect of different pain-related manipulations on opioid self-administration, choice, and reinstatement of drug seeking

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Abstract: Background: Pain increases the risk of opioid addiction and opioid-induced pain relief may function as a negative reinforcer to increase opioid taking. However, experimental pain-related manipulations generally do not increase intravenous opioid self-administration in rodents. This discrepancy may reflect insufficient learning of pain-relief contingencies or the confounding impact of pain-related motor impairment. Here we determined if pairing noxious stimuli with opioid self-administration would promote pain-related reinstatement of opioid

seeking or increase opioid choice over food.
Methods: In Experiment 1, rats self-administered fentanyl in the presence or absence of repeated intraplantar capsaicin injections in distinct contexts to model context-specific exposure to acute cutaneous inflammatory pain. After capsaicin-free extinction in both contexts, we tested if capsaicin reinstated fentanyl seeking. In Experiment 2, rats self-administered heroin after intraperitoneal (i.p.) lactic acid injections to model acute visceral inflammatory pain. After acid-free extinction, we tested if lactic acid would reinstate heroin seeking. In Experiment 3, we tested if treatment with either repeated lactic acid or intraplantar Complete Freund's Adjuvant (CFA; to model sustained inflammatory pain) would increase fentanyl choice over a food reinforcer.
Results: In Experiments 1 and 2, neither capsaicin nor lactic acid reinstated opioid seeking after extinction, and lactic acid did not increase heroin-induced reinstatement. In Experiment 3, lactic acid and CFA decreased reinforcement rate without affecting fentanyl choice.
Conclusions: These results extend the range of conditions across which pain-related manipulations fail to increase opioid seeking in rats and suggest that enhanced opioid-addiction risk in humans with chronic pain involve factors other than enhanced opioid reinforcement and relapse.
Supported by P30DA0333034, F32DA047026, 1FI2GM128603, and Intramural Research Program of NIDA

Disclosures: D.J. Reiner: None. **E. Townsend:** None. **J. Menendez Orihuel:** None. **S. Applebey:** None. **S. Claypool:** None. **M. Banks:** None. **Y. Shaham:** None. **S.S. Negus:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.14

Topic: G.09. Drugs of Abuse and Addiction

Support: DA25267
DA48353

Title: In vitro and in vivo pharmacological comparison of a synthetic analogue of the primary Mitragyna speciosa (kratom) alkaloid mitragynine

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Abstract: With the ongoing opioid epidemic, opioid-dependent individuals have turned to the Southeast Asian indigenous tree, *Mitragyna Speciosa* (Kratom), to curb the undesirable opioid-induced adverse effects associated with continual use of opioids. Mitragynine is the major bioactive constituent in the plant, accounting for up to 66% of crude alkaloid extract, and exerts an important pharmacological effect to mitigate the opioid-induced adverse effects via the μ-opioid (MOR) and α_2-adrenergic ($A\alpha_2R$) receptors. To better understand the structure-activity

relationship of mitragynine at these targets, structurally simplified analogues were synthesized and assessed for affinity and efficacy at opioid and α_{2C}-adrenergic receptors *in vitro* and *in vivo*. In a radioligand receptor binding assay, affinities (Ki values) of mitragynine at human μ-, κ-, δ-opioid, and α_{2C}-adrenergic receptors were 709 ([^{3}H]DAMGO), 1,530 ([^{3}H]U69,593), 6,800 ([^{3}H]DADLE), and 4,040 nM ([^{3}H]RX821002), respectively. Among the mitragynine analogues, affinities of MLC-1, a C_1-modified analog, at μ-, κ-, δ-opioid, and α_{2C}-adrenergic receptors were 2.1-fold lower, 1.8-fold higher, 2.9-fold lower, and 2.5-fold higher compared to mitragynine. In a [^{35}S]GTPγS functional assay at MOR, Emax values of mitragynine and MLC-1 were less than 4.0% relative to DAMGO. In rats trained to discriminate mitragynine (32 mg/kg, i.p.), MLC-1 (17.8 mg/kg, i.p.), the MOR agonist morphine (up to 56 mg/kg, i.p.), and the Aα_2R agonist lofexidine (up to 0.56 mg/kg, i.p.) produced 77.2%, 70.4%, and 74.1% mitragynine-lever responding, respectively. In rats trained to discriminate morphine (3.2 mg/kg, i.p.), MLC-1 (up to 32 mg/kg), mitragynine (up to 56 mg/kg, i.p.) and lofexidine produced 31.9%, 56.6%, and 23.9% morphine-lever responding, respectively. In a hotplate assay at 52°C, maximum possible antinociceptive effects of morphine, mitragynine, MLC-1, and lofexidine were 89.4%, 1.1%, 25.6%, and 43.3%, respectively. In these rats, maximum hypothermic effects of lofexidine, mitragynine, MLC-1 and morphine were 4.9°C, 1.0°C, 1.5°C, and 0.18°C, respectively. The present results suggest that C_1- modification of mitragynine results in no dramatic change in *in vivo* activity at μ-opioid and α_{2C}-adrenergic receptors; however, a slight shift toward the Aα_2R activity was observed. Further structural simplification of the mitragynine scaffold is required to understand the structure-activity relationship at both μ-opioid and α_{2C}-adrenergic receptors.

Disclosures: **M.L. Crowley:** None. **S. Obeng:** None. **V.L.C. Pallares:** None. **F. León:** None. **L.R. Gamez-Jimenez:** None. **T. Hiranita:** None. **L.R. McMahon:** None. **C.R. McCurdy:** None.

Digital Abstract Session

P299. Opioid Adaptive Behavior

Program #/Poster #: P299.15

Topic: G.09. Drugs of Abuse and Addiction

Support: MRC Grant MR/N02530X/1

Title: Behavioural and neurobiological basis of the individual vulnerability to develop compulsive heroin seeking

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Abstract: Addiction has been hypothesised to result from a transition from volitional, recreational drug use to compulsive drug seeking seen in vulnerable individuals. The neural and behavioural factors underlying the vulnerability to switch to compulsive drug use for stimulant

drugs are increasingly being understood. Here we present a new procedure to measure compulsive heroin seeking behaviour in order to identify behavioural and molecular markers of the vulnerability to develop this characteristic of heroin addiction that is a major burden on individual and society. In this novel procedure, heroin seeking in a drug-free state, maintained by heroin-associated cue presentations over prolonged periods, was challenged by the contingent presentation of electric footshocks in a large cohort of rats with an extended history of heroin seeking and use. Marked individual differences in the tendency to seek heroin compulsively enabled the investigation of the behavioural and molecular endophenotypes of this vulnerability. In a series of longitudinal studies, drug naive rats were tested for several addiction-relevant traits including anxiety, decision making, sensation and novelty seeking as well as reward (sweetness) preference, prior to being trained to seek heroin under the influence of the conditioned reinforcing properties of heroin-paired cues for several weeks (a second-order schedule of reinforcement). The compulsive nature of heroin seeking behaviour was then measured over several sessions during which mild footshock punishment was delivered contingent on seeking responses prior to, but not during, the administration of heroin. An individual's tendency to engage in vigorous cue-controlled heroin seeking and to resist punishment was shown to be predicted by both a high anxiety trait and sign tracking phenotype. This individual vulnerability was associated with an alteration in the adenosinergic system in the nucleus accumbens. Rats identified as highly compulsive had higher mRNA and protein levels of the adenosine 2A receptor in the ventral striatum as compared to low compulsive rats. The expression of adenosinergic proteins was further shown to predict compulsive heroin seeking at the population level. Together, these data indicate that high anxiety and a propensity to ascribe incentive value to conditioned stimuli predict the vulnerability to develop compulsive heroin seeking behaviour that may be mediated by alterations in the adenosinergic system in the ventral striatum. These data offer new avenues to developing both preventative and potential therapeutic targets to decrease compulsive heroin seeking, a key characteristic of heroin addiction.

Disclosures: **M. Fouyssac:** None. **M. Puaud:** None. **M. Siddle:** None. **B.J. Everitt:** None. **D. Belin:** None.

Digital Abstract Session

P300. Mechanisms of Opioid Action

Program #/Poster #: P300.01

Topic: G.09. Drugs of Abuse and Addiction

Support: 20-ERD-009

Title: Cellular, molecular, and functional changes in the brain following acute and chronic fentanyl exposure: Effect of age and gender

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Abstract: Background: The CDC has reported that 70, 237 people have died from a drug overdose and out of those drug overdose deaths, two thirds involved an opioid, like Fentanyl. Fentanyl is one of the most frequently used synthetic opioids in medicine where it has established itself as an analgesic for patients undergoing surgery, and in the proper management of chronic pain. Unfortunately, due to its heroin-like euphoric effects, in the past two decades fentanyl has found itself involved in a number of illicit and lethal drug overdosing cases. Fentanyl, like morphine, binds to mu-opioid receptors (MOR) in many brain regions, and this binding event can lead to addiction, impairment of cognitive functions, and inhibit nociception, arousal, and respiration. Clinical evidence suggests gender differences between children and adults in response to opioids, such as: significant ventilation depression in young female children; higher rates of opioid abuse in men and greater negative side effects (e.g., nausea and vomiting) in women. However, it is not clear how gender differences contribute to the differential response observed in acute and chronic exposure to synthetic opioids, such as fentanyl. Methods/Results: In this study, we examined the effects of acute (i.e., single dose) and sub-chronic (i.e., daily dosing for 5 days) exposure to 50 μg/kg of fentanyl to the brains of female and male postnatal (P14-18) and adult (6-8 weeks) rats. Immunohistochemistry and bulk RNA-sequencing was used to characterize the cellular and molecular changes in the cortex, hippocampus and cerebellum. To examine the effects of opioid use on neuronal function, neurons from postnatal and adult rat brains sub-chronically exposed to fentanyl were harvested and cultured on a multi-electrode array to monitor neural network formation and maturation. Conclusion: Collectively, the findings of this study will determine whether age and sexual dimorphism following acute and sub-chronic fentanyl exposure can be determined by cellular, molecular, and functional changes in the brain. Evaluating these factors will be important to identify whether the age and sex of the warfighter, treated with fentanyl, is susceptible to the adversive effects of the synthetic drug.
This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC 52-07N A27344 through LDRD award 20-ER D-009. (LLNL-ABS-807371)

Disclosures: D. Lam: None. **A. Sebastian:** None. **S.K.G. Peters:** None. **M.E. Mendez:** None. **N.R. Hum:** None. **C.A. Valdez:** None. **N.O. Fischer:** None. **G.G. Loots:** None. **H.A. Enright:** None.

Digital Abstract Session

P300. Mechanisms of Opioid Action

Program #/Poster #: P300.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01AA027214
NIH Grant F32AA026488

NIH Grant T32AA007462
NIH Grant UL1TR002529

Title: Mu opioid receptors in vGluT2-expressing glutamatergic neurons modulate opioid reward

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Abstract: Much research has focused on the role of mu opioid receptor (MOR)-mediated regulation of GABA transmission in opioid reward. MORs also regulate glutamate transmission and much less is known about the role of these MORs in drug reward. Previously, we found that MORs inhibit glutamate transmission at thalamostriatal synapses that express the type 2 vesicular glutamate transporter (vGluT2). To explore the role of MORs in vGluT2-expressing neurons in opioid-related behaviors, we created a transgenic mouse that lacks MORs in vGluT2-expressing neurons (MORflox-vGluT2cre). A variety of behavioral assessments were performed on adult male and female MORflox-vGluT2Cre positive (+) knockout mice with cre negative (-) littermate controls, with a focus on opioid reward. MORflox-vGluT2cre mice do not acquire conditioned place preference for a low doses (0.05, 0.5 mg/kg) of the opioid, oxycodone, but acquire conditioned place aversion for a higher dose (5 mg/kg), whereas control mice acquire conditioned place preference for all tested doses. In two-bottle choice oral consumption assessment, MORflox-vGluT2cre mice consume less oxycodone and have reduced preference for oxycodone over water, compared to controls. MORflox-vGluT2cre mice also lack oxycodone-induced locomotor stimulation, but show no basal differences in locomotor activity, compared to controls. Interestingly, MORflox-vGluT2cre mice display baseline withdrawal-like responses following the development of oxycodone dependence that are not seen in controls. In addition, unlike controls, withdrawal-like responses do not increase following challenge with the opioid antagonist, naloxone. Other MOR-mediated behaviors, including oxycodone-induced antinociception and sucrose consumption, are intact in MORflox-vGluT2cre mice. Additionally, MORflox-vGluT2cre mice do not differ from controls in basal or oxycodone-induced plasma corticosterone levels, a measure of stress. These data reveal that MOR modulation of glutamate transmission is a critical component of opioid reward. Current research is focused on identifying the neurocircuitry of MOR modulation of glutamate transmission and role of these neurocircuits in opioid reward. We selectively deleted MORs in the thalamus, a region with a high density of vGluT2 neurons, by stereotaxically injecting AAV.CamKii.Cre.GFP into the thalamus of 6-week-old male and female MORflox mice. Control mice were injected with AAV.CamKii.GFP. Deleting thalamic MORs did not affect acquisition of oxycodone conditioned place preference, suggesting this population of MORs does not play a significant role in opioid reward.

Disclosures: K.C. Reeves: None. **M. Kube:** None. **G.G. Grecco:** None. **B. Fritz:** None. **B. Munoz:** None. **F. Yin:** None. **Y. Gao:** None. **D. Haggerty:** None. **H. Hoffman:** None. **B.K. Atwood:** None.

Digital Abstract Session

P300. Mechanisms of Opioid Action

Program #/Poster #: P300.03

Topic: G.09. Drugs of Abuse and Addiction

Support: TAMU 2019 T3 President's Excellence Fund

Title: Detecting individual differences in response to opioids using Raman spectroscopy

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Abstract: Both personality and social environment were suggested to influence the development of opioid use disorder (OUD). Similarly, we demonstrated using rodent models that both social environment and individuals' sociability levels influence the response to opioids. Mice can be divided, based on their sociability levels, into Socially Avoiding (SA) and Socially Exploring (SE). We previously demonstrated that SE mice are more sensitive to the sensitizing effects of opioids. In contrast, SA mice are more sensitive to opioids' effects on stress and pain. This suggests that the positive reward system is likely mediating abuse escalation in SE individuals, while the negative reinforcement is likely mediating abuse escalation in SA individuals. Although opioid use disorder is a serious and relapsing mental health disease, in contrast to other chronic disorders, there are no objective diagnostic tools for early detection or to monitor disease progression. Raman spectroscopy is used in other biomedical fields for both research and as a diagnostic tool, but is underutilized for the study of OUD. In this project, we have demonstrated that by using Raman spectroscopy, we can detect baseline differences between SA and SE mice, as well as in their responses to morphine. SA and SE mice were treated with saline or morphine. Different brain areas were examined, including the amygdala, nucleus accumbens, ventral tegmental area, prefrontal cortex, and hippocampus. We observed molecular differences between saline-treated SA and SE mice. This indicates that individual differences in sociability levels could possibility be detected and assessed using Raman spectroscopy. Repeated exposure to opioids altered brain molecular composition in both SA and SE mice. However, differential responses were observed in the response to morphine in SA and SE mice. These results suggest that SA and SE individuals have differential behavioral and molecular responses to opioids, and that treatment for OUD should be tailored to individuals based on their personalities and social interactions style. Thus, these methodologies are expected to provide insights into potential need to individualize the care for OUD patients, as well as markers to be used for objective diagnosis of the development of trajectory of OUD in humans.

Disclosures: C.A. Madison: None. **S. Chavva:** None. **S. Mabbott:** A. Employment/Salary (full or part-time):; Texas A&M. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; T3 Research

Grant, TAMU. **S. Eitan:** A. Employment/Salary (full or part-time):; Texas A&M. **P. Wellman:** A. Employment/Salary (full or part-time):; Texas A&M.

Digital Abstract Session

P300. Mechanisms of Opioid Action

Program #/Poster #: P300.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant MH106500
UMSOM Dean's Challenge Award ACCEL-Med

Title: Increased fentanyl consumption in male and female C57BL/6 mice after chronic witness defeat stress

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Abstract: Synthetic opioids, like fentanyl, have accelerated rising opioid related drug overdose. Thus, there is a critical need to investigate factors that may facilitate the development of opioid use disorder. Social stress is linked to escalated cocaine and alcohol consumption in preclinical research. However, Chronic Social Defeat Stress (CSDS), a paradigm used to achieve social stress, often produces social and physical stress. Since opioids have inherent analgesic properties, it is important to delineate between analgesic and rewarding effects when examining the potential stress-sensitization of opioid consumption. Chronic Witness Defeat Stress (CWDS), a validated paradigm that only produces social stress, allows examination of stress-sensitized opioid consumption. The goal was to evaluate stress induced changes in fentanyl consumption using CWDS and escalating fentanyl two-bottle choice paradigms. Male and female C57BL/6 mice completed 10 days of CWDS, in which subjects indirectly experienced the direct social defeat of a male C57BL/6 intruder mouse by a male CD1 aggressor. A social interaction (SI) test was completed to determine susceptible (avoidant behavior) and resilient (approach behavior) groups. Afterward, mice completed a 15 day escalating fentanyl two-bottle choice paradigm that comprised of three distinct periods of forced fentanyl access, each characterized by a higher dose of fentanyl (i.e. 5, 10, and 15 μg/mL). Periods of forced fentanyl access were followed by a choice day of voluntary fentanyl consumption wherein mice had access to fentanyl and water. A second SI test was completed to assess susceptible and resilient behavioral groups after fentanyl exposure. Overall, CWDS elicited stress-induced increases in fentanyl consumption. Resilient and susceptible male mice consumed more fentanyl in all three forced access periods compared to controls. Female mice voluntarily consumed more fentanyl compared to resilient mice and had higher fentanyl preferences compared to control and resilient mice during the third choice day. Susceptible male mice subjected to CSDS preferred and consumed more fentanyl compared to resilient mice during the second period of forced and voluntary fentanyl access. Thus, CWDS and CSDS paradigms can yield stress induced changes in fentanyl consumption. Resilient male and female mice also displayed a decrease in social interaction after completion of the fentanyl

two-bottle choice paradigm, suggesting that fentanyl exposure itself can negatively impact social behavior. Overall, our findings suggest that social stress can increase vulnerability to opioid misuse in susceptible and resilient populations.

Disclosures: **D. Franco:** None. **M.E. Fox:** None. **A. Wulff:** None. **M.K. Lobo:** None.

Digital Abstract Session

P300. Mechanisms of Opioid Action

Program #/Poster #: P300.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH-MBRS SCORE-1SC2DA047809-02
NIH-RISE-R25GM061838

Title: Hippocampal, amygdalar and accumbal BDNF expression in morphine extinction

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Abstract: Opiate addiction has quickly become a health crisis in the United States. The Council of economic advisors estimates that the opioid crisis will have a cost of 2.5 trillion dollars from 2018 to 2022. Efforts to reduce the number of opiate addicts have led to the development of new treatment options, such as medication replacement therapies (methadone, buprenorphine) and behavioral (exposure) therapies. Despite their relative success, a high number of opiate addicts' relapse, and many die of drug-related overdoses. We used preclinical models of addiction to understand the molecular mechanisms underlying opioid-seeking behaviors. Previously, we created an mRNA profile of the Ventral Striatum/ Nucleus Accumbens (VS/NAc) of rats who had undergone morphine extinction. We found an increase in bdnf mRNA in animals who successfully extinguished morphine place preference. Although BDNF has been associated with extinction of addictive behaviors, whether this increase at the messenger level could be translated into active protein expression, remains to be determined. Here, we measured BDNF expression in the VS/NAc of animals that underwent extinction of morphine place preference by using Western blots. In accordance with our previous finding, we found an increase in BDNF protein expression in VS/NAc of animals in the extinction group. Given that BDNF expression in VS/NAc is naturally low, we questioned whether this BDNF could be axonally transported as well, from other brain regions of the reward circuit ie., hippocampus and amygdala. In the hippocampus we found a significant increase of BDNF in animals that successfully extinguished morphine place preference, while in the amygdala, we found this increase not only in animals of the extinction group, but also in animals showing extinction failure. The observed increase in BDNF expression in the VS/NAc validates our previous findings and suggests BDNF's viability as a potential future pharmacological target for the extinction of opioids.

Disclosures: **M.E. Lloret Torres:** None. **R.N. Ayala-Pagan:** None. **F.J. Martinez:** None. **J.L. Barreto-Estrada:** None. **L.A. Gonzales-Martinez:** None.

Digital Abstract Session

P300. Mechanisms of Opioid Action

Program #/Poster #: P300.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R00-MH105549
Rising STARs Award from University of Texas System
NARSAD Young Investigator Grant from Brain & Behavior Research Foundation
NIH R01 MH120136

Title: Neuronal correlates of opioid-induced risk-taking behavior in the prelimbic cortex.

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Abstract: Opioid misuse is associated with impaired risk-related decision making. However, the neural correlates of opioid-induced risky motivated behavior are unclear. Working with the hypothesis that altered prefrontal cortex activity underlies opioid-induced risk taking, we employed an approach-avoidance conflict model using a modified conditioned place preference protocol combined with a fear-inducing predator odor. Adult male Long-Evans rats implanted with single-unit electrodes in the prelimbic cortex (PL) were injected with saline or the opioid drug morphine and placed in the side of the apparatus least preferred at baseline. Conditioning with saline (n=18) or morphine (n=20) occurred on alternating days over a 10-day period. After 72 h of forced abstinence, morphine-treated rats showed increased preference for the drug-paired side when compared to saline controls ($p<0.01$). Immediately following preference testing, a conflict test began in which a predator odor stimulus (cat saliva) was placed in the previously drug-paired side. During conflict, saline-paired rats demonstrated a clear aversion to the paired side. In contrast, morphine-paired rats continued to enter the paired side despite the presence of predator odor, suggesting an increase in risk-taking behavior. Single-unit recordings from PL neurons revealed a significant suppression in neural activity after acute administration of morphine (22%), but not saline (6%, $p<0.05$). During the last day of conditioning, PL cells no longer exhibited drug-induced suppression of firing rates, suggesting that repeated morphine administration causes a physiological adaptation in PL activity. During the conflict test, the spontaneous firing rates of most PL cells in the saline group were inhibited (53%) rather than excited (7%; $p<0.05$), though this pronounced inhibition-to-excitation ratio was not observed in the morphine group (35% inhibited vs. 33% excited; $p=1.0$). Together, our results indicate that repeated opioid exposure leads to enhanced expression of reward-related memory and a neuronal

adaptation to opioid effects in PL. Additionally, repeated administration of opioids increases risk-taking responses during motivational conflict and such behavior correlates with decreased inhibitory tone in PL neurons.

Disclosures: C.B. Quave: None. **A.M. Vasquez:** None. **E.P. Bora:** None. **C.L. Chidomere:** None. **D.S. Engelke:** None. **F.H. Do Monte:** None.

Digital Abstract Session

P300. Mechanisms of Opioid Action

Program #/Poster #: P300.07

Topic: G.09. Drugs of Abuse and Addiction

Support: 1P01DA041307-01
R01CA142115-02
NIH S10 OD019948

Title: Cannabinoids influence on respiratory function and their impact on morphine induced respiratory depression

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Abstract: CANNABINOIDS INFLUENCE ON RESPIRATORY FUNCTION AND THEIR IMPACT ON MORPHINE INDUCED RESPIRATORY DEPRESSION Beth M. Wiese[1], Erika Liktor-Busa[1], Aidan Levine[1], Sarah A. Couture[1], Spyros P. Nikas[2], Lipin Ji[2], Yingpeng Liu[2], Kenneth Mackie[3], Alexandros Makriyannis[2], Tally M. Largent-Milnes[1], Todd W. Vanderah[1]
[1]Department of Pharmacology, University of Arizona, 1501 N Campbell Ave., Life Sciences North Rm 621, Tucson, AZ, 85724, USA. [2]Chemistry and Chemical Biology, Bouve College Health Sciences – Center for Drug Discovery, 116 Mugar Life Sciences Building, Northeastern University, 360 Huntington Ave., Boston, MA, 02115, USA. [3]Department of Psychological and Brain Sciences, Indiana University Bloomington, Multidisciplinary Science Building II 120, 702 North Walnut Grove Ave., Bloomington, IN 47405-2204.The current wide-spread scope of the opioid epidemic continues to be defined by an escalating number of fatalities resulting from accidental opioid overdoses. Respiratory depression is usually the cause of a fatal opioid overdose, when the neurons of the preBötzinger complex, a nucleus in the brainstem that controls reflexive inspiration, become desynchronized due to mu opioid receptor activation. Prior literature has shown cannabinoid receptor agonism may enhance opioid effects, yet it is unknown whether cannabinoids enhance or mitigate opioid-induced respiratory depression. In this study we sought to define the role of the cannabinoid receptor 2, CB2R, in respiratory function using selective agonists to determine the effects of CB2 activation on respiratory depression alone and

when combined with morphine sulfate (MS) in CD1 mice. Utilizing whole body plethysmography, selective CB2 agonism with AM2301 did not induce respiratory depression nor did the phytocannabinoids, THC, CBD, and BCP. In contrast, a selective synthetic CB1 cannabinoid, AM356, induced respiratory depression. Moreover, AM2301 the CB2 agonist, significantly prevented morphine-induced respiratory depression when drugs were administered in combination. These findings show mitigation of opioid induced respiratory depression can be achieved with activation of CB2Rs, and not CB1Rs. These data support CB2R agonists as an adjunct to opioid therapy, reducing the harmful, and potentially lethal, opioid-induced respiratory depression.

Disclosures: B.M. Wiese: None. **E. Liktor-Busa:** None. **A. Levine:** None. **S. Couture:** None. **T. Largent-Milnes:** None. **T. Vanderah:** None. **S. Nikas:** None. **A. Makriyannis:** None. **L. Ji:** None. **Y. Liu:** None. **K. Mackie:** None.

Digital Abstract Session

P300. Mechanisms of Opioid Action

Program #/Poster #: P300.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NS027881
DA034748

Title: Chronic morphine exposure induces cell type specific changes in the intrinsic electrophysiological properties of mouse hypocretin/orexin (H/O) neurons

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Abstract: H/O neurons regulate arousal, feeding, and reward – a point recently punctuated by the discovery that human heroin addicts have H/O neurons that are ~22% smaller and 54% more numerous than those in age-matched subjects (Thannickal et al. 2018). To investigate how chronic opioid exposure functionally impacts H/O neurons, we made whole-cell recordings from H/O-EGFP neurons in acute brain slices from male mice (9-18 w) treated with either morphine (50 mg/kg/day) or saline for 14 days. With investigators blind to treatment for all experiments, we first used standard immunohistochemistry to verify that morphine altered soma size of H/O-EGFP neurons, since only 40-70 % of H/O neurons express EGFP in these mice. We found that the average somatic cross-sectional area was 17% smaller in morphine treated mice (n=4, ~100 neurons/mouse) compared to saline treated mice (n = 4, ~90 neurons/mouse; $p < 0.05$). We next examined sEPSCs and the intrinsic properties of H/O-EGFP neurons (n~30 neurons, from 6 mice in each treatment group). We found that morphine treatment did not change the proportion of D- or H- type H/O neurons (saline n=18/13 D/H type, morphine n=17/14 D/H type) which we

distinguished by characteristic membrane responses dominated by A-type K^+ current in H-cells (Schöne et al. 2011). Morphine treatment also did not alter spontaneous firing, sEPSC amplitude, input resistance or cell capacitance, although there was a trend toward increased capacitance and decreased sEPSC amplitudes for H cells ($p = 0.07$). In contrast, morphine treatment selectively altered spiking in H cells. These neurons showed an ~43% increase in rheobase, an ~10% decrease in action potential amplitude and an afterhyperpolarization minimum that was ~3 mV less negative (t-test, $p < 0.05$) than in H-cells from saline treated mice. A specific decrease in H-cell excitability was further indicated by an analysis of repetitive firing which found that H- but not D- cells had F-I relations that were right-shifted and reduced in slope following morphine. Moreover, the firing patterns for H-cells, but not D-cells shifted from one of continuous firing throughout the current pulse toward more clustered and inconsistent firing. Collectively, these data underscore the functional heterogeneity among H/O neurons and indicate that in addition to inducing structural plasticity, chronic morphine differentially decreases excitability of H-type H/O neurons. This implies their encoding and transmission of information is impaired following chronic morphine exposure and suggests that alterations in the electrophysiological properties of H-type H/O neurons may contribute to opiate addiction.

Disclosures: E.A. Berry: None. **S. Javed:** None. **R. McGregor:** None. **M. Ishibashi:** None. **J.M. Siegel:** None. **C.S. Leonard:** None.

Digital Abstract Session

P300. Mechanisms of Opioid Action

Program #/Poster #: P300.09

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Chemogenetic Inhibition of Midbrain Dopamine Neurons During Object Exploration Enhances Future Novel Object Discrimination

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Abstract: It is well established that dopamine (DA) neurons in the ventral midbrain signal novelty by increasing their bursting activity, while decreasing it after repeated inconsequential stimulus presentations. Thus, the lack of DA neuron burst activity is a physiological signature that a particular stimulus has become familiar. Here, we tested the hypothesis that decreasing DA neuron activity while animals explore objects for the first time enhances familiarization and improves subsequent novel object discrimination.
Novel object recognition is dependent on the amount of pre-exposure to the familiar object. We show that two familiarization sessions spread out over two days produce robust novelty discrimination, while only one familiarization session is not sufficient to cause novelty discrimination during the novel object recognition task. The lack of exploration on the novel object is thought to reflect a stage of equal attention allocated to the familiar and novel stimulus,

which is interpreted as a weakened memory of the previously seen familiar stimulus. To investigate whether decreasing DA neuron activity prompts gain-of-function in novelty recognition, we chemogenetically inhibited DA neurons in the ventral tegmental area (VTA) during the familiarization session of the novel object recognition paradigm that results in poor novelty discrimination.
We injected an AAV *flp*-dependent hM4D into the VTA of TH-*flp* transgenic mice to selectively inhibit TH-positive DA neurons. The selectivity of the viral strategy was verified via immunohistochemistry staining. The virus was then activated via injection of Clozapine N-oxide dihydrochloride (CNO; i.p.) 30 minutes before trial. We found that inhibiting DA neurons during the familiarization phase resulted in enhanced discrimination during the novelty task in experimental animals compared to control animals. To confirm these results were due to an effect on familiarization processes, we conducted further trials in which DA neurons were only inhibited after the familiarization phase and during subsequent novel object recognition session. Under this manipulation, we did not see an improvement in discrimination. These results suggest that suppressing DA activity ameliorates novelty discrimination by enhancing familiarity.

Disclosures: **S. Fleury:** None. **S. Mingote:** None. **U. Gether:** None. **A. Sørensen:** None.

Digital Abstract Session

P301. Nicotine: Circuitry, Cognition, Behavior, and Physiological Effects

Program #/Poster #: P301.01

Topic: G.09. Drugs of Abuse and Addiction

Support: HHMI Gilliam Fellowship
NSF GRFP
Tobacco-Related Disease Research Program

Title: Characterizing a brainstem to midbrain circuit involved in nicotine aversion

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Abstract: Decades of research have demonstrated a crucial role of ventral tegmental area (VTA) dopamine (DA) neurons in mediating the brain's response to natural rewards and the reinforcing effects of addictive drugs. However, recent studies have revealed that the VTA is a heterogeneous population of neurons, with DA cells in the medial VTA signaling aversion. Here, we leverage the dose-dependent properties of nicotine to understand how the midbrain DA system responds to an addictive drug that is rewarding at low doses and aversive at high doses. Using anatomical tracing, electrophysiology, and in vivo fiber photometry, we demonstrate that an aversive dose of nicotine strongly activates an inhibitory input from the laterodorsal tegmentum (LDT) to the VTA and that this functional input can inhibit VTA DA cells to affect downstream DA release in the nucleus accumbens (NAc). Aversive nicotine both inhibits a pathway that signals reward by decreasing DA release in the lateral shell of the NAc (NAcLat)

and activates a pathway that signals aversion by increasing DA release in the ventromedial shell of the NAc (vNAcMed). Importantly, ablating LDT GABA neurons alters the DA release patterns in response to an aversive dose of nicotine. Together, these results introduce a circuit mechanism for nicotine aversion that involves subcircuits regulating both reward and aversion and implicates a long-range brainstem input to the midbrain as a mediator of this effect.

Disclosures: C. Liu: None. **A.J. Tose:** None. **Y. Zhu:** None. **J.W. de Jong:** None. **S. Lammel:** None. **K.T. Beier:** None.

Digital Abstract Session

P301. Nicotine: Circuitry, Cognition, Behavior, and Physiological Effects

Program #/Poster #: P301.02

Topic: G.09. Drugs of Abuse and Addiction

Support: N01DA-13-8908

Title: Potential Application of FSNY-1 as Smoking-Cessation Drug

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[2]Pharmacol. and Neurosci., [3]Dept. of Pharmacol. and Neurosci., [1]Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: A novel compound, FSNY-1 was tested for its ability to inhibit the biphasic psychomotor stimulant and depressant effects of nicotine in mice. Different groups of young mice were pretreated with FSNY-1 or the positive controls varenicline and mecamylamine and placed into chambers for recording of locomotor activity after an injection of 1.6 mg/kg nicotine. A negative control group was pretreated with the peripheral ganglion-blocking agent, hexamethonium, prior to nicotine injection. FSNY-1 and hexamethonium selectively blocked the stimulant phase of nicotine's effect occurring 30-60 minutes following injection, whereas mecamylamine attenuated both the depressant (0-30 min following injection) and stimulant phases of nicotine's action. Mecamylamine fully reversed both phases of nicotine's action and did not affect locomotor activity when given alone at the effective dose. Varenicline partially reversed the depressant effect and fully reversed the stimulant effect of nicotine but had a significant depressant effect when injected alone at the effective doses. Based on these results, it is possible that FSNY-1 will be useful in the treatment of nicotine dependence with fewer side effects than varenicline. It is significant that hexamethonium, which does not cross the blood-brain barrier, had a profile identical to FSNY-1. This outcome supports the conclusion that ability to block autonomic outflow may be a sufficient condition for clinical efficacy. Thus, inhibition of the peripheral actions of nicotine may represent a significant target for new drug development.

Disclosures: B. Bunce: None. **R. Hill:** A. Employment/Salary (full or part-time):; University of North Texas HSC. **M.J. Forster:** A. Employment/Salary (full or part-time):; University of North Texas HSC. B. Contracted Research/Research Grant (principal investigator for a drug study,

collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; N01DA-13-8908. **R.A. Shetty:** A. Employment/Salary (full or part-time):; University of North Texas HSC.

Digital Abstract Session

P301. Nicotine: Circuitry, Cognition, Behavior, and Physiological Effects

Program #/Poster #: P301.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant

Title: Insulin resistance enhances the reinforcing effects of nicotine in a sex-dependent manner.

Authors: *S. ORTEGON[1], B. CRUZ[1], F. MATOS[1], A. RODRIGUEZ[1], K. P. URIBE[1], K. I. GALINDO[1], K. M. SERAFINE[1], A. NAZARIAN[2], L. E. O'DELL[1];
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Abstract: Presenting author: Author e-mail: Sebastian Ortegon Sortegon@miners.utep.edu**Title:**Insulin resistance enhances the reinforcing effects of nicotine in a sex-dependent manner.**Authors:**S. Ortegon; B. Cruz; F. Matos-Ocasio; A. Rodriguez-Crespo; K. P. Uribe; K. I. Galindo; K. M. Serafine; A. Nazarian; L. E. O'Dell. Department of Psychology, The University of Texas at El Paso.**Abstract:**Clinical studies have shown that persons with diabetes are more susceptible to the tobacco use; however, it is unclear whether this due to greater pleasurable effects of nicotine. Our laboratory employs rodent models of diabetes to study the behavioral effects of nicotine. The present study used female and male rodents to examine sex differences in the reinforcing effects nicotine following a chronic high-fat diet (HFD) regimen that induced insulin resistance. Briefly, adult female and male rats received a HFD regimen for 8 weeks and controls received a regular diet (RD). Separate groups of HFD-treated rats received a low dose of streptozotocin (STZ; 25 mg/kg/sc) to facilitate insulin resistance. To determine the magnitude of insulin resistance, all rats first received an insulin injection and glucose levels were assessed 15, 30, 60, 120, and 180 minutes later. The rats were then given extended (23 hour) access to intravenous self-administration (IVSA) of 3 increasing doses of nicotine. Each nicotine dose was delivered for 4 consecutive days with 3 intermittent days of drug abstinence. Our major finding was that the HFD regimen induced insulin resistance, which enhanced the reinforcing effects of nicotine relative to their respective RD controls. Regarding sex differences, the magnitude of this effect was greater in female versus male rats that were insulin resistant. Together, our results suggest that insulin resistance, a hallmark feature of diabetes, enhances the reinforcing effects of nicotine particularly in females. These data imply that greater vulnerability to tobacco use in persons with diabetes is due to enhanced rewarding effects of nicotine.

Disclosures: **S. Ortegon:** None. **B. Cruz:** None. **F. Matos:** None. **A. Rodriguez:** None. **K.P. Uribe:** None. **K.I. Galindo:** None. **K.M. Serafine:** None. **A. Nazarian:** None. **L.E. O'Dell:** None.

Digital Abstract Session

P301. Nicotine: Circuitry, Cognition, Behavior, and Physiological Effects

Program #/Poster #: P301.04

Topic: G.09. Drugs of Abuse and Addiction

Support: SC2DA052119
RO1DA021274

Title: Short-term increases in risky choice following nicotine vapor exposure in rats

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Abstract: Increased frequency of use, higher nicotine concentrations, and unique additive chemicals associated with electronic nicotine delivery systems (i.e., e-cigarettes) results in different pharmacokinetics and pharmacodynamics for nicotine, relative to that seen with conventional cigarettes. Unfortunately, targeted advertising, addition of palatable flavors, and misconceptions about safety has led to a dramatic increase in recreational use of e-cigarettes. Exposure to nicotine injections in rats has been shown to impair decision making; however, the effects of nicotine vapor administration on risky choice remains unknown. The goal of this research is to explore changes in risky decision making following single and repeated exposure to nicotine vapor. Twenty-four male Sprague Dawley rats were trained in the probability discounting task until stable. Following training, rats were exposed to either a control dose of 0 mg/ml nicotine vapor or 24 mg/ml nicotine vapor for 10 consecutive days, with testing in the probability discounting task occurring immediately after daily exposures. Results show that rats receiving single or repeated nicotine vapor exposures show immediate, but not long-term, increases in risky choice. Testing in a response perseveration task further demonstrated that nicotine vapor exposure does not increase perseverative responding. Research investigating the long-term effect of e-cigarette vapor exposure on cost-benefit decision making, such as that described in this proposal, will provide much needed information on the cognitive and behavioral effects of e-cigarettes.

Disclosures: **P. Giner:** None. **L. Maynez-Anchondo:** None. **S. Ortegon:** None. **M. Blanco:** None. **A.E. Liley:** None. **K.P. Uribe:** None. **N.W. Simon:** None. **I.A. Mendez:** None.

Digital Abstract Session

P301. Nicotine: Circuitry, Cognition, Behavior, and Physiological Effects

Program #/Poster #: P301.05

Topic: G.09. Drugs of Abuse and Addiction

Support: SC2DA052119
R01DA021274

Title: Blocking nicotinic receptors following repeated nicotine vapor exposure induces withdrawal and anxiety-like behavior in rats

Authors: M. MARTINEZ[1], V. ESPINOSA[1], O. LIRA[2], P. GINER[2], K. P. URIBE[1], F. MATOS-OCASIO[2], L. E. O'DELL[1], *I. A. MENDEZ[2];
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Abstract: In recent years, there has been a dramatic increase in the consumption of nicotine vapor through the use electronic nicotine delivery systems, particularly among adolescents. Pre-clinical studies investigating the rewarding and withdrawal effects of nicotine have primarily used injections to administer nicotine liquid. Currently, there are only a handful of published studies investigating the effects of nicotine vapor inhalation on nicotine reward and withdrawal in rodents. The goal of this study is to explore physical withdrawal signs and anxiety-like behavior following exposure to nicotine vapor in rats. Fifty-six male Sprague Dawley rats were exposed to either 0, 12, or 24 mg/ml nicotine vapor for 9 days. For all groups, blood samples were collected on day 6 of vapor exposure to assess levels of cotinine, a nicotine metabolite. Physical withdrawal signs and anxiety-like behavior, following subcutaneous injections of the nicotinic receptor antagonist mecamylamine, were assessed on day 7 and day 9 of vapor exposure, respectively. Additionally, 30 female Wistar rats were exposed to either 0, 12, or 24 mg/ml nicotine vapor for 14 days, with physical withdrawal signs and anxiety-like behavior following subcutaneous injections of mecamylamine tested on day 14. An increase in cotinine levels, physical withdrawal signs, and anxiety-like behavior was seen in male groups exposed to 12 and 24 mg/ml nicotine vapor, relative to the 0 mg/ml controls. Physical withdrawal signs and anxiety-like behavior was also seen in female groups exposed to 12 mg/ml nicotine vapor, relative to the 0 mg/ml controls. The findings suggest that repeated exposure to nicotine vapor leads to a physical withdrawal syndrome similar to that seen with more traditional routes of administration, in male and female rats. The proposed work will offer a foundation for future pre-clinical studies to identify the neurobiological mechanisms driving dependence following repeated nicotine vapor exposure.

Disclosures: **M. Martinez:** None. **V. Espinosa:** None. **O. Lira:** None. **P. Giner:** None. **K.P. Uribe:** None. **F. Matos-Ocasio:** None. **L.E. O'Dell:** None. **I.A. Mendez:** None.

Digital Abstract Session

P301. Nicotine: Circuitry, Cognition, Behavior, and Physiological Effects

Program #/Poster #: P301.06

Topic: G.09. Drugs of Abuse and Addiction

Support: T32 DA 17637-14

Title: Characterization of the effects of oral nicotine administration and abstinence on anxiety-like behavior and the orexinergic system: role of Chrna4 and Chrna5

Authors: *H. L. MATHEWS[1], J. D. JONES[2], M. KONDRATENKO[2], M. BROWN[2], S. ALLSUP[2], J. A. STITZEL[3];
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Abstract: Relative to non-smokers, smokers are more likely to report symptoms of anxiety. Although nicotine has been reported to produce anxiolytic effects, smoking may increase the risk for anxiety disorders. Withdrawal from nicotine is also associated with increased anxiety. In this study, we investigated the effect of nicotine consumption and abstinence on anxiety-like behavior and the orexinergic system in C57BLJ/6 mice as well as *Chrna4* and *Chrna5* null mutant mice. Mice were assigned to one of 5 groups: pre-nicotine baseline, nicotine administration day 8, nicotine vehicle day 8, nicotine abstinence day 1, and abstinence vehicle day 1. Mice in nicotine groups received 200ug/ml of nicotine in a 0.2% saccharin drinking solution for 14 days, and nicotine abstinence was initiated by omitting the nicotine from the vehicle solution. Vehicle groups received 0.2% saccharin in water. Anxiety-like behavior data was assessed using an elevated zero maze at either ZT16 or ZT20 (4 and 8 hours into the active phase, respectively). In C57 mice, anxiety-like behavior was not altered by time of day. Relative to C57 mice, α4(-/-) mice displayed increased number of open entries at ZT 20, suggesting decreased anxiety. In α5(-/-) mice, anxiety-like behavior was decreased at ZT16 relative to ZT20. Neither an effect of nicotine administration nor abstinence was seen for anxiety-like behavior in C57, α4(-/-), or α5(-/-) mice. Following behavioral testing, prepro-orexin (PPO) and orexin receptor 2 (OX2R) protein levels were quantified in the hypothalamus. At baseline, PPO expression was reduced at ZT20, relative to ZT16, in C57 mice. The opposite pattern was seen in α4(-/-) mice and there was no time of day effect in α5(-/-) mice. In contrast, no clear effect of nicotine consumption or abstinence on PPO levels were detected for any genotype. Interestingly, the relationship between nicotine intake and PPO levels differed by genotype; in C57 mice, there was a negative correlation, in α4(-/-) mice there was a trend for a positive correlation, and there was no relationship between nicotine intake and PPO levels in α5(-/-) mice. OX2R protein expression at baseline did not differ across timepoints for C57 or α5(-/-) mice. But, similar to the results for PPO, α4(-/-) mice did exhibit increased OX2R expression at ZT16 relative to ZT20. No clear effect of nicotine treatment or abstinence were observed for OX2R expression in any genotype.

Disclosures: **H.L. Mathews:** None. **J.D. Jones:** None. **M. Kondratenko:** None. **M. Brown:** None. **S. Allsup:** None. **J.A. Stitzel:** None.

Digital Abstract Session

P301. Nicotine: Circuitry, Cognition, Behavior, and Physiological Effects

Program #/Poster #: P301.07

Topic: G.09. Drugs of Abuse and Addiction

Support: ACS RSG-16-023-01 - CPPB

Title: Brain activation during self-regulation to smoking cues

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Abstract: Nearly 60-80% of smokers who attempt to quit smoking are unable to quit. Difficulty quitting smoking may be due to an imbalance between brain regions that help smokers control their impulses to smoke and brain regions that drive smokers towards rewarding activities like smoking. Therefore interventions used to help smokers quit smoking should aim to strengthen brain regions that help smokers control their impulses to counteract this drive towards smoking reward. The current study examines the impact of practicing skills typically taught to help smokers quit smoking on these key brain regions. In this study, participants are randomized to practice self-regulation by delaying their first cigarette of the day for 2 weeks. The objective of the current analysis, was to identify differences in brain activation when smokers actively regulated their respons to smoking cues compared to not regulating their response. Baseline data for all participants (n = 79) varied in terms of motivation to quit (range = 0 to 10; mean = 4.85; SD = 2.60). During the regulation of craving task participants were asked to regulate their responses to smoking and non-smoking appetitive cues by thinking about negative consequences of consuming the item or not regulate by thinking about positive consequences of consuming the item. Whole brain analysis identified increased activation in the left dlPFC when participants actively regulated their response ($p_{corrected} < .001$). After viewing each image, participants were asked to rate how much they wanted the item on a scale from 1 to 5. Smokers indicated that they wanted smoking items more than non-smoking items ($p < .001$) and that they wanted items less when they were regulating their response ($p < .001$). Future analyses will examine brain changes following a brief intervention during which smokers were asked to delay their first cigarette of the day. These results suggest that effortful regulation when viewing smoking cues results in increased engagement of regulation regions and lower desire for cigarettes. Findings from this study will be used to inform the development of future smoking cessation approaches to help smokers successfully quit smoking by targeting self-regulation brain regions.

Disclosures: **L.E. Martin:** None. **M.G. Brucks:** None. **A.T. Fox:** None. **V.B. Papa:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.01

Topic: H.01. Attention

Support: European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie (grant agreement no. 885672 – DYSCEYE- 7T)
European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programmes (grant agreement no. 801715 – PUPILTRAITS)
European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programmes (grant agreement no. 832813 – GenPercept)

Title: Numerosity drives fast saccades in humans

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Abstract: Evolution has endowed us with the ability to shift our gaze to faces and animals with exceptional speed. These rapid automatic oculomotor responses are called fast saccades and take only slightly more than a hundred milliseconds. Discriminating the number of foe or prey may also have an evolutionary advantage. In this study fourteen adults (six males, 29 ± 5 yo) were asked to saccade towards the more numerous of two arrays. Saccadic rection-times showed bimodal distributions. In spite of similar and very high accuracy, slow saccades were more frequently elicited in response to very low and very high numerosities; however, fast saccades were more frequent for intermediate numerosities. For the latter, accurate saccades were as fast as 190 ms. Numerosity estimation is thought to rely on a relatively complex neural circuit, comprising several relays of information through parietal and pre-frontal cortex. The short saccadic-reaction times we observe suggest that number is detected by a primitive system that operates on feed-forward signals. In a separate experiment we collected reaction-times for vocal responses using the same task. Vocal responses were slower and homogeneous across numerical ranges. Reaction-times for vocal responses correlated only with the slow, not the fast saccades, suggesting the existence of different systems. Vocal and saccadic responses to very low and intermediate numerosities correlated with arithmetical abilities. This suggests that the saliency of the numerical information may favor numerical operations to be performed on this automatically detected visual feature, setting up the basis for math.

Disclosures: **E. Castaldi:** None. **D. Burr:** None. **M. Turi:** None. **P. Binda:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.02

Topic: H.01. Attention

Support: R21MH120784
5T32NS073553-10

Title: The vast differences between prosaccade and antisaccade performance are largely explained by a sign change in exogenous attention

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Abstract: When we move our eyes, oculomotor circuits weigh both the physical salience of the objects in view and current behavioral goals, but how? The antisaccade task pits these exogenous and endogenous influences against each other, and performance is highly sensitive to cognitive and developmental deficits. Resolving the contributions of these distinct attentional mechanisms is critical, but requires temporal resolution far greater than that of traditional tasks. Here we use novel, urgent variants of classic anti- and prosaccade tasks to uniquely reveal how exogenous and endogenous mechanisms interact in time to dictate performance. In these urgent tasks, the go signal that tells the subject to respond is given before the visual cue that specifies the correct choice. This way, motor plans are initiated early, and time permitting, later arriving perceptual information guides them toward the correct alternative. Thus, subjects guess on some trials and make informed choices on others. Accuracy varies with available processing time to yield a novel psychometric measure — the tachometric curve — with the necessary temporal precision. The behavioral results from 10 human participants are replicated in quantitative detail by a race-to-threshold model in which two opposing motor plans compete to drive the eye movement to the right or the left. Initially, these plans advance at randomly-drawn rates, but later, when perceptual information arrives to the circuit, they are accelerated or decelerated. The exogenous signal arrives earlier (~80 ms after cue onset) and rapidly accelerates the plan toward the cue, regardless of task instructions; the endogenous signal arrives (~40 ms) later to accelerate the correct plan and decelerate the incorrect one as determined by the task. This task dependence is a crucial distinction. By essentially flipping the sign of the exogenous signal, the same mechanisms readily account for the drastically different performance curves in the two tasks. In antisaccade trials, the early exogenous pull opposes the task goal causing performance to drop into a 40 ms attentional "vortex." In prosaccade trials, congruence of exogenous and endogenous influences facilitates performance at precisely the same time point. By imposing urgency, we have effectively resolved the exogenous and endogenous components that drive saccade target selection and have developed a framework for the neural mechanisms responsible for making the choice.

Disclosures: A.T. Goldstein: None. **E. Salinas:** None. **T.R. Stanford:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.03

Topic: H.01. Attention

Support: ISF Individual Research Grant 1485/18 to SGD

Title: Individual differences in eye movement strategies during a parafoveal attentional task are associated with differences in performance

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Abstract: Attention and eye movements have been shown to rely on joint mechanisms. Shifting attention to a peripheral target could be hidden (covert attention) or precede a saccade (pre-saccadic attention). Pre-saccadic attention was found to enhance performance at the saccade target location just before the eyes move more effectively than covert attention. This was found for low and high-level visual tasks when participants are instructed to perform saccades or to hold fixation. Here we were interested in the individual differences in the tendency to employ either covert or pre-saccadic attentional strategies under uninstructed conditions during a parafoveal attentional task. A group of 31 young adults with normal vision participated in center-periphery discrimination tasks of faces, of inverted faces and of houses, and completed an ADHD-related questionnaire. In each task, participants were instructed to fixate while viewing a central study stimulus which was followed by a target stimulus that briefly appeared (for 200ms) at one of 13 foveal to parafoveal locations (up to 4°). Despite being instructed to fixate, we found that each individual consistently adopted one of two classes of eye-movement patterns, and this individually adopted pattern was consistent across experiments in 24 of 25 participants. One group (n=13) kept central fixation throughout the trial (“fixators”) employing covert attention. The other group (n=18) performed saccades towards the targets (“hoppers”) employing pre-saccadic attention. These post-stimulus saccades in the hoppers group were in the target’s direction and proportional to its distance but were both too short to reach it and too late to land on it (landed after stimulus offset). The “hoppers” showed enhanced performance at parafoveal locations compared to the “fixators” for faces and houses, but not inverted faces, demonstrating a selective gain of pre-saccadic attention. These differences in eye movement patterns across observers were not explained by attention related ADHD measures or by the decline of VA with eccentricity. Our results suggest that individual differences in internally-driven eye movement patterns while attending the visual field may be an active (even if unaware) “choice” related to individual traits.

Disclosures: **O. Kreichman:** None. **Y. Bonneh:** None. **S. Gilaie-Dotan:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.04

Topic: H.01. Attention

Support: ANII (Uruguay) PD_NAC_2018_1_150365

Title: Visual spatial attention modulations over auditory neural processing emerge with high audiovisual precision and are foreground size dependent

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Abstract: Synchronization of concurrent auditory and visual events strongly facilitates their integration into unified audiovisual (AV) objects. Further, fully in-sync AV dynamics has been shown to assist bimodal object-based attentional selection. It is unclear how do unimodal selective biases, such as visuospatial attention, transfer cross-modally in AV perception. A possibility is that temporal precision, rather than synchronization, more generally represents a driving factor since perfect temporal matching is often not required for integration. Support for this view may come from proposals that perceptual uncertainty estimation is a relevant computational mechanism for selective attention.
The study addresses whether temporal AV precision but also unimodal sources of uncertainty (e.g. visuospatial) influence the cross-modal transfer of attentional biases. We tested the hypothesis that changes to auditory processing under visuospatial selection are dependent on bimodal but also unimodal uncertainty factors.
Using electroencephalography (EEG), we estimated temporal response function (TRF) models of the auditory processing of tone pips. These sounds were randomly associated to visual contrast changes (‘flips’) spread over a circular dartboard display. Participants (N=30; 9 male; mean age 24; sex differences not addressed) were asked to exclusively attend to visual flips within display subsets (half versus quarter dartboard), while judging for AV precision differences in a two-interval forced choice task. Because pips and flips were never synchronous, the role that presentation order (i.e. pip leads flip and vice versa) has on the timing of cross-modal interactions was further investigated.
TRF estimates show that high AV precision between auditory and visual onset times determines the presence of cross-modal modulations. Visual foreground size additionally determined transfer effects only at low positional uncertainty (quarter dartboard), enabling the visual priming of subsequent tones as relevant for auditory segregation in line with top-down processing timescales. Auditory uncertainty (i.e. when pips lead flips) on the other hand determined susceptibility of early tone encoding to automatic changes by incoming visual update processing. The findings provide a hierarchical account of the role of uni- and cross-modal sources of uncertainty on the neural encoding of sound dynamics in a multimodal attention task.

Disclosures: **F. Cervantes Constantino:** None. **T. Sánchez-Costa:** None. **G.A. Cipriani:** None. **A. Carboni:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.05

Topic: H.01. Attention

Support: Grant IN217918 from DGAPA-UNAM to AERC
Grant IN215218 from DGAPA-UNAM to OPG
Grant IA205218 from DGAPA-UNAM to MMD

Title: Attentional demand and consistency of stimuli differentially affect the hierarchical visual processing

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Abstract: The ability to process the global and local elements of a visualscene might be modulated by attentional demand and consistency of stimuli. Herewe used Event-related potentials to evaluate if the attentional demand andconsistency might modulate the visual hierarchical processing. Thirty-ninehealthy young participants solved the Navon paradigm (i. e., local lettersforming a global one) under two attentional demand conditions (low, i. e.,discriminating between H and S or between 4 and 5; vs. high, i. e., discriminatingbetween H and 4 or between S and 5). Participants answered global (i. e.,identify the global stimulus) and local (i. e., identify the local stimulus)tasks in separate blocks, depending on the demand condition; 50% in each blockwas consistent (i. e., global and local elements coincided). The globalinterference (i. e., larger reaction times for local than for global trials)was higher during the low than the high demand condition. The mean amplitude ofthe P2 component at parieto-occipital sites was higher for local than forglobal processing. At the parietal electrodes, posterior P2 had a more positivemean amplitude, and N2 a less negative mean amplitude, for high demand than forlow demand in the attention task. On the other hand, the P300 latency andreaction times showed that the global processing during the low demand task wasprocessed faster than the global processing during the high demand task,regardless of the consistency of the stimulus. Later, came the processing ofthe local consistent stimulus in the low demand task followed by those in thehigh demand one. Finally, the slowest processing was for local inconsistentstimuli, regardless of the task demand. Our results showed that first, theforest is processed before the trees, followed by a reanalysis of the forestand the trees that depend on the similarity of the trees or the difficulty fordiscriminating the local elements.

Disclosures: **A.E. Ruiz-Contreras:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.06

Topic: H.01. Attention

Title: Feature-selective modulations of neural priority maps in human cortex

Authors: *D. THAYER, T. SPRAGUE;
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Abstract: Priority maps are representations of visual space which determine the relative importance of a given location. Computational priority map models suggest that spatial maps for individual features are integrated into a single map that is modulated by both bottom-up image salience and top-down attention (Itti & Koch, 2001). At the neural level, previous work has identified neural regions that are sensitive to color (hV4/VO1; Brewer et al., 2005) as well as motion (hMT+/TO1/TO2; Amano et al., 2009), making these regions strong candidates for 'feature maps'. Furthermore, when presented with a stimulus consisting of several features, attending to the preferred feature of a region results in a greater fMRI response (McMains et al., 2007; Runeson et al., 2013). In the present study, we hypothesize that multivariate activation patterns across retinotopic feature-selective cortical regions form spatial 'feature dimension maps', which combine to guide attentional priority. If these regions act as feature dimension maps, there will be greater activation corresponding to the location of a stimulus when attending to the preferred feature of a given region (e.g., within color-responsive hV4 when attending color). To test this, participants viewed a peripheral colored moving-dot stimulus presented at a random location. They were precued to attend either the color or motion direction of the stimulus and discriminate which individual feature value was most prevalent (e.g., is red or blue more prevalent when attending color). Additionally, regions of interest were independently defined via retinotopic mapping procedures. We applied a multivariate spatial inverted encoding model to reconstruct spatial maps of the visual field from each retinotopic ROI. Consistent with previous univariate results, we found that stimulus representations within reconstructed priority maps were stronger in color selective regions when color was attended compared to motion, and representations in motion selective regions were stronger when motion was attended compared to color. These findings suggest that feature-selective cortical regions support spatial dimension maps, and that top-down attention can reweight activation profiles across these maps.

Disclosures: D. Thayer: None. **T. Sprague:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.07

Topic: H.01. Attention

Support: NSF

Title: Electrophysiological measures reveal distinct contributions of exogenous and endogenous mechanisms of attention in visual working memory

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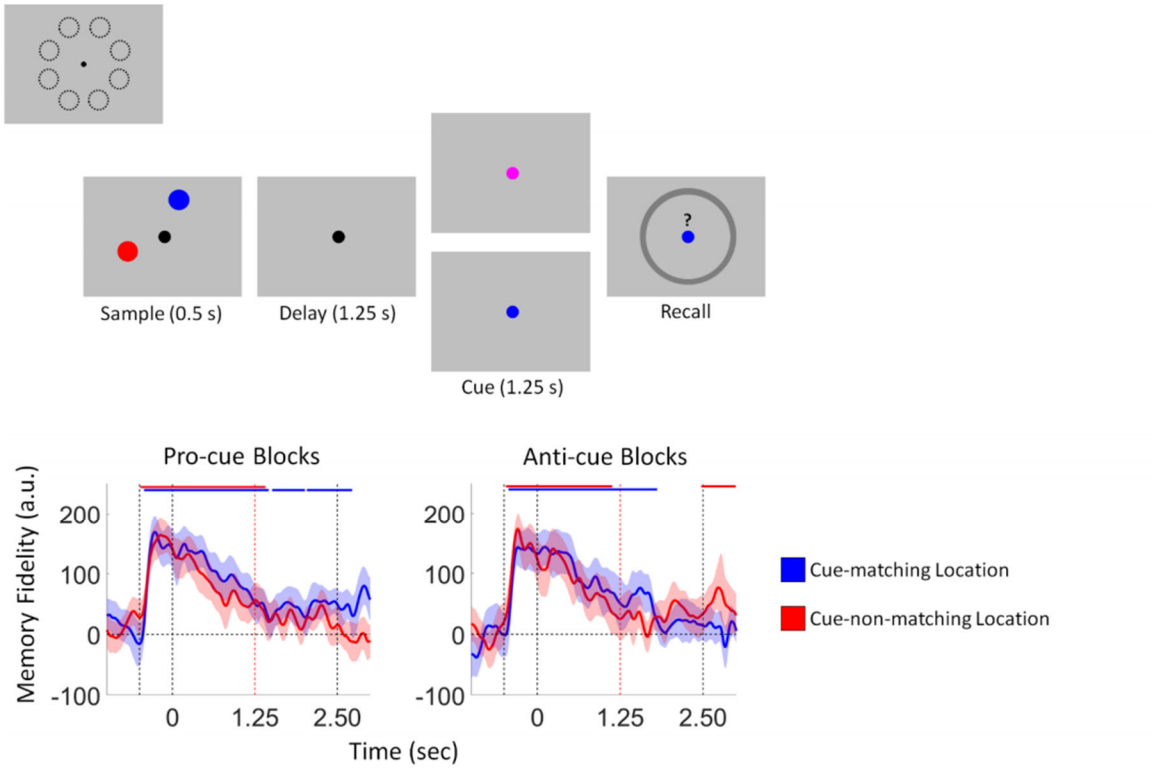
Abstract: Working memory (WM) performance can be enhanced by an informative cue presented during storage. This effect - termed a retrocue benefit - can be used to explore how participants prioritize information stored in memory for behavioral output. There is general agreement that both voluntary and involuntary attention contribute to retrocue benefits, but it is unclear whether they do so via the same mechanisms. Here we tested this possibility by examining the effects of voluntary and involuntary attention shifts on location-specific working memory representations reconstructed from human brain activity (EEG). 40 human volunteers (both sexes) performed a retrospectively cued spatial WM task requiring them to remember the positions of two stimuli over a delay. To disentangle the effects of voluntary and involuntary attention and retrocue benefits, each participant completed task blocks with cues that identified the to-be-reported location (i.e., pro-cues) and task blocks with cues identified the task-irrelevant location (i.e., anti-cues). Both pro-cues and anti-cues led to improvements in memory performance relative to no-cue blocks. However, EEG recordings revealed noticeable differences in the quality of reconstructed spatial WM representations during pro- and anti-cue blocks. Specifically, during pro-cue blocks, we observed a strengthening of the cue-matching representation coupled with a reduction in the strength of the cue-non-matching representation, replicating earlier findings from our laboratory (e.g., Ester, Nouri, & Rodriguez, J. Neurosci 2018). However, during anti-cue blocks, we observed a transient increase in the strength of the cue-matching representation immediately after cue onset, followed by a rapid reduction in its strength. This effect was coupled with a gradual increase in the strength of the cue-non-matching representation. Collectively, our findings suggest that both voluntary and involuntary attention influence the prioritization of information in WM, but most likely recruit different mechanisms to do so.



Disclosures: **A. Nouri:** None. **E.F. Ester:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.08

Topic: H.01. Attention

Support: DGAPA-UNAM Grant IN217918
DGAPA-UNAM Grant IN215218
DGAPA-UNAM Grant IA205218

Title: Electrophysiological markers of selective attention are affected by the competition of target and distractors in the same visual hemifield

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Abstract: Attentional control is the ability to enhance and suppress information for a goal-directed behaviour. The N2pc and Pd event-related potential components are markers for the

enhancement and suppression of information, respectively. N2pc has a more negative mean amplitude when a target is present than when it is not. Pd has a more positive mean amplitude when a distractor is present than when it is not. We evaluated the competition between a target and a distractor presented in the same or different visual hemifields. Sixty-two healthy young participants indicated the absence or presence of a target stimulus in a visual display. There were four types of trials (each trial consisted in six vertical green rectangles array): Homogeneous (no-singleton), Target, Non-target and Target+Non-Target. These last trials had two singletons: the target and a distractor. In 50% of the trials, they were in different visual hemifields. The mean amplitude difference between the presentation of the target and distractor in the same vs. different hemifield was analysed. No Pd was observed. N2pc was enhanced by the presentation of the singletons in different visual hemifields. This suggests that the attentional system allocates more resources to the target when the distractor is distant because there is an involuntary capture that needs to be overridden; when the singletons are in the same hemifield, the disengagement process is not necessary due to an effect of proximity. Another possibility is that the attentional resources are distributed between the singletons; when they are separate, N2pc is not as prominent because attentional resources are allocated to the non-target in the opposite hemifield. Whereas when target and distractor are in the same hemifield, the prominence of N2pc is given by the addition of the processing of both stimuli. In conclusion, the electrophysiological marker for selective attention modulates the processing of the singletons in the same visual hemifield.

Disclosures: **J.A. Franco-Rodriguez:** None. **T.V. Roman-Lopez:** None. **M. Mendez Diaz:** None. **O. Prospero-Garcia:** None. **A.E. Ruiz-Contreras:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.09

Topic: H.01. Attention

Support: MH117991

Title: Frontoparietal control of willed attention

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Abstract: Cueing paradigms are commonly used to study the neural mechanisms of visual spatial attention control. In these paradigms, each trial starts with an external cue, which instructs the subject to pay covert attention to a spatial location in order to process an impending stimulus. Neuroimaging studies have consistently shown that the dorsal attention network (DAN) is activated following the cue. Recent work has introduced a new form of cueing which asks the subject to spontaneously decide which spatial location to attend (willed attention). We examined the neural substrate of willed attention control by analyzing fMRI data recorded at two

institutions (UF and UC Davis) using the same willed attention paradigm. In addition to instructional cues, a choice cue was included, which prompted the subject to choose the side of the visual field to attend. Applying the general linear model we found that both instructional cues and choice cues activated the DAN. Choice cues additionally activated frontoparietal regions including the dorsal anterior cingulate cortex (dACC), anterior insula (AI), dorsal lateral prefrontal cortex (DLPFC) and inferior parietal lobule (IPL). An MVPA analysis showed that for neural activity evoked by choice cues, decoding accuracy in these frontoparietal regions was significantly above chance level, whereas for instructional cues, decoding accuracy was at chance level. These results, consistent across the two datasets, suggest that willed visual spatial attention is controlled by three major brain networks: the salience network (dACC and AI), the central executive network (DLPFC and IPL), and the DAN.

Disclosures: Q. Yang: None. **S. Meyyappan:** None. **M. Ding:** None. **G.R. Mangun:** None. **J.J. Bengson:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.10

Topic: H.01. Attention

Support: NIH grant MH117991

Title: Patterns of pre-cue alpha power predict the decision about where to attend in willed attention

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Abstract: In voluntary cued spatial attention paradigms, each trial starts with a cue instructing the subject to focus attention on a spatial location in order to process an impending stimulus. In contrast to cued attention, attention can also be allocated by purely internal decisions (willed attention), such as when subjects are asked to spontaneously decide where to attend on each trial. Bengson et al. (2014) reported that lateralized alpha power over occipital-parietal cortex immediately before a decision predicted where subjects would attend. Here, we used multivariate pattern analysis (MVPA) of EEG in Bengson's data set and a replication recorded at UF. In addition to instructional cues, 33% of trials had a choice cue that prompted the subject to choose the side of the visual field to attend on that trial; inter-trial intervals varied widely (2-8 s) and unpredictably. To assess the time course of alpha power patterns that predicted where subjects would attend, we performed time window-by-window decoding over the 3000 ms pre-choice cue interval. Above chance (56.42% for UF and 56.57% for UCD datasets) decoding of alpha power prior to the choice cues was observed only immediately prior to the cue, and not earlier in time,

replicating Bengson using decoding. These results demonstrate that decisions about where to attend are influenced by the pattern of alpha power only in the few hundred milliseconds immediately before a decision, supporting the idea that independent of goal-directed or bottom-up sensory factors, spontaneous brain states influence how humans focus attention.
Keywords: decoding, multivariate pattern analysis, pre-cue alpha power, willed attention

Disclosures: S. Kim: None. **S. Meyyappan:** None. **J. Bengson:** None. **G. Mangun:** None. **M. Ding:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.11

Topic: H.01. Attention

Title: An activation likelihood estimation meta-analysis of attentional bias to emotional information

Authors: *L. FANG, R. SYLVAIN, N. STRAND, J. M. CARLSON;
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Abstract: When in an environment overwhelmed with information, we tend to allocate our limited cognitive resources to certain objects. In particular, selectively attending to emotional information plays a crucial role in our day-to-day lives. Although there are a number of narrative and systematic reviews on attentional bias to emotional information, meta-analysis concerning its neural correlates is still scarce. The current study aimed to identify brain regions that consistently activate during attentional bias across different paradigms, age groups, and clinical status by using an Activation Likelihood Estimation (ALE) meta-analysis method (Eickhoff et al., 2009; Turkeltaub et al., 2002). Relevant studies were identified by conducting a systematic database search for peer-reviewed articles published between 1994 and 2020 on Google Scholar and PubMed. A total of 98 experiments, 826 foci, and 3090 participants were included in the final meta-analysis. The main ALE-analysis of all studies revealed the regions that are consistently involved in attentional bias to emotional stimuli at an uncorrected $p < .001$ threshold with a minimal cluster size of 100 mm3, such as inferior frontal gyrus, medial frontal gyrus, parietal lobule, temporal gyrus, occipital gyrus, as well as right amygdala, left insula, left precuneus, anterior cingulate cortex and paracingulate gyri, supplementary motor area, right lingual gyrus, and left thalamus. Additional ALE-analyses were also conducted by examining different paradigms (i.e., dot-probe task and emotional stroop task), age groups (adults and non-adults), and clinical status (patients and healthy controls). No significant clusters were found for contrast analyses. However, when the ALE-analyses were conducted separately, some regions were identified as specific to paradigm or group type. Our findings provide empirical support across studies for current theoretical models of attentional bias, which suggest that both subcortical regions that are sensitive to emotional features and cortical regions that associated with cognitive control engage in attentional bias to emotional information. In addition, the identifications on

specific groups also deepen our understanding of the involvement of different regions and corresponding cognitive processes in attentional bias in different assessment paradigms, age groups, and clinical status.

Disclosures: **L. Fang:** None. **J.M. Carlson:** None. **R. Sylvain:** None. **N. Strand:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.12

Topic: H.01. Attention

Support: KAKEN Grant 18H05089

Title: The emotional words temporally capture the spatial attention

Authors: ***H. ONISHI**, R. GUAN, H. NAGAMURA, M. HISHITANI, S. MURAI, K. I. KOBAYASHI;
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Abstract: Our cognitive function was largely modulated by internal emotional factors, which is triggered by external stimuli relevant to specific emotional meaning. Selective attention, for instance, is one of the important functions which is closely affected by the external emotional stimulus. A large number of previous studies revealed that positive emotional stimulus attracted participants' attention to space where the stimulus was presented compared to non-emotional stimulus. These findings were conducted using a visually guided emotional cue. It's important to study other than the visual stimuli for pursuing the generality between modalities. Here we investigated such behavioral mechanisms using auditory emotional stimulus during a Posner cueing task. To understand emotional effects on spatial shift on attention, we used short Japanese spoken words as cue stimuli, each contained positive, neutral, or negative emotional meaning. Reaction time in the trials, which is the target and cue stimulus presented in the same space (valid trial), was significantly faster compared to trials in which the two stimuli were emitted in different space (invalid trial). Moreover, the right stimulus was more attracted to their attention compared to the left stimulus, which is consistent with the notion of right hemispheric involvement specific to language processing. However, there is no apparent difference between emotional cue types in reaction time. To address how long the emotional effect toward sustained attention, we compared the reaction time in each different emotional cue stimulus in previous trials. Our data showed that when a positive stimulus followed a negative stimulus, attention was not directed where the positive stimulus was presented but was sustained where the negative stimulus was presented. The results indicate that negative auditory stimulus sustains their spatial attention in consequent trials compared to the positive or neutral stimulus. This suggests that the emotional effect on our attentional shift is the maintenance of spatial attention, whereas the attentional capturing has no clear difference between emotional types.

Disclosures: **H. Onishi:** None. **R. Guan:** None. **H. Nagamura:** None. **M. Hishitani:** None. **S. Murai:** None. **K.I. Kobayashi:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.13

Topic: H.01. Attention

Support: NSF Grant DRL-1660548

Title: Characterizing Physiological Synchrony of the Eyes, Mind and Heart

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Abstract: Movies are known to synchronize physiological signals across viewers. This has been demonstrated for neural signals, eye movements, heart rate, and others. But how exactly does synchronization of different physiological signals relate to one another, and is there a common factor? Here we simultaneously recorded neural signals, heart rate, respiration, eye movements, pupil size and head movements while study participants watched informative videos. The most robust synchronization was found for neural signals, eye movements and pupil size, and to a lesser extent, heart rate and head movements, while respiration did not measurably synchronize in any participant. Synchronous fluctuations of these signals were on a time scale of seconds to minutes, with the exception of neural signals, which synchronized across a broader range of timescales. Subjects with high synchronization in neural signals, eye movements, pupil size, or heart rate also showed high synchrony in the other signals, suggesting that synchronization is modulated by a common underlying factor. The principal component in these data was highly predictive of the ability of participants to recall information presented in the video. Additionally, for all signals, synchronization was modulated by attention, with the strongest effect for eye movement synchronization. Together, these results suggest that cognitive processing of the video mediates physiological responses of the body.

Disclosures: **J. Madsen:** None. **L. Parra:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.14

Topic: H.01. Attention

Support: Brighton and Sussex Medical School Clinical Imaging Science Centre
University of Sussex School of Psychology

Title: I can see what you are thinking: establishing objective markers for indexing the occurrence of spontaneous involuntary thoughts

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Abstract: Involuntary thoughts are a common feature of daily life and play a key role in many clinical disorders from addiction to ADHD. Here we establish a novel approach for studying the neural mechanisms of such thoughts, using a planted 'marker' thought. Twenty-four subjects (14 females, age range 19-33 years, mean age = 24 years, SD = 4.35 years) were first familiarized with the faces of two characters from the board game 'Clue', during a value-training visual search task in which presentation of the faces was associated with a financial reward. Inside the fMRI scanner, we recorded each participant's fusiform face area (FFA) response while viewing faces. In a separate sustained attention task, participants were instructed to suppress thoughts of the faces. Intermittent thought probes measured whether participants experienced involuntary thoughts about the faces either immediately before the probe, or during the preceding task block (~ 30 seconds). First, a univariate analysis based on an FFA region of interest (obtained while viewing faces), revealed significant group level FFA activation during the two seconds immediately preceding probes at which subjects reported thinking of the faces (M=0.85, SD=0.77) versus being on-task (M=0.21, SD=0.12), $t(13) = -2.98$, $p=.01$. We next employed Representational Similarity Analysis (RSA) analysis to identify the occurrence of spontaneous thoughts across the task block. This analysis compares the multivoxel pattern of activity during face viewing to each timepoint during the sustained attention task. We examined both mean similarity across a block, as well as the number of 'discrete reactivation events' (timepoints showing similarity higher than baseline noise). Mean similarity between the sustained attention blocks and the face templates were significantly higher during blocks where participants reported thinking about a particular face, versus those in which they reported being on task (Face 1, $t(10) = -4.8$, $p = .001$; Face 2, $t(9) = -3.6$, $p=.006$). There were also more reactivation events during blocks in which face thoughts were reported versus on task (Face 1, $t(10) = -6.8$, $p < .001$; Face 2, $t(9) = -3.9$, $p=.004$). We hence demonstrate that spontaneously arising thoughts elicit reliable stimulus-specific patterns of neural activity that resemble those involved in perception, and that can be identified using both univariate and multivariate approaches. Our findings also imply that discrete reactivation events may be used as a 'probe-free' method of identifying when such thoughts occur.

Disclosures: **P. Manguele:** None. **S. Forster:** None. **C. Bird:** None. **C. Racey:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.15

Topic: H.04. Executive Functions

Support: CONACyT scholarship 828871/621180).

Title: Error detection in children exposed to general anesthesia during early childhood: an ERP study.

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Abstract: Error detection in children exposed to general anesthesia during early childhood: an ERP study.
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Disclosures A. Abundis-Gutierrez: None. **A. Vazquez-Moreno**: None. **L. Ríos-Muñoz**: None. **F. Jáuregui-Huerta**: None.
Abstract: Some studies have pointed out the possible adverse neurocognitive effects of exposure to general anesthesia during early childhood. Animal models have shown detrimental effects on brain cell survival and cognitive functions. Human data is not conclusive: some studies have found that anesthesia is related to risk of neurodevelopmental disorders, while other studies showed no difference in cognitive function between exposed and unexposed children. However, as far as we know, there is no study that have addressed the impact of general anesthesia on neurodevelopment by means of brain electrophysiology. The aim of this research was to evaluate the electrical brain activity related to error detection in two groups of preschool children: with and without history of exposure to general anesthesia (n=26). Participants observed animal puzzles being formed either correctly or incorrectly (head of another animal), while their electrical brain activity was recorded. Observation of incorrect configurations produced a significantly larger P3b on the unexposed children, while the anesthesia group showed a difference between correct and incorrect conditions by means of a negative deflection around 450 ms. at central midline. Patterns of brain activity observed on the exposed group are more alike to those observed on toddlers using the same experimental task. From a developmental perspective, our results suggest that exposure to general anesthesia during early childhood could be related to a certain maturation gap in the processing and detection of errors that may be preceded by an attention deficiency. Our results point out the need for more research in this topic, since general anesthesia is in many cases applied to facilitate medical procedures that do not compromise children's life and could be managed using other alternatives to achieve children cooperation.

Disclosures: A. Abundis-Gutiérrez: None. **A. Vázquez-Moreno:** None. **L.M. Ríos:** None. **F. Jauregui-Huerta:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.16

Topic: H.01. Attention

Support: 2017M3C7A1029485

Title: Spatial characteristics of EEG activities of idiopathic REM sleep behavior disorder patients during visuospatial attention revealed by explainable machine learning

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Abstract: Introduction: Idiopathic REM sleep behaviour disorder (iRBD) is an early sign of synucleinopathies, including Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. iRBD is known to be associated with visuospatial impairments. In this study, we tried to reveal spatial the distinctive characteristics of neural activities of iRBD patients based on interpretable machine learning technique. **Methods**: Nineteen healthy subjects (63.47 ± 7.37 years) and 16 drug-naive iRBD patients (64.94 ± 6.92 years) participated in this study. We recorded 60-channel event-related potentials (ERPs) during the Posner task. The ERP amplitudes at 60 channels within 230 ms to 330 ms after probe item were calculated and used as input to a convolutional neural network (CNN) classifier. Layer-wise relevance propagation (LRP) and class activation mapping (CAM) were applied to investigate the relevance of spatial location topographical for distinguishing patients from healthy controls. **Results**: The classification accuracy was 87.81 ± 0.79 % (10-fold cross validation, repeated 10 times). The heatmaps obtained from LRP and CAM showed relevant features for distinguishing iRBD patients, which were mainly located in frontal, centroparietal, occipital regions. **Discussion**: The iRBD patients could be successfully detected based on single trial ERP topographies with high accuracy using a CNN classifier. Furthermore, we could find out disease-specific spatial features of ERP using interpretable machine learning approach which were hardly to be found from statistical modelling. The most relevant features were localized in centroparietal and occipital sites, which may reflect visuospatial attention dysfunction associated with iRBD. Considering that visual dysfunction is regarded as an important diagnostic marker for synucleinopathies including PD, our results support that visual attention problem is pervasive in iRBD which is considered as the prodromal stage of neurodegeneration.

Disclosures: H. Kim: None. **P. Seo:** None. **K. Cha:** None. **J. Byun:** None. **J. Sunwoo:** None. **K. Jung:** None. **K. Kim:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.17

Topic: H.01. Attention

Support: NIBIB P41EB018783

Title: Tagging up! Calibrating a P300-based Home Brain-Computer Interface (BCI) System

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Abstract: People affected by neuromuscular disorders such as amyotrophic lateral sclerosis (ALS), brainstem stroke, and high-level spinal cord injury need alternative communication and control methods. A brain-computer interface (BCI) using the P300 event-related potential (ERP) allows people with ALS to communicate and control computer applications (Wolpaw et al., Neurology 2018). At present, system calibration requires that the person complete a BCI test session. This pass-fail step relies on day-of candidate readiness, established system parameterization (including a stylized application), and test-set results of $\geq$70%. The present study tests the hypothesis that calibrating the system with data from actual system use, independent of the application, increases the likelihood of success and improves system reliability. Eleven participants (6 men, one with advanced ALS), age 17-60 yr, completed one session with the BCI Home System. We recorded 8 EEG channels from frontal, central, and posterior scalp locations. The person sat in a chair or at a 30^{o} in bed ~100 cm from a 24" monitor for one 1-hr session. A session has 3 stages: a 21-selection training set (S1), a 7-selection test set (S2), and an 81-selection picture-matching set (S3). In S1 and S2, Users viewed a 4x7 (28-item) on-screen keyboard, including all English letters arranged alphabetically, and received visual cues for each selection. For S3, Users viewed a 3X4 (12-item) or a 2X4 (8-item) matrix that included whole words and pictures. At the beginning of S3, the person was instructed how to complete the picture-matching task. Each selection in S1-3 required the person to count up to 14 faces as they flashed over the target item. S1 and S2 selections contained a priori information on the correct selection (AP). S3 data were tagged post hoc (PH). A stepwise linear discriminant function selected and weighted the EEG features to determine the desired selection. Every person completed all stages and averaged >95% accuracy on the Matching Task (S3). Offline analysis (5-fold cross correlation) yielded average accuracies of the 10 people without disability were 92.2$\pm$3.6 and 95.7$\pm$2.8 ($p>.01$) for the AP and PH trials, respectively. The results for the person with were 89.7$\pm$2.2 and 87.0$\pm$5.0 ($p>.21$). These results suggest that an automated calibration routine is an effective alternative for initial assessment of potential BCI Users. This new method could help clinicians, reduce evaluation time, and increase the numbers of people who might benefit from the BCI Home System. We are now automating the method.

Disclosures: **K.M. Fitzpatrick:** None. **V. Gupta:** None. **C.S. Carmack:** None. **K. Gosmanova:** None. **D. Goldberg:** None. **G. Townsend:** None. **T.M. Vaughan:** None. **J.R. Wolpaw:** None.

Digital Abstract Session

P302. Mechanisms of Attention: Human Studies

Program #/Poster #: P302.18

Topic: H.01. Attention

Support: BLS Biological Life Sciences Fellow
NSF Neuroscience Fellow
Thompson Center for Autism & Neurodevelopmental Disorders

Title: The Effects of Methylphenidate on Verbal Creativity, Verbal Fluency, and Convergent Thinking Tasks in Adults with Attention-Deficit/Hyperactivity Disorder

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Abstract: Introduction: A common pharmacological treatment for attention-deficit/hyperactivity disorder (ADHD) is methylphenidate (MPH), which is a psychostimulant that acts as a norepinephrine-dopamine reuptake inhibitor. Both the noradrenergic and dopaminergic systems have been linked to aspects of creativity (Flaherty, 2005; Alexander et al., 2007). Anecdotal reports from patients with ADHD suggest that their creativity is impaired by MPH. MPH does not generally impair creativity among those without ADHD, but effects in those with ADHD is not known. Therefore, this study examined effects of MPH on convergent and divergent tasks associated with creativity in adults with ADHD. **Method:** Seventeen adults aged 18-40 with a diagnosis of ADHD and prescribed MPH participated in the study. Participants attended two sessions in a counterbalanced order, once after taking the prescribed amount of MPH, and once after withholding their MPH. Participants completed problem-solving tasks (anagrams and compound remote associates) and generative tasks (letter fluency, semantic fluency, and Verbal Torrance Test for Creative Thinking, or VTTCT) in a counterbalanced fashion.
Results: There was a significant increase in the number of words generated on the semantic fluency task, $t(16)=3.27$, $p=0.005$ for the MPH session. In addition, there was a significant increase in the MPH session for the originality scores generated on the VTTCT, $t(8) = 1.867$, $p=0.049$, and a trend toward significance was found for the overall VTTCT battery average score, ($p=0.056$). However, there was no effect on problem solving tasks.
Conclusions: In adults with ADHD, MPH enhanced verbal fluency and divergent thinking

abilities, but had no effect on convergent thinking abilities. Furthermore, MPH appeared to enhance the originality aspect of verbal creativity. Therefore, MPH appears to have positive effects on divergent task performance in adults with ADHD. but not convergent tasks. Future studies are needed to understand the roles of norepinephrine and dopamine in this effect.
Keywords: methylphenidate; attention deficit disorder with hyperactivity; creativity; dopamine; norepinephrine

Disclosures: **C. Appling:** None. **A. Gonzalez:** None. **A. Schaeffer:** None. **A. Zand:** None. **D. Wang:** None. **A. Sam:** None. **A. Tosh:** A. Employment/Salary (full or part-time):; University of Missouri, Columbia. **B. Ferguson:** None. **D.Q. Beversdorf:** None.

Digital Abstract Session

P303. Networks For Attentional Control

Program #/Poster #: P303.01

Topic: H.01. Attention

Support: CIHR
Epilepsy Canada

Title: Disinhibition of frontal cortical circuits underlies inattention and cognitive rigidity associated with Cacna1a loss-of-function mutations

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Abstract: Context. Loss-of-function mutations in the *CACNA1A* gene, encoding the α1 subunit of voltage-gated Cav2.1 channels, result in epilepsy and neurocognitive impairments, including attention deficits, intellectual deficiency and autism, as we observed in a cohort of 16 individuals. We showed previously that a homozygous deletion of *Cacna1a* in mice forebrain GABAergic interneurons (IN) leads to epilepsy through disruption of synaptic efficiency from parvalbumin-positive (PV) INs. However, the mechanisms underlying the associated cognitive deficits are unknown, especially in the context of haploinsufficiency.**Hypothesis.** We propose that impaired perisomatic inhibition in frontal circuits underlies the cognitive deficits in *Cacna1a* heterozygous mutants.**Methods.** We generated mutant mice carrying a targeted heterozygous deletion of *Cacna1a* restricted to PV neuronal populations ($PV^{cre};Cacna1a^{c/+}$) or to cortical pyramidal cells (PCs) ($Emx1^{cre}$; $Cacna1a^{c/+}$), which we characterized using patch-clamp electrophysiology, video-EEG and behavioural assays.**Results:** $PV^{cre};Cacna1a^{c/+}$ mutant mice display reduced synaptic efficiency from PV-INs on PCs in both the medial prefrontal (mPFC) and orbitofrontal (OFC) cortices. In addition, they show enhanced seizure susceptibility to PTZ, impulsivity, attention deficits and cognitive rigidity. Interestingly, no change in PC synaptic function or in behaviour were observed in $Emx1^{cre}$; $Cacna1a^{c/+}$ mutants, suggesting

compensation in PCs. To further dissect the contribution of frontal cortical sub-circuits, we injected Cre-expressing AAVs in the mPFC or the OFC. Targeted deletion of *Cacna1a* in the mPFC induces attention deficits, while a deletion in the OFC results in cognitive rigidity. Further, the attention deficits and the cognitive rigidity in $PV^{cre};Cacna1a^{c/+}$ mutants can be rescued by a selective chemogenic activation of PV-INs in the mPFC and the OFC, respectively.**Impact:** In summary, our results demonstrate that haploinsufficiency of *Cacna1a* disrupts perisomatic inhibition in frontal circuits, leading to a range of potentially reversible neurocognitive deficits.

Disclosures: A. Lupien-Meilleur: None. **X. Jiang:** None. **M. Lachance:** None. **V. Taschereau-Dumouchel:** None. **L. Gagnon:** None. **C. Vanasse:** None. **J. Lacaille:** None. **E. Rossignol:** None.

Digital Abstract Session

P303. Networks For Attentional Control

Program #/Poster #: P303.02

Topic: H.01. Attention

Support: James McDonnell Foundation

Title: Spatial attention influences bursting in pulvinar subdivisions

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Abstract: Thalamic neurons are capable of firing single action potentials (tonically) or trains of action potentials that ride atop a calcium influx (bursting). Previous work showed that higher-order thalamic regions, like the pulvinar, have a higher burst-propensity than first-order thalamic regions, like the lateral geniculate nucleus (LGN). The pulvinar, however, is a heterogeneous structure and it is currently unknown whether burst propensity differs across subregions of the pulvinar. Moreover, the pulvinar plays a role in selective spatial attention and it is not known whether spatial attention modulates burst propensity in the pulvinar. We recorded from the pulvinar of two macaques using linear multielectrode arrays and assigned electrodes to dorsal and ventral pulvinar based on receptive field mapping responses. We recorded well-separable single-unit activity (N = 104, 62 in dorsal and 42 in ventral pulvinar) while the animals performed a spatial-attention task. We compared individual units to firing rate matched Poisson simulated data and showed that ~60% of pulvinar units (62 out of 104) show burst percentages more extreme than simulated data. Moreover, this analysis shows that ~53% of dorsal vs. ~69% of ventral pulvinar units burst more than expected under purely Poisson firing. Next, we assessed differences in bursting between attention conditions. We used a burst refractory index in visually responsive cells (N = 24) and found significantly less bursting ($p < .05$ with permutation test) in the attend-in than in the attend-out condition. Importantly, these findings were not caused by differences in firing rate between attention conditions. Taken together, these findings suggest

that pulvinar bursting is more pronounced when spatial attention is directed outside of the receptive field. Decreased bursting under the influence of spatial attention has been previously described in cortical regions such as V4 and MST. Here, we extend these findings to the primate higher-order thalamus.

Disclosures: T. van Viegen: None. **R. Ly:** None. **S. Kastner:** None.

Digital Abstract Session

P303. Networks For Attentional Control

Program #/Poster #: P303.03

Topic: H.01. Attention

Title: Functional differentiation in the rat posterior parietal cortex: Implications for controlled and stimulus driven attention

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Abstract: The posterior parietal cortex (PPC) in rodents and primates is important for visuospatial attention. The human PPC shows functional differentiation such that dorsal areas are implicated in top-down, controlled attention, and ventral areas are implicated in bottom-up, stimulus driven attention. Whether the rat PPC also shows functional differentiation is unknown. The details of anatomical and functional homology of the PPC across different species is needs to be further examined. In the present study, we provided evidence from functional neuroanatomy and in vivo electrophysiology to address this open question. Using anterograde and retrograde tract-tracing methods, we first examined connectivity with other structures implicated in visuospatial attention including the postrhinal cortex (POR) and the the lateral posterior nucleus of the thalamus (LPn) considered the rodent homolog of the primate pulvinar. We showed that the LPn projects to the entire PPC, preferentially targeting more ventral areas. All parts of the PPC and POR are reciprocally connected with the strongest connections evident between ventral PPC and caudal POR. Next, we simultaneously recorded neuronal activity in several subregions of the PPC as rats performed a visuospatial attention task that engaged in both bottom-up and top-down attention. Our previous study showed that the dorsal PPC is involved in multiple cognitive process in the translation of perception to action. In this study, we showed that ventral PPC cells responded to stimulus onset more rapidly than dorsal PPC cells suggesting involvement in stimulus-driven, bottom-up attention. The slower time course of neuronal response latency in the dorsal PPC might reflect top-down control to guide appropriate actions. These data suggest that the rat PPC, like the primate PPC, is differentiated into at least two functional subregions, which interact closely during visuospatial attention processing.

Disclosures: F. Yang: None. **R.D. Burwell:** None.

Digital Abstract Session

P303. Networks For Attentional Control

Program #/Poster #: P303.04

Topic: H.01. Attention

Support: R01 MH119523
F31 MH121010

Title: Post-error Recruitment of Frontal-Sensory Cortical Projections Promotes Attention in Mice

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Abstract: Goal-directed behavior, such as attention, requires a cognitive control system which not only monitors contextually relevant internal states and external events, but also implements adjustment in informational processing and behavior. Across species, attention is regulated by direct projections from the frontal cortex to sensory areas. Here, we aim to identify the conditions that recruit frontal-sensory projections from the anterior cingulate area to the visual cortex (ACAvis) to influence attentional behavior in mice. We performed circuit-specific fiber photometry and optogenetics to monitor and manipulate ACAvis activity in mice performing freely moving attention behavior during the 5-choice serial reaction time task (5CSRTT) with an automated touchscreen system. The 5CSRTT requires mice to sustain and divide their attention across five response windows during a 5s delay in anticipation of the random presentation of brief stimulus at one of the five locations. First, we sought to characterize the time course and circumstances of ACAvis neuron recruitment during attention. ACAvis neuron activity did not vary depending on whether the current trial was a correct choice, when attention was properly allocated, or an error. Instead, ACAvis neuron recruitment depended on the result of the previous trial as ACAvis neurons demonstrated elevated activity on the trial immediately following error trials compared to correct, suggesting that ACAvis neurons encode an error monitoring signal. The summation of ACAvis neuron activity alone did not fully explain error correction behavior, leading us to examine whether specific patterns of ACAvis neuron activity contribute to post-error performance. We then performed circuit-selective optogenetic modulation of channelrhodopsin-expressing ACAvis neurons while mice performed the 5CSRTT and found temporal-, frequency-, and history-dependent improvement of attention. We then aimed to dissect the temporal requirement of ACAvis projections during error correction behavior in the 5CSRTT independently of local ACA activity. Projection-selective optogenetic suppression of ACAvis terminals in visual cortex using inhibitory opsin halorhodopsin revealed key time points during 5CSRTT showed in which ACAvis circuit plays causal role in post-error attention. Our

data identify prior error history as a key internal condition to recruit top-down frontal-sensory cortical projections and drive post-error attention enhancement. Our findings may provide circuit-based insight into the pathophysiology and intervention strategy for impaired visual attention in neuropsychiatric disorders.

Disclosures: **K.J. Norman:** None. **H. Koike:** None. **J.S. Riceberg:** None. **J. Bateh:** None. **S. Lopez:** None. **K. Caro:** None. **A. Liang:** None. **K. Yamamuro:** None. **M. Flanigan:** None. **Y. Garkun:** None. **E. Nabel:** None. **D. Brady:** None. **C. Cho:** None. **M. Sadahiro:** None. **K. Yoshitake:** None. **P. Maccario:** None. **M. Demars:** None. **S.J. Russo:** None. **M.G. Baxter:** None. **M.L. Shapiro:** None. **P. Rudebeck:** None. **H. Morishita:** None.

Digital Abstract Session

P303. Networks For Attentional Control

Program #/Poster #: P303.05

Topic: H.01. Attention

Support: HK Research Grants Council GRF 14606417
HK Research Grants Council GRF 14602418

Title: Tms on the inferior frontal cortex (ifc) during standard processing abolished subsequent mismatch negativity (mmn) to deviants: The role of the ifc in establishing a prediction model

Authors: ***C.-Y. TSE**[1], T.-Y. LUI[2], Y.-H. SHUM[2], X.-Z. XIAO[2], Y. WANG[1];
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Abstract: The human brain constantly monitors the environment and able to detect unexpected changes even without conscious awareness (i.e., the automatic or pre-attentive change detection). According to the prediction model hypothesis, the inferior frontal cortex (IFC) is involved in extracting and establishing the prediction model from the frequently presented standards, or re-instating the prediction model for detecting deviant. Our previous studies (Tse et al., 2018; Xiao et al., 2020) demonstrated a causal functional connection of IFC and STC in pre-attentive change detection by applying Transcranial Magnetic Stimulation (TMS) to disturb the functioning of IFC while the later STC mismatch response was measured with event-related optical signals. However, evidence supporting the IFC's contribution to the prediction model is typically inferred from studies examining the deviance detection process. The current study examined the functional role of IFC in establishing the prediction model independent of deviance processing by disrupting the functioning of the IFC in processing a train of standard with Transcranial Magnetic Stimulation (TMS) and measuring the subsequent TMS effect on MMN responses to pitch deviants. Specifically, the MMN response preceded by a 3-standard train and with TMS pulses applied to the IFC at the initial 2 standard positions (2W1O; 2 standards with TMS followed by 1 standard without TMS) was compared to that of a 6-standard train with IFC TMS at the initial 2 positions (2W4O), a 6-standard train with IFC TMS at the initial 5 positions

(5W1O), and a 9-standard train with IFC TMS at the initial 5 positions (5W4O). An abolishment of MMN response to deviant was only observed when TMS was delivered to the IFC of the 2W1O condition, while the MMN responses were preserved in other train length conditions with IFC TMS, or in all the train length conditions when TMS at the vertex or TMS pulse sound was presented. These results demonstrated that IFC was involved in processing the standards and critical for pre-attentive detection of subsequent deviants. These results are also consistent with the prediction model hypothesis on the functional role of IFC.

Disclosures: C. Tse: None. **T. Lui:** None. **Y. Shum:** None. **X. Xiao:** None. **Y. Wang:** None.

Digital Abstract Session

P303. Networks For Attentional Control

Program #/Poster #: P303.06

Topic: H.01. Attention

Support: Natural Sciences and Engineering Research Council Discovery Grant
Canadian Foundation for Innovation Leaders Opportunity Fund infrastructure grant

Title: Laterality of individual differences in emotion and cognition networks: Evidence from spontaneous neural activity

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Abstract: Objective: Emotion-cognition interactions are mediated through communication between ventral affective emotion processing system (VAS), and dorsal executive system (DES). While each of these networks and their interactions have been studied during task performance, their intrinsic connectivity patterns (resting state functional connectivity between regions of interest [ROIs]) and their association with emotion regulation scores have not been investigated. Graphical models are useful for exploring the complex neural networks as this method is informative about unique functional connectivity between ROIs. **Methods**. Five-minute simultaneous resting state EEG-fMRI recordings were acquired from 19 healthy adults (18-31 years old, 13 females). A set of standard neuropsychological tests in the emotional domain (Scale of Emotional Arousability [SEA], Emotional Contagion Scale [ECS], Emotional Regulation Questionnaire [ERQ]), and attentional domain (Barratt Impulsiveness Scale [BIS]) were collected before the neuroimaging session. Nineteen ROIs that previously have been shown to be

involved in emotion-cognition interactions were chosen. Using the sLORETA toolbox, the scalp EEG activity was transformed to MNI source space, and brain activity was calculated within each ROI. Partial correlation and conditional independence between each ROI pair was calculated to find a sparse network in each hemisphere. **Results:** Although the left and the right hemisphere networks were similar, there were more connections within the DES in the left hemisphere (left dorsolateral prefrontal cortex (dlPFC) and medial prefrontal cortex; and left supramarginal gyrus and insula) and more connections within the VAS in the right hemisphere (amygdala and right fusiform gyrus) supporting brain laterality characteristics. Connectivity within the VAS (left ventrolateral prefrontal cortex (vlPFC) and left amygdala) was significantly correlated with various emotional domains scores from ERQ (expressive suppression), and SEA (general emotionality and timidity scores) scales. Functional connectivity between the left VAS and DES (left vlPFC and the left dlPFC) was significantly correlated with BIS scores in the attentional domain (total score, first and second order attentional score, and cognitive impulsiveness score). **Conclusion:** The findings of this study provide new insights into the lateralization of brain circuitry, namely right-lateralization of connections within the VAS and left-lateralization of connections within the DES. Moreover, they emphasize the importance of integration between these two networks for emotional regulation abilities.

Disclosures: R. Bakhtiari: None. **A.T. Shafer:** None. **I. Cribben:** None. **K. Cheema:** None. **M. Moore:** None. **F. Dolcos:** None. **A. Singhal:** None.

Digital Abstract Session

P303. Networks For Attentional Control

Program #/Poster #: P303.07

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH grant R01 AG054513

Title: The role of Neurons, Glia, and Synapses in Defining Hierarchical Structure of Resting State Functional Connectivity Networks

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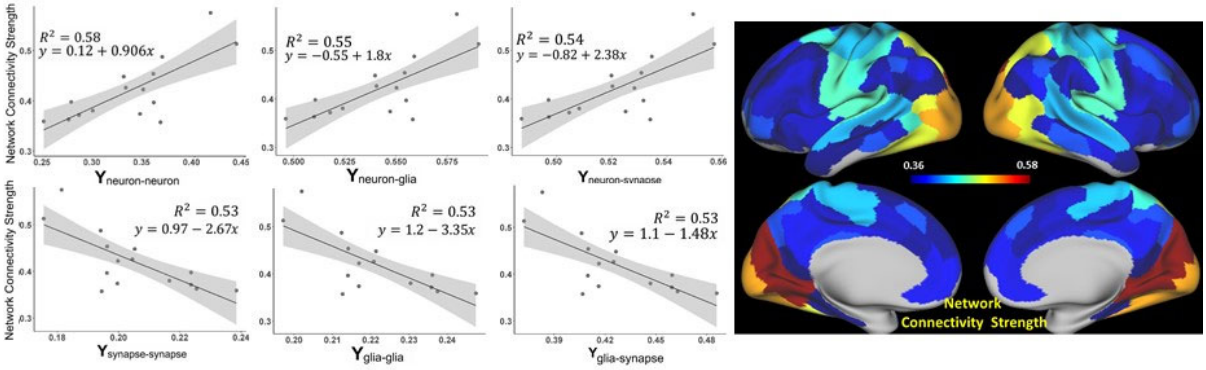
Abstract: While significant progress has been achieved in studying resting state functional networks in a healthy human brain and in a wide range of clinical and neurological conditions, many questions related to their relationship to organization brain cellular circuits remain open. In this study we use genetically-enabled quantitative Gradient Recalled Echo MRI (qGRE) technique (Ulrich and Yablonskiy, MRM **75**: 606-615, 2016) for in vivo quantitative mapping of brain cellular circuits and resting state data from Human Connectome Project to explore how components of the human brain cellular circuits relate to the organization of the resting state functional networks. The qGRE data analysis allows in vivo mapping of tissue cellular specific (*R2t**) GRE signal relaxation (here "*t*" means *tissue*) in the human brain cortical GM that is

directly related to the brain tissue cellular composition (Wen, et al, PNAS **115**(41): E9727-E9736, 2018). The functional connectivity data were parcellated to 17 resting-state networks (Yeo, et al, J Neurophysiol **106**(3): 1125-1165, 2011). Our results show that the synchrony of the *short-range* brain connections is mostly governed by the neuron-based circuits while the *long-range* network connectivity is governed not only by the strength of the neuron-neuron connections, but also neuron-glia, and neuron-synapse cross-talk between network regions that are most prominent in the infra-slow frequency range (0.01-0.16Hz) of brain activity. These mechanisms lead to a rather broad distribution of resting state functional networks' properties. We found that the visual networks have the highest neuronal density (but lowest density of glial cells and synapses), the strongest coherence of BOLD signal and the strongest intra-network connectivity (Figure). The Default Mode Network (DMN) that plays a very prominent role in the overall organization of the brain in health and disease is positioned near the opposite part of the spectrum with relatively low coherence of the BOLD signal but a remarkable diversity of cellular circuits.



Disclosures: **S. Kahali:** None. **M.E. Raichle:** None. **D.A. Yablonskiy:** None.

Digital Abstract Session

P303. Networks For Attentional Control

Program #/Poster #: P303.08

Topic: D.07. Vision

Support: NIH National Eye Institute R01 EY019693

Title: Image-computable attention model of texture segmentation across the visual field

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Abstract: Goal. Attention modulates texture segmentation, our human capacity to isolate objects from their surroundings across the visual field. The cortical computations that underlie their interaction are unknown. We developed a model to explain several signatures of texture segmentation and of its attentional modulation across eccentricity: (i) the central performance

drop, which is the parafoveal advantage for segmentation over foveal locations; (ii) that involuntary attention improves segmentation in the periphery but impairs it at central locations; (iii) that voluntary attention improves segmentation across eccentricity.
Approach. We developed an image-computable model to explain all of these phenomena. The model's components are motivated by canonical motifs that underlie information processing across the visual hierarchy, e.g., normalization and spatial summation. Specifically, segmentation relied on the interaction between normalization and attentional modulation with distinct operating regimes for voluntary and involuntary attention systems. Attention operated before normalization, as per the Reynolds-Heeger (2009) normalization model of attention (NMA), to adjust sensitivity for coarse and/or fine visual details. The model operated on grayscale texture images to reproduce performance across many eccentricities from ten psychophysical experiments—involuntary attention was manipulated in six and voluntary attention in four experiments.
Results. (i) The model generated the central performance drop through spatial summation of normalized sensory inputs, which determined the model's baseline sensitivity to visual detail. (ii) Involuntary attention effects were generated by enhancing sensitivity to a narrow range of visual details (high spatial frequencies), which were too fine to distinguish the textural target at central locations, but progressively overlapped the target toward the periphery. (iii) Voluntary attention effects were mediated by a uniform enhancement of sensitivity to coarse and fine details (broad range of spatial frequencies), which encompassed the target.
Conclusion. Our results highlight and explain how involuntary and voluntary attention differentially shape texture segmentation, and extend NMA to capture attention effects on contrast and texture sensitivity across eccentricity. The model provides a quantitative framework for assessing interactions between attention and perception for complex visual stimuli.

Disclosures: M. Jigo: None. **D.J. Heeger:** None. **M. Carrasco:** None.

Digital Abstract Session

P304. Neuromodulation of Attention

Program #/Poster #: P304.01

Topic: H.01. Attention

Support: JSPS KAKENHI 19K16302

Title: Locus coeruleus modulates perception in the auditory cortex during zebra finch song learning

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Abstract: Juvenile zebra finches learn to sing by vocal communications with adult tutors. While juveniles learn poorly from passive exposure to song playback, song playback paired with task performance causes effective song learning. This suggests that internal states of juveniles, such

as attention or motivation regulate song learning. Here we investigated neuronal activities in the attention control brain region, the nucleus locus coeruleus (LC), when juvenile zebra finches listened to tutor singing via social interaction by using chronic extracellular single-unit recording. LC neurons increased their firing rates in response to tutor song (TUT) playbacks and increased more upon tutor singing. Moreover, LC neurons sustained greater responses to TUT playbacks after hearing hours of tutor singing (mean response strength (RS) before: 1.6±0.4 s, after: 4.5±0.9 (spikes/s), n=16).LC neurons send their axons to the zebra finch higher auditory cortex, the caudomedial nidopallium (NCM), where tutor song memories are thought to form. Previously we showed a fraction of NCM neurons that respond selectively to TUT playback. As seen in LC neurons, TUT selective NCM neurons showed a greater response to tutor singing than to TUT playback and sustained greater responses to TUT playback after hearing hours of tutor singing (mean RS before: 0.4 ± 0.2, after: 4 ± 0.67 (spikes/s), n=24). To ask whether LC neurons directly influence song perception in the NCM, we recorded NCM neuronal activity with optogenetic LC terminal inactivation while juveniles interacted with tutors. We found a subset of silent narrow spiking NCM neurons which showed no auditory responses to TUT playback but responded to LC terminal inactivation (mean RS TUT playback: 0, LC inactivation: 3.8 ± 0.64 (spikes/s), n=10). In contrast, broad spiking NCM neurons, which responded to TUT playbacks, showed reduced responses to tutor singing when paired with LC terminal inactivation (mean RS to TUT playback: 5.6 ± 0.82, tutor singing with LC inactivation: 3.4± 0.73 (spikes/s), n=10). Those neurons showed a reduced response to TUT, but not to the other song playbacks after listening to two hours of tutor singing with LC terminal inactivation. Juveniles, which were tutored for three days with LC terminal inactivation every time a tutor sang, failed to copy tutor songs, while control juveniles that tutored without LC inactivation learned tutor songs well. Taken together, our data suggest that increased attention by social interaction with a tutor modulates song perception in the NCM via LC neuronal activities; this might be an underlying neuronal mechanism for attention control of song learning.

Disclosures: J. Katic: None. **Y. Morohashi:** None. **Y. Yazaki-Sugiyama:** None.

Digital Abstract Session

P304. Neuromodulation of Attention

Program #/Poster #: P304.02

Topic: H.01. Attention

Title: The involvement of glutamate in sustained attention: Effects of the NMDA co-agonist, D-serine, on the skew of initiation time distributions in rats.

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Abstract: Many people live with impaired sustained attention, including individuals with ADHD, schizophrenia, depression, and age-related cognitive decline. The most common treatments for impaired attention include stimulant medications; however, such drugs are

associated with negative side-effects. Stimulant medications improve attention at least partially through effects on the dopamine and norepinephrine (NE) systems. Safer treatments might be developed by targeting other neurotransmitter systems that support sustained attention. For example, glutamate is a major excitatory neurotransmitter that is thought to function in concert with NE to promote cognitive processes like memory and perception (Mather et al., 2016, Behav Brain Sci (39), e200). We tested the involvement of glutamate in sustained attention by administering D-serine, a co-agonist that promotes the activation of NMDA receptor complexes. Male Sprague-Dawley rats (n=16) were tested in a two-choice reaction time task (2CRTT). Power analysis was conducted with an expected effect size from past data. In the 2CRTT, rats nosepoke in a central aperture for 0.3 - 6.0 sec. A stimulus light then comes on above either a left or right aperture. Rats must exit the central aperture and nosepoke beneath the illuminated light. Water is given for a correct response within a performance-based time limit. Each reaction time is broken into initiation time (IT) and movement time (MT) to isolate attentional processes. IT is the time from stimulus presentation until the rat initiates a response. IT mode represents attentive sensorimotor processing speed, while IT distribution skew (devmode; mean – mode) is thought to reflect lapses in attention (Leth-Steensen et al., 2000, Acta Psychologica (104), 167-190; Sabol et al., 2003, Behav Pharm (7), 489-500). All rats received two full dose-response series of six D-serine doses (saline, 10, 50, 100, 150, and 300 mg/kg, i.p.) in a counterbalanced repeated-measures design. Drug was administered 30 minutes before testing. D-serine dose-dependently reduced IT devmode without affecting IT mode. Reduced IT devmode is thought to indicate improved sustained attention, as rats were more able to maintain fast ITs throughout test sessions. The lack of effect on IT mode suggests no effect on sensorimotor processing speed when animals were attentive. D-serine's effects on IT devmode are the first indication from a rat model that sustained attention can be improved by increasing the NMDA-mediated effects of glutamate. This translational work could lead to the development of safer treatments that improve quality of life in a wider range of people living with impaired attention.

Disclosures: Z.V. Redding: None. **K.E. Sabol:** None.

Digital Abstract Session

P304. Neuromodulation of Attention

Program #/Poster #: P304.03

Topic: H.01. Attention

Support: AG050518

Title: Sb-334867, a selective orexin-1 receptor antagonist, alleviates dizocilpine-induced attentional impairments in an nmda receptor hypofunction model of schizophrenia

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Abstract: Schizophrenia is a complex and often-debilitating neuropsychiatric condition that is associated with impaired attentional processing and performance. Failure to maintain attention is thought to result from a hyper-reactive basal forebrain cholinergic system that cannot support increasing attentional load, and available antipsychotics often fail to address this pervasive symptom. Acute systemic administration of psychotomimetic NMDA receptor antagonists, which reproduce the NMDA receptor hypofunction observed in schizophrenia, markedly elevates cortical acetylcholine release and disrupts posterior attention systems. Both orexin (hypocretin) receptor subtypes (orexin-1 and orexin-2) are amply expressed on corticopetal cholinergic neurons, suggesting that their suppression may lessen basal forebrain excitability and reduce attentional deficits stemming from a hypercholinergic state. A previous experiment in our lab showed that rats trained in a sustained attention signal detection task performed worse and responded less when given the NMDA receptor antagonist dizocilpine (MK-801: 0 and 0.1 ip), and these impairments were reversed when dizocilpine was co-administered with the dual orexin receptor antagonist filorexant (MK-6096: 0, 0.1, and 1.0 mM ICV), suggesting orexin receptor antagonism attenuated dizocilpine-induced overstimulation of cortical regions critical for sustained vigilance. To parse the unique role of the orexin-1 receptor, rats in the present experiment received either saline or dizocilpine injections in tandem with the orexin-1 receptor antagonist SB-334867 (0, 3, and 6 µg ICV) prior to attention task performance across six sessions. As was previously found, dizocilpine diminished signal detection accuracy, particularly during and following a flashing visual distracter, and increased the number of omitted trials. Similarly with filorexant, both doses of SB-334867 ameliorated dizocilpine-induced signal detection deficits and significantly reduced errors of omission. Thus, we propose that the orexin-1 receptor is an important target for minimizing the attentional deficits associated with NMDA receptor antagonism.

Disclosures: E.B. Maness: None. **J.A. Burk:** None.

Digital Abstract Session

P304. Neuromodulation of Attention

Program #/Poster #: P304.04

Topic: H.01. Attention

Support: Weston Family Foundation

Title: Evaluating the potential for remediation of attentional deficits in 3xTg-AD mice by 3α-diol

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Abstract: Neurosteroids and their brain-synthesized metabolites have been shown to promote neural cell survival, but their levels are significantly reduced in patients with Alzheimer's disease

(AD). Recent work has suggested that the androgen-derived neurosteroid metabolite 5α-androstane-3α,17ß-diol (3α-diol) may specifically contribute to this neuroprotection, as well as to sex differences in the incidence of AD. This study aimed to determine the effect of long-term 3α-diol treatment on the development of neuropathology and cognitive dysfunction associated with a triple-transgenic mouse model of AD (3xTg-AD). To test the hypothesis that 3α-diol will slow cognitive decline and the progression of the neuropathology of AD in the mouse model used, the 5-Choice Serial Reaction Time Task (5-CSRTT) was utilized. This task is a rodent behavioural paradigm designed to measure the effects of drugs and other manipulations, including genetic, on attentional performance and stimulus control. The following data concerns a cohort of male and female mice aged 6 months implanted with slow release silastic capsules loaded with either 3α-diol or vehicle at 3 months of age. Consistent with our past work, we found that 3xTg-AD mice had significantly lower accuracy than wild-type mice. 3xTg-AD mice also had longer correct response latencies than wild-type mice. In terms of reward collection latency, no genotype or treatment effect was found, but male mice took longer to collect the reward in comparison to their female counterparts. Thus, we confirm the sensitivity of the 5-CSRTT to 3xTg-AD attentional impairment, but provide no evidence to indicate a protective effect of 3α-diol. Further research is required to assess the potential benefits of 3α-diol at later ages and on other cognitive tests. If 3α-diol has significant neuroprotective properties in the central nervous system, in vivo, it could lay the foundation for potential clinical applications as a therapy to slow the progression of AD.

Disclosures: J. Visscher: None. **B.D. Winters:** None. **N.J. MacLusky:** None. **N. Kuwahara:** None. **S. Bhullar:** None. **J. Felix:** None. **J. Zakaria:** None. **C. Connery:** None.

Digital Abstract Session

P304. Neuromodulation of Attention

Program #/Poster #: P304.05

Topic: C.10. Brain Injury and Trauma

Support: CDMRP Grant W81XWH-13
DoD Grant W81XWH-16-2-0023

Title: Using FAST language and music to optimize attentional engagement for Disorders of Consciousness with severe TBI.

Authors: *N. CHAUDHRY[1,2], S. LIVENGOOD[2,3], J. HIGGINS[4], E. WALSH[2], A. GUERNON[2,5], R. ALSAKAJI[2], S. KLETZEL[2], T. PARRISH[4], T. PAPE[2,3];
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Abstract: Background: Disorders of Consciousness (DoC) is a neurological disconnection syndrome, hallmarked by a lack of volitional attention, with few treatment options. Effective treatments are further challenged by heterogeneous lesion profiles associated with severe TBI (sTBI). Familiar Auditory Sensory Training (FAST) was designed to exercise the DoC brain through mechanisms of salience, attention, and the broad cortical activations associated with autobiographical memory recall. To optimize attentional engagement for remaining neurological function, we created FAST-L and FAST-M based on the premise that language/music are comparable in complexity, hierarchical structure, and the type of autobiographical memories they invoke, while engaging a left/right hemisphere processing bias. We compared fMRI volumetric activation to contrasts of FAST-L versus FAST-M in three networks associated with attentional control: the dorsal attention network (DAN), associated with top-down control; the ventral attention salience network (VAN) associated with bottom-up reflexive attention; and the Fronto-Parietal Control Network (FPCN) associated with mediating top-down and bottom-up attention.
Hypothesis: We hypothesized that more left/right diffuse axonal injury (DAI) would predict more attentional engagement to FAST-M/FAST-L, respectively in the VAN, DAN and FPCN.
Methods: In a task-fMRI block design, 3 DoC-sTBI subjects (left DAI, vegetative state: N=2; Right DAI, minimally conscious sate: N=1; 1 Female, 2 Males; mean age = 27, SD = 9.5). Subjects listened to 16 alternating 30s blocks of FAST-L and FAST-M. fMRIprep and AFNI were used to warp, smooth, denoise and censor the data in MNI space. A 40 voxel cluster analysis was run, with a threshold of 3.3, uncorrected p=0.001.
Results: Pilot data analyses supported our hypothesis and revealed that DAI x stimuli-type predicted maximal voxels of activation of VAN, DAN and FPCN, with one caveat. Consistent with our hypotheses, left/right hemisphere DAI was associated with maximal activation of the DAN and FPCN in response to FAST-M/FAST-L respectively. The VAN followed a similar DAI x stimuli-type interaction; however, all 3 subjects showed maximal activation in response to the stimuli-type targeting the damaged hemisphere (e.g., Right DAI showed max VAN response to FAST-M), suggesting the damaged hemisphere may be working harder to reflexively process its optimal stimuli-type.
Conclusions: Pilot data show that both FAST-L/FAST-M can be used to maximize engagement of attention networks in the DoC-sTBI brain, based on DAI profile.

Disclosures: N. Chaudhry: None. **S. Livengood:** None. **J. Higgins:** None. **E. Walsh:** None. **A. Guernon:** None. **R. Alsakaji:** None. **S. Kletzel:** None. **T. Parrish:** None. **T. Pape:** None.

Digital Abstract Session

P305. Models of Human Perception

Program #/Poster #: P305.01

Topic: H.02. Perception and Imagery

Support: DFG Grant SH 166/3-2
DFG Grant GL 342/3-2

Title: Another dead salmon? The measurement flaw of serial dependence in human perception

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Abstract: Human perception is susceptible to various types of contextual biases, such as the range, distribution, and sequential exposure of the stimuli. Recent studies explain those biases as consequences of an optimal estimation process that uses additional contexts and prior knowledge to achieve an overall improvement in estimation reliability (e.g., Jazayeri & Shadlen, 2010, Glasauer & Petzschner 2011). Recently, there is a surge of interest in one type of contextual bias - serial dependence (e.g., Fischer & Whitney 2014, Bliss et al. 2017; Clifford et al. 2018). Serial dependence refers to the current perceptual judgment being influenced by past exposure stimuli. Investigation of serial dependence is not new, it has been shown about a half century ago (e.g., Holland & Lockhead 1968). The novelty is the way of how the serial dependence is measured in recent studies: the perceptual error, measured by the difference between the perceived and the given physical stimulus in the current trial, is then expressed as a function of the stimulus difference between the previous and the current trial (we refer to this as relative measure). This measure, however, is flawed due to mathematical coupling (Archie 1981, Curran-Everett 2010) given that the current stimulus appears in the estimation of both variables - the perceptual error and the stimulus difference. We show that random arbitrary or constant responses can also yield a bogus strong serial dependence in such relative measure - another dead salmon echoing the famous 'dead salmon case' in fMRI analysis (Bennett et al., 2009). Therefore, this relative measure cannot provide a meaningful quantification of serial dependence. Rather, the 'old' measure proposed by early studies (e.g., Holland & Lockhead 1968) - the current error as a function of the stimulus in the previous trial - can reflect a true serial dependence. We further show that iterative updating models (Glasauer & Petzschner 2011; Dyjas et al. 2012; Cicchini et al. 2018), which use past stimulus information for estimating the current stimulus and updating the prior, can make specific and testable predictions about the serial dependence and its relation to the classical central tendency effect, an effect of overestimation of small magnitudes and underestimation of large magnitudes. We reveal that the serial dependence and the central tendency are causally related: if information from previous trials is used as prior for the current perceptual estimate, a central tendency bias will occur as a consequence.

Disclosures: S. Glasauer: None. **Z. Shi:** None.

Digital Abstract Session

P305. Models of Human Perception

Program #/Poster #: P305.02

Topic: H.02. Perception and Imagery

Title: The effect of prior knowledge on visual processing of agents: Time-resolved representational similarity analysis on EEG data

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Abstract: Recognition of agents during action observation is a crucial skill for many species including humans. Visual processing of agents is considered to take place in the pSTS node of the Action Observation Network; however, its temporal course and the factors that affect it still remain unknown. We investigated how prior knowledge about agents affects temporal characteristics of neural processing. We conducted 2 EEG experiments with human subjects. In both experiments (12 female, 15 male in total), subjects were presented videos and still images of 3 agents who performed 8 different actions as EEG was recorded. In the first experiment (Prior experiment), subjects (n=19) were familiarized with the stimuli and informed about the identity of the agents prior to the experiment. In the second experiment (Naive experiment), subjects (n=16) were naive to the identity of the agents. After standard preprocessing, we linked the EEG data in a time-resolved manner to a categorical Agent Model using representational similarity analysis (Kendall Tau correlation). We also used 3 additional models that capture the non-target variables within presented stimuli, a categorical Action model and two low-level visual models: pixel-wise intensity and pixel-wise motion energy. Finally, we did multiple regression analysis at each time point to determine how much each individual model could independently predict the neural data; thus, enabling us to regress out the effect of non-target variables on the correlation between the Agent Representational Dissimilarity Matrix (RDM) and the neural RDM (Bonferroni corrected for all time points and conditions, $p<0.05$). Our results show that the most informative channels about agent information were the ones over the occipital and parietal cortex. In those channels, there was a specific time range when agent information was available but it depended on whether the subjects were naive about the identity of the agents and the presentation mode. The comparison between Prior and Naive experiments showed that when subjects had no prior knowledge about agents, agent information became available for a longer duration during processing of still images (66-418 ms) than videos (90-130 ms). However, when subjects had prior knowledge about the agents, agent information was present for similar durations in both stimulus presentation modes: 82-196 ms for still images or 90-172 ms for videos. We conclude that prior information affects temporal characteristics of visual processing of agent perception by modulating the onset and duration of when agent information is available.

Disclosures: S. Er: None. **H.O. Elmas:** None. **A.P. Saygin:** None. **B.A. Urgen:** None.

Digital Abstract Session

P305. Models of Human Perception

Program #/Poster #: P305.03

Topic: H.02. Perception and Imagery

Support: ISF Individual Research Grant 1485/18 to SGD

Title: Perceptually biased distance judgments of closer lower visual field information coincide with statistical properties of the visual environment

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Abstract: Human visual perception is often biased and this is sometimes attributed to misinterpretations of visual scenes based on visual priors that may reflect statistical properties of the visual environment. Following a recent model we proposed that suggests differences between the processing of upper and lower visual field information, here we hypothesized that during natural behavior visual inputs from upper and lower visual fields are significantly different and that this difference may bias perceptual decisions. Specifically we assumed that objects in the lower visual field are closer to the eyes than those in the upper visual field, and that distance estimations would therefore be biased for closer distance judgements in the lower visual field. Visual inputs (world and depth videos) were recorded during "in the wild" behavior via Intel Realsense d415 depth and world camera that is mounted on a glasses-like device (PupilLabs eye tracker) such that the camera lenses were right above the eyes. Data was collected during naturalistic behavior in 4 different types of environments (walking in indoor or outdoor environments, passenger in the front seat of a driving car, or while occupied with electronic devices). In all environments we found consistent evidence (across time and space) that lower visual field was closer to the eyes (e.g. in outdoors and car >95%, screens ~80%, and indoors ~75% of the time). We then examined the possibility that perceptual judgments may be biased in accordance with these statistical differences. 190 participants (aged 18-73 with normal vision) were presented with world images with 2 marked points, one in the lower half and one in the upper half of the image, and they were asked to judge which point was closer to the camera that took the picture. The number of pictures with closer upper point was equal to the number of pictures with closer lower point, with the critical condition being the pictures with equidistant points. While there were more errors made towards lower point being closer across all conditions, in the critical equidistant condition, more than half of the decisions (~56%) were of lower point being closer, which was 5 times the amount reported for upper point being closer (~11%). These judgements were not influenced by age, gender, or the height of the participants. Our results reveal a new perceptual bias for distance judgments where objects in the lower visual field are perceived as closer to the eyes than those in the upper visual field. This perceptual distance bias can be explained by our initial finding that in the visual environment objects in the lower visual field are significantly closer to our eyes than those in the upper visual field.

Disclosures: Y. Zilbertzan: None. **S. Gilaie-Dotan:** None.

Digital Abstract Session

P305. Models of Human Perception

Program #/Poster #: P305.04

Topic: H.02. Perception and Imagery

Title: Left inferior parietal cortex represents subjective stimulus visibility

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Abstract: One of the major debates in neural correlates of visual consciousness concerns which brain regions are the neural bases of the subjective stimulus visibility and how it anatomically relates with visual attention. Stimulus visibility fluctuated even though visual stimulus was physically identical. These dynamics were reported to be involved in the posterior parietal cortex (PPC) in previous studies. Moreover, although visual attention has been believed to be dissociated from subjective visibility, neural substrates of visual attention located in PPC, resulting in the conflation of specific localized regions corresponds to subjective visibility. Here, we investigated which regions in PPC significantly contribute to the representation of subjective visibility independent of visual attention. We used classical Posner cueing task to measure visual attention and applied backward masking to render cue stimulus bedimmed. Participants were instructed to discriminate the location where the tilted target stimulus (in left or right) was presented. They also reported subjective visibility of cue stimulus in four graded scales (invisible to clearly seen). We then constructed four regions of interest (ROIs) in PPC, which is widely covered the PPC to address the information containing subjective visibility, and performed multivariate pattern classification of the BOLD signal to test whether the hemodynamic responses in each ROI represent subjective visibility. Our results demonstrated that only the left inferior parietal lobule (IPL) had the information of subjective visibility. On the other hand, univariate analysis for subjective visibility revealed that the region involved in left IPL was significantly correlated with subjective visibility. Moreover, the hemodynamic responses in the left IPL did not predict the behavioral response latency, indicating that the left IPL response was independent of the visual attentional effect. These results suggest that the left IPL is involved in processing subjective visibility.

Disclosures: **M. Hishitani:** None. **Y. Osako:** None. **S. Murai:** None. **H. Nagamura:** None. **H. Onishi:** None. **K.I. Kobayasi:** None.

Digital Abstract Session

P305. Models of Human Perception

Program #/Poster #: P305.05

Topic: H.02. Perception and Imagery

Support: HK Research Grants Council GRF 14606417
HK Research Grants Council ECS 24401314

Title: The Fronto-occipital Network in Pre-attentive Detection of Visual Changes: An Event-related Optical Signal (EROS) Study

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Abstract: Pre-attentive change detection refers to the ability of human brains to monitor and detect changes in the environment even without attention. Previous studies showed that the Inferior Frontal Cortex (IFC) and the Superior Temporal Cortex (STC) were part of the brain network in pre-attentive detection of auditory changes. Specifically, an early IFC followed by STC then late IFC (i.e., early IFC-STC-late IFC) activation pattern was observed in detecting ambiguous auditory changes. As proposed by the prediction violation framework, the early IFC is related to reinstating the prediction model before the change detection process in the STC; when a prediction violation is detected, STC generates a prediction error that triggers the prediction model updating process which is shown as the late IFC activity. However, it remains unclear whether this fronto-sensory network organization is specific to detecting auditory changes or can also be applied to detecting visual changes. To address this question, the current study investigates the fronto-occipital (OC) network in pre-attentive detection of visual changes by using the Event-related optical signal (EROS). As EROS measures the change in optical properties of the brain associated with neuronal activity, it is able to localize brain activities temporally and spatially in the millisecond and sub-centimeter range. Twenty-four participants were recruited and presented with a visual passive oddball paradigm consisted of a simple physical feature change (i.e., orientation change of a bar array) and an abstract regularity violation (i.e., violating the clockwise rotation pattern among the standard bar arrays). Based on the number of preceding standards, each of the physical and abstract change deviants were further divided into the short train and the long train conditions. A shorter standard train or an abstract regularity among the standard is less likely to provide sufficient information to establish a stable prediction model. In detecting physical changes, the EROS results showed an early IFC-OC pattern to deviant preceded by a short standard train, while an OC-late IFC pattern when preceded by a long train. In detecting abstract changes, no EROS match response was observed for deviant preceded with a short train; however, an early IFC-OC- late IFC pattern was observed for deviant preceded with a long train. These results demonstrated 1) the spatial-temporal dynamics in pre-attentive detection of visual changes is similar to that in detecting auditory changes; and 2) activation patterns that was consistent with the prediction violation framework.

Disclosures: Y. Shum: None. **X. Xiao:** None. **Y. Wang:** None. **C. Tse:** None.

Digital Abstract Session

P305. Models of Human Perception

Program #/Poster #: P305.06

Topic: H.02. Perception and Imagery

Support: NIH grant R01 EY023915
Marie Sklodowska-Curie grant H2020-MSCA-IF-2017-795807

Title: Spatiotemporal population receptive field modeling of human visual cortex

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Abstract: We experience visual scenes containing objects that change over space and time. To interpret these dynamic changes, our visual system requires representations of both space and time. However, previous population receptive field (pRF) implementations model the spatial aspects of the neural response, but not its temporal aspects. Here, we developed a novel spatiotemporal pRF encoding model that characterizes neural responses to space and time-varying stimuli. The new model predicts fMRI responses to stimuli that vary spatially over the visual field and temporally from milliseconds to seconds. To evaluate if different spatiotemporal models predict different fMRI responses to the same stimulus, we developed a software framework that (i) generates synthetic fMRI data from a stimulus and a spatiotemporal pRF model, (ii) solves spatiotemporal pRF parameters, and (iii) tests the validity of the solution with respect to the ground truth parameters used to synthesize the spatiotemporal pRF. We show that (i) the software accurately solves the parameters of the spatiotemporal pRF validated against the ground-truth data and (ii) both 2-channel (Stigliani et al., 2019) and delayed normalization (Zhou et al., 2019) spatiotemporal pRF models predict stronger responses to traveling wave stimuli using brief (33ms) stimuli compared to the standard pRF model. Then, we tested how well spatiotemporal pRF models predict experimental fMRI data. The fMRI data were collected while participants (n=5) viewed traveling wave stimuli that traversed the visual field, where stimuli within each location varied in their temporal properties from 30 stimuli, each for 33ms to 1 stimulus for 5s. We found that both the 2-channel and delayed normalization spatiotemporal pRF models predict fMRI response better than the standard pRF model for a brief, transient visual stimuli in each of the retinotopic visual areas (V1, V2, V3, V3a, hV4, LO1, LO2, TO1, TO2). Our experiments with synthetic and experimental data suggest that it is important to use spatiotemporal pRF modeling to improve predictions of neural responses in human visual cortex for a wide range of spatial and temporal stimuli.

Disclosures: I. Kim: None. **G. Lerma-Usabiaga:** None. **W. Shim:** None. **K. Grill-Spector:** None.

Digital Abstract Session

P305. Models of Human Perception

Program #/Poster #: P305.07

Topic: H.02. Perception and Imagery

Support: Science and Technology Commission of Shanghai Municipality Grant No. 201409002800

National Natural Science Foundation of China Grant No. 32071060
Joint Research Institute for Science and Society Incubating Project

Title: Posterior parietal cortex neurons distinguish memorised past from what is perceived as reality

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Abstract: Humans can discriminate whether a piece of information is being held internally as memories or externally as coming from the outside world. The posterior parietal cortex (PPC), as an information accumulator for conscious perception, is hypothesized to monitor such distinction between the memorized and perceived information. We recorded electrophysiology data from the dorso-medial posterior parietal cortex on an adult monkey while it performed a temporal-order judgement (TOJ) task. Critically, in the delay/maintenance period of the TOJ task, we presented a congruent image in 1/4 of the trials (i.e., extracted from the video that they had just encoded), in another 1/4 of the trials with an incongruent image (i.e., extracted from other videos), and in 1/2 of the trials without any distractor as baseline (no-picture). We acquired single-unit spike data (66 neurons) from 6 daily sessions (440 trials in total). The monkey's TOJ accuracy was 82.5% ± 38% with RT at 977.9 ms ± 353.5ms. By fitting mixed effect models, RT for TOJ in the congruent condition was longer than that in the no-picture condition ($t(418.34)=2.077$, $p=.038$), and that RT in the incongruent condition was also marginally longer than the no-picture condition ($p = .13$), whereas there was no difference between congruent and incongruent condition ($p = .67$). With the neuronal data, we focused on the 500-ms period in which images were presented during the memory maintenance period. For each neuron, we performed receiver operating characteristic (ROC) analyses to measure how discriminable the distributions of its firing rates to each condition might differ and compared their area under the curve (AROC) with chance level. We found that 16 (~24.2%) of the neurons discriminate congruent and incongruent conditions from no-picture condition. Among them, two-thirds of them (10 neurons, ~15% of total) can further discriminate the incongruent condition from the congruent condition (despite the lack of differences in RT). In addition, another 9 neurons (~13.6%) discriminate the incongruent condition from congruent and no-picture conditions. These results indicate that primate PPC neurons are responsive to the external stimuli while the animal performed a memory task, and more importantly, being able to monitor the congruency of such external stimuli and evaluate them against the memorized content in working memory. We conclude that such neuronal correlates for distinguishing memorized vs. perceived content underline our ability in separating imagined/memorized information from external reality. Our work carries implications for clinical symptoms such as hallucination that is observed in psychosis.

Disclosures: **Y. Zheng:** None. **L. Wang:** None. **L. Huang:** None. **H. Lau:** None. **S. Kwok:** None.

Digital Abstract Session

P305. Models of Human Perception

Program #/Poster #: P305.08

Topic: D.07. Vision

Support: Douglas Hospital Research Center-McGill University

Title: The physics of our perceptual-phenomenal-conscious world can be detected:Replicating for the second time the small impacts of the percepts of a partner on the event-related brain potentials of participants

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Abstract: As neuroscientists, we know that we are not seeing stimuli. We know that stimuli trigger outputs from our sensors. We know that these outputs are processed by the brain, which then *creates* percepts. However, these percepts look so real to us that we do as if they were the actual objects we are dealing with. We thus tested whether percepts have their own physics to which we are sensitive to. To achieve that goal, we capitalized on two things: first, on the distance we experience between us and the object-percept that is created by our brain, because this distance suggests that the physics at stake have some nonlocal properties (McFadden, 2020); second, we capitalized on the similarity this physics should have across people. Taken together, these two things mean that, at least in some conditions, the activity of a brain might be sensitive to the percepts of others and could thus include traces of that sensitivity. To look for such traces, we recorded the electrical activity (EEG) of the brains of partners during trials where the photography of a face was presented to participants. Importantly, the design was reciprocal. At each trial, partners were also, and at the same time, presented with a face and having their EEG recorded. These EEGs were used to compute the event-related potentials (ERPs) evoked by those faces in partners and participants. Most importantly, none saw the face presented to the other. Despite this privacy of the presentations, slightly larger late posterior positivities (LPPs) were found in the grand (n = 29) average ERPs when the two face-photographs differed than in the grand average ERPs obtained when they were the same. These slightly larger LPPs seem to be replicable. They appear in each of the two subgroups used. Importantly, it was announced to participants, before the block of trials, that they were about to see images that differ from those simultaneously presented to their partner. The results obtained thus confirm those found by Bouten et al's (2014) and by Haffar et al's (2018) who also observed slightly larger LPPs when the two images of each trial were the same than when these images differed after it had been announced that they will always differ. This is thus the third block-design study that supports the claim that percepts have nonlocal physics, in addition to that of the local neuronal activities responsible for their production. Future studies should thus search for the nature of this physics and test, for instance, McFadden's (2020) electromagnetic hypothesis. In the meantime, cognitive neuroscientists could test whether the sensitivity to percepts of others could be a factor of the similarity of percepts existing across individuals (Hofstadter, 2007).

Disclosures: J. Debruille: None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.01

Topic: H.03. Decision Making

Support: NIH Grant MH110391
NIH Grant NS104818
NIH Grant DA051155

Title: Identification of perceptual inaccuracy as a source of suboptimal behavior in a mouse foraging task using reinforcement learning

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Abstract: In the classical view of economic choices, subjects make rational decisions evaluating the costs and benefits of options in order to maximize their overall income. Nonetheless, subjects often fail to reach optimal outcomes. Many studies have shown that the overt values of options drive the direction of decisions, however few have identified sources of suboptimality in animals' decision-making process. Additionally, many questions remain to be answered as to which contexts contribute the most to deviation from an optimal solution and the extent of these effects. In order to tackle these questions, we devised a decision-making task for mice, in which cost and benefit parameters could be independently and flexibly adjusted and for which a tractable optimal solution was known. We then evaluated the optimality of mice in the task and compared their performance across sessions with different cost and benefit parameters finding that the cost parameter had the largest impact on optimality. Lastly, we identify possible sources of suboptimality of mice in the task by comparing their behavior to that of reinforcement learning (RL) agents. We find that only RL models that include a non-linear cost function and take into account estimation error in numerosity (or alternatively time) according to the Weber-Fechner law can reproduce the behavior of the mice. Taken together, our results suggest that although the mouse brain may be employing a normative decision-strategy in this task, perceptual constraints limit the ability of the mice to perform optimally and these constraints affect behavior differentially across sessions with different experimental parameters.

Disclosures: B.N. Ballintyn: None. **N. Schneider:** None. **H. Pi:** None. **D.B. Katz:** None. **P. Miller:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.02

Topic: H.03. Decision Making

Support: NIH Grant R01DA047870
NIH Grant R01NS104834

Title: Predicting matching behavior based on the consistency of response to reward feedback

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Abstract: For decades, behavioral neuroscientists have used the matching law (i.e., choice fractions match reward fractions) to quantify how animals distribute their responses between multiple options in response to reinforcement they receive from the environment. More recently, multiple reinforcement learning (RL) models have been developed to understand how global (in time) matching behavior emerges as a result of local learning rules. Despite reasonable success of these models in capturing choice on a trial-by-trial basis, they are not good predictors of overall matching and its variability. To address this issue, we analyzed data from a probabilistic reversal learning task with a dynamic reward schedule in mice (N = 18 animals, 528 sessions, 3764 blocks) and developed new metrics to capture consistency in response to reward feedback based on the concept of conditional entropy in information theory. In this task, mice chose between licking the left or right tubes that deliver water with certain probabilities. Reward probabilities associated with the two actions changed at random time points without any signal to the animals. Consistent with previous studies, we found that mice exhibit significant and highly variable undermatching behavior. Moreover, we found that our entropy-based metrics could accurately predict deviation from matching on a block-by-block basis, explaining up to 50% and 72% of the variance individually and together. We next used differences between the predictions of existing RL models and observed data in terms of our new metrics to identify missing mechanisms crucial for matching behavior. Using this approach, we developed a new model that combines a slow RL component with a fast win-stay lose-switch (WSLS) component and uses expected uncertainty to arbitrate between the two. The new model improved fit of choice behavior and captured our metrics and undermatching more accurately, whereby revealing new mechanisms for adaptive behavior. Together, our results provide novel entropy-based metrics that can predict one of the most fundamental behavioral laws and can be used to refine reinforcement learning models and reveal neural mechanisms underlying adaptive behavior.

Disclosures: E. Trepka: None. **M. Spitmaan:** None. **B.A. Bari:** None. **J.Y. Cohen:** None. **A. Soltani:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.03

Topic: H.03. Decision Making

Support: ERC H2020-EU.1.1. #683209

Title: Rat trajectories in 2AFC task endorse parallel processing of motor planning and evidence accumulation

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Abstract: Standard decision-making models postulate that a response is triggered once the accumulated evidence reaches a decision bound. We have recently shown that rats in a free reaction time (RT) auditory discrimination task trigger their response before stimulus is processed or even presented in a considerable fraction of trials. However, accuracy in those expressed responses is influenced by stimulus strength, suggesting they are not guesses but sensory informed choices. A new model explained this phenomenon by proposing the parallel processing of response initiation and evidence accumulation processes [1]. Here we expand on this model by characterizing the fine dynamics of the decision making process from the precise response trajectories exhibited by our subjects.

We extracted subject's response orienting trajectories while performing the task and quantified the extent to which they are modulated by stimulus strength, expectations based on trial history and RT. Average trajectories showing the decision coordinate as a function of time exhibit a sigmoidal shape which we quantified by the time at which they crossed a decision threshold right before the side ports and the peak velocity with which they crossed.

Using catch trials with no stimulus we found that prior expectations modulated trajectories in absence of stimulus by reducing the crossing time of the decision thresholds and increasing the peak velocity. This prior effect decreased with RT.

In regular trials, stimulus strength also modulated average trajectories' peak velocity. The point in the trajectory where we could first detect the impact of the stimulus decreased with RT until it plateaued at 80 ms after movement onset, what constitutes an upper bound of the motor efferent latency. For express responses in which the initial trajectory was independent of the stimulus, the modulation onset increased by 40 ms, which set an upper bound of the sensory afferent latency. These results suggest that priors determine an a priori trajectory which is updated en route by the stimulus.

Finally, we explored trials in which expectations were incongruent with the presented stimulus and found a significant fraction of trajectories in which the initial choice was reversed. Several additional analyses indicated that these trajectories may represent changes of mind: their rate increased with prior-stimulus incongruency, decreased with RT and were mostly correcting reversals.

Together, our results illustrate how response trajectories reveal the timely superposition of two processes, prior and sensory evidence, which compete asynchronously to dictate the subject's choices.

1. Hernández-Navarro et al. Psyarxiv 2020

Disclosures: J. Pastor-Ciurana: None. **L. Hernández-Navarro:** None. **L. Bektic:** None. **D. Duque:** None. **A. Hyafil:** None. **J. de la Rocha:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.04

Topic: H.03. Decision Making

Support: BES-2011-049131
IJCI-2016-29358
SAF2015-70324-R
RTI2018-099750-B-I00
PSI2015-74644-JIN
RYC-2017-23231
ERC-2015-CoG - 683209 Priors

Title: Neural signatures of competing proactive and reactive processes during perceptual decisions in dorsomedial striatum

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Abstract: Standard models of perceptual decision-making postulate that responses are triggered in reaction to the stimulus, when the accumulated evidence reaches a decision threshold[1]. This excludes however informed responses that are generated proactively at a time independent of stimulus[2]. Here we find that, in a free reaction time (RT) auditory discrimination task in rats [Fig. 1a], the timing of the fastest responses does not depend on the stimulus [b], although the choices do [c]. This unveils the coexistence of reactive and proactive responses for all RTs, suggesting that choice selection and motor initiation, commonly viewed as serial processes, are decoupled in general. We capture this behavior by a novel model in which proactive and reactive responses are triggered when either of two competing processes, respectively Action Initiation (AI) or Evidence Accumulation (EA), reaches a bound [d-e]. In all responses, the choice is ultimately informed by the EA. To investigate the neural substrate of the AI, we performed population recordings in the dorsomedial striatum (DMS) of 4 rats (n= 652 single units). Preliminary analysis revealed that a large fraction of neurons (~40%) encoded choice, from response initiation to next trial response. Another subpopulation of neurons (~20%) displayed stimulus-independent ramping activity before response onset whose slope correlated with RT, suggestive of the AI ramping process [f]. Overall, these results fundamentally extend standard models of evidence accumulation in decision making by showing that proactive and reactive processes compete for the generation of responses, and that DMS may play an important role in generating the proactive AI process.

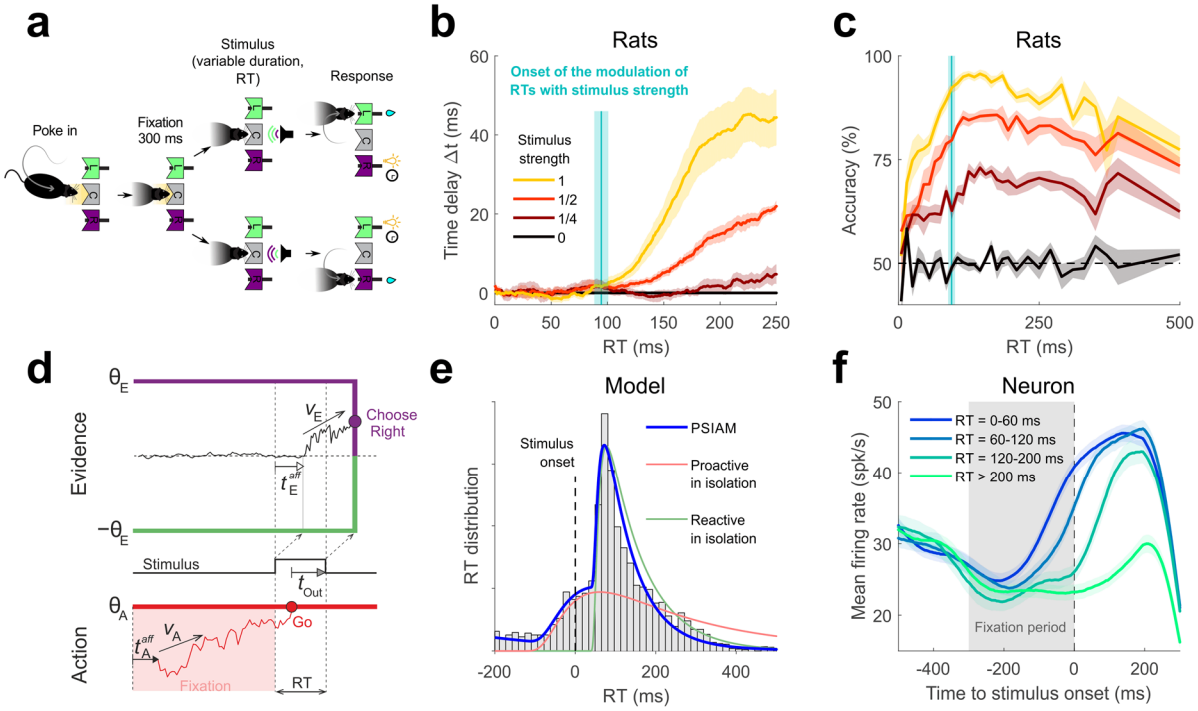

Figure 1. a, Task sketch. b, Delay between RT cumulative distributions, mean across 10 rats, shaded areas are s.e.m. c, Tachometric curves; legend as in b. d, Sketch of the model with an example proactive response. e, Model fit to RTs for an example rat. f, PSTHs by RT bin for an example neuron in DMS, shaded areas are s.e.m.

1 Gold et al. Annu. Rev. Neurosci (2007)
2 Haith et al. J. Neurosci (2016)

Disclosures: L. Hernández-Navarro: None. **A. Hermoso-Mendizabal:** None. **D. Duque:** None. **J. de la Rocha:** None. **A. Hyafil:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.05

Topic: H.03. Decision Making

Support: NIH Grant RO1-NS113104-01
KU PREP R25-GM078441

Title: Distinct Social Interactions Elicits Shifts in Functional Connectivity in the Social Decision-Making Network

Authors: *K. R. GOSSMAN, B. DYKSTRA, B. H. GARCIA, A. P. SWOPES, A. S. SMITH; Univ. of Kansas, Lawrence, KS

Abstract: Intimate relationships rely on an individual's ability to display affiliative behaviors toward a partner and rejection of potential partner. It is theorized that these context-appropriate decisions are processed through the Social Decision-Making Network (SDM). Specifically, the SDM hypothesis suggests that the expression of a given social behavior is reflected by the overall activity of a network of brain structures rather than activity of any single structure. While the SDM network was proposed on functional grounds, most of the supporting research is restricted to structural connections and behaviorally-associated regional activity. In the present study, we sought to assess the organization of regions in the SDM network using c-Fos, a neuronal marker for activity, to generate functional connectivity models during specific social interactions in a socially monogamous rodent, prairie voles (*Microtus ochrogaster*). Males from established male-female pairs were exposed to one of four resident interactions: no interaction (control), female partner, novel female, or novel male. Male voles displayed robust partner-directed affiliation and stranger-directed aggression. These social interactions increased levels of the immediate early gene, c-Fos, in select brain regions associated with the SDM network. Pearson's correlation matrices displayed distinct coactivity patterns between individual brain regions based on the social exposure. The correlations presented are exhibited as edges between regions within the network models. Hierarchical clustering was used to group regions with similar c-Fos activity during each social encounter. These clusters of regions form modules that make up separate interrelated units in the functional connectivity models. Our functional connectivity models display unique connectivity and organization based on the social encounter. Our partner functional connectivity model presents similarities to the SDM network, along with connections between regions associated with affiliative behaviors. However, both stranger female and male networks exhibit distinct architecture from one another and the SDM network. Stranger networks demonstrate potential interregional connections that may be associated with threat, aversion, and aggression. This highlights that functional connectivity maps of the SDM network differ during distinct social encounters, which may underlie the display of context-appropriate social behaviors.

Disclosures: K.R. Gossman: None. **B. Dykstra:** None. **B.H. Garcia:** None. **A.P. Swopes:** None. **A.S. Smith:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.06

Topic: H.03. Decision Making

Title: B-rats and v-rats: understanding biological rats through a comparison with virtual rats

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Abstract: Recent neuroscientific approaches suggest ways of generating hypotheses of neural computation in animals, guided by their task-performing models. First, both the animal and its model are trained on the same operant task. Next their performance and learning dynamics are compared. Finally, if they are found to be sufficiently similar, the decision-making module of the artificial agent can be used to generate hypotheses regarding the neural computations in the animal. However, the comparison between the biological and the artificial agents is not thoroughly investigated or standardized.
We analyzed a sound discrimination task in freely behaving rats. The biological data consists of 21,000 trials that spanned the learning process of one rat from its first introduction to the task and up to performance saturation. The virtual rat (V-Rat) is modeled as a point inside the arena, with constraints on its acceleration and turn speed. The V-rats decision-making is performed by an artificial neural network (4-layered multi-layer perceptron, 300 neurons in total) that emits an action based on the observed state. The V-rat was trained through reinforcement learning and converged to a near-optimal policy after few thousands of trials. We quantify V-Rat optimality along the learning process by comparing it with an optimal performer, an exact solution of the optimal control problem.
To compare the two agents, the state of each of them is described at any moment by a behavioral feature vector. Several consecutive feature vectors form a 'behavioral motif' that captures complex temporal dynamic in the agent behavior. We therefore compared corpuses of motives of the two agents. We found that the behavior of both agents is largely shaped by the task constraints. Moreover, we applied motif analysis to phenotype the behavior of individual V-Rats and quantified the population variation, explaining it by the unique realization of the exploration process and the initial network weights.

Disclosures: **A. Kazakov:** None. **I. Nelken:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.07

Topic: H.03. Decision Making

Support: NIH NIDA K01 DA036659
Behavioral and Neural Sciences Graduate Program Graduate Assistantship

Title: Monkey See, Monkey Choose: A Nonhuman Primate Model of Gaze Biases in Economic Choice

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Abstract: In economic decision making individuals must choose between items on the basis of their perceived value. For both human and nonhuman primates, these decisions are typically

carried while the locus of gaze is rapidly shifted between available options. Recent studies in humans have shown that these shifts in gaze actively bias choice. In particular, choices tend to be biased in favor of the items that are viewed first, last, or for the overall longest duration in a given trial. This suggests a possible role for gaze in the value computations that underlie decision making. To date, however, the neural mechanisms that subserve the relationship between gaze and choice are still largely unknown. A critical step towards identifying these mechanisms is the development of a suitable animal model of this behavior. To this end, we have created a novel value-based choice task for macaque monkeys that captures the essential features of the human paradigms in which gaze biases have been observed. Using this task, we observed gaze biases in the monkeys that were both qualitatively and quantitatively similar to those exhibited by humans. In addition, we found that these results are well explained using a computational framework previously used to describe gaze biases in humans—the first time this framework has been used to explore value-based choice in nonhuman primates. Together, these findings suggest that this novel task is ideally suited to study the neural mechanisms underlying the relationship between gaze and value-based choice.

Disclosures: S.M. Lupkin: None. **V.B. McGinty:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.08

Topic: H.03. Decision Making

Title: Inferential reasoning in monkeys

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Abstract: When entering a new restaurant, we use contextual cues to infer whether it is a sit-down or a fast food restaurant. Once this is determined, we can apply previously learned rules for each context to guide our behavior. To understand how the brain infers the current rule, we trained a monkey to perform a task with multiple latent rules. The monkey was trained on three different rules, each requiring to attend and respond to a single feature of a two-dimensional stimulus. Changes in the rule were not cued, forcing the animal to determine which rule was in effect on each block. The animal performed the task well, quickly switching between the rules. However, its performance was reduced for stimuli associated with incongruent responses across the rules. Moreover, the monkey's initial performance improved after a correct response to such an incongruent stimulus, but degraded if the stimulus was instead congruent. To understand the mechanism supporting behavior, we compared two classes of models: an incremental learner, relearning each rule from tabula rasa after a switch, and an inference model inferring the rule in effect through Bayesian update over known latent rules. Only the inference model captured the

key qualitative features of the data, in particular differences in performance and learning following stimuli that either had congruent or incongruent responses across the rules. Together, these results suggest monkeys can use inferential reasoning when determining how to behave.

Disclosures: F. Bouchacourt: None. **S. Tafazoli:** None. **M.G. Mattar:** None. **N. Daw:** None. **T. Buschman:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.09

Topic: H.03. Decision Making

Title: Expected uncertainty drives phasic and tonic exploration during probabilistic learning.

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Abstract: In changing environments, adaptive decision-making requires balancing when to choose familiar, known options with when to explore new, unknown options. This balancing act, known as the explore-exploit tradeoff, is critical to how we make choices that can maximize reward. Specifically, exploration supports optimal decision-making by reducing the uncertainty associated with certain choices. Exploration is often seen as phasic, where the decision to explore depends on peaks in uncertainty that signal when the benefit of exploring is greatest. However, exploration can also be tonic, occurring more regularly in time. While tonic exploration has been demonstrated in settings where uncertainty is limited to discrete, unexpected rule changes , it is unclear how tonic exploration relates to expected uncertainty from stochastic reward outcomes. Here, we used a Bayesian modeling approach to examine monkeys' choice behavior during a two-arm reversal learning bandit task (Costa et al., 2015) where the monkeys learned to reverse probabilistic reward associations under three different reward schedules: 60/40%, 70/30%, and 80/20%. We found that the monkeys' committed more spontaneous errors (i.e. lapses) during the reversal phase of the task in 60/40% blocks compared to 70/30% or 80/20% blocks. Across all three schedules ($F(2,96)=0.512$, $p=0.601$), however, we found that lapses were negatively correlated with how the monkeys' accuracy in detecting reversals in the reward contingencies. This suggests that a common process contributes to both phasic and tonic exploration (Ebitz et al. 2019). This relationship is not affected by the administration of haloperidol or levodopa ($F(2,16)=3.81$, $p=0.617$). Further, we find that tonic exploration is directed rather than random, as lapses can be accurately predicted by Bayesian estimates of unpredictability ($t(2)=7.66$, $p=0.0166$) and choice consistency ($t(2)=-7.33$, $p=0.0186$). Our results demonstrate how in dynamic learning environments tonic exploration complements phasic exploration to reduce uncertainty and maximize reward.

Disclosures: S.R. Bindas: None. **V.D. Costa:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.10

Topic: H.03. Decision Making

Title: Past reward biases decision process in auditory detection task

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Abstract: The perceptual decision depends not only on sensory input but also on internal factors (e.g., motivation, emotion, and past experience). For example, when we want to play the slot, we tend to choose the same slot machine that we won in the past. Previous studies in visually guided decision task reported that participants showed various reward-history-dependent bias such as win-stay, lose-switch, and lose-stay strategy, even when the optimal decision is independent of their internal history. This indicates that our decision process is largely modulated by reward-associated history. However, how reward history biases the decision process remained unclear. Here, we investigated how reward-associated history modulates behavioral strategies by using an auditory detection task, and we fitted participants' decision to bounded-accumulation decision models to reveal which component of the task and internal factors biases their decision process. In our task, the auditory stimuli were 2300-3000 ms duration sounds consisted of two streams (1000 or 500 Hz) of tone-bursts with background white noise. A 1000 and 500 Hz stream were synthesized by a 155 and 99 ms duration of the tone bursts and a 93 and 74 ms inter-tone interval, respectively. Participants were instructed to detect which of two auditory streams disappeared. In case that they correctly detected the change, they were randomly given 0, 1, or 5 points. They were also instructed to maximize their points and to respond as soon as possible. We found that previous reward, not current reward, significantly slowed reaction time depending on the size of the past trial reward. To obtain the interaction between the past trial reward and the decision process, we then fitted the drift diffusion model to behavioral data. We found that the model with reward-history-dependent drift bias better explained the data than the model without reward-associated history factor and the starting point bias model of reward history. These results indicate that reward history degrades the speed to reach detection threshold rather than shifts the starting point of the perceptual evidence accumulation, suggesting that the reward-associated history affect our decision process as suppressor of accumulating evidence.

Disclosures: H. Nagamura: None. **E. Sakaue:** None. **H. Onishi:** None. **M. Hishitani:** None. **S. Murai:** None. **Y. Osako:** None. **K.I. Kobayashi:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.11

Topic: H.03. Decision Making

Support: John Templeton Foundation Grant 61454
NIH Grant MH121093

Title: Habits can arise from model-based planning due to latent cause inference

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Abstract: Computational models have separately explored two distinct aspects of conditioning. First, findings that instrumental responses are sometimes sensitive-but sometimes insensitive-to reinforcer devaluation have been argued to reflect the use of two learning mechanisms, model-based vs. model-free (Daw et al. 2005). For instance, findings that overtrained animals habitually persist in responding for devalued reinforcers may reflect model-free learning, which is blind to post-training changes in outcome value.
Second, generalization between contexts in classical conditioning has been studied using latent cause inference (LCI) models (Gershman et al. 2010). These theories describe how animals can group experiences into different covert "causes", depending on their overlap, leading to differential generalization across them. For instance, if acquisition and extinction training are attributed to different causes, extinguished conditioned responses reappear when animals reinstate the acquisition cause.
We present a new model combining elements of both lines, to explore the consequences of LCI for instrumental conditioning. The model nests state-action-state model-based instrumental learning under a Dirichlet-process mixture model for trial clustering. Surprisingly, despite omitting model-free learning, this reproduces key aspects of habits: persistent instrumental responding in extinction for a devalued reinforcer, even when a consumption test verifies the efficacy of aversion conditioning. The model is inspired by and captures the patterns of results from recent experiments (Bouton et al. under review) showing that response sensitivity to reinforcer devaluation depends on the similarity between aversion conditioning and acquisition/test contexts. The model captures value-insensitive responding even using model-based evaluation within each cause due to failure to generalize aversion learning to the latent cause where the instrumental test is inferred to occur. Aversion conditioning experience generalizes more readily during the consumption test because of greater feature overlap (e.g. the presence of the outcome itself). Although these results do not rule out a contribution of model-free learning, they point to the importance of state inference in instrumental learning.

Disclosures: S.M. Allan: None. **N. Garrett:** None. **N.D. Daw:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.12

Topic: H.03. Decision Making

Support: NSF 1658303
NSF 1850849

Title: A macro-level perspective on evidence accumulation during decision making

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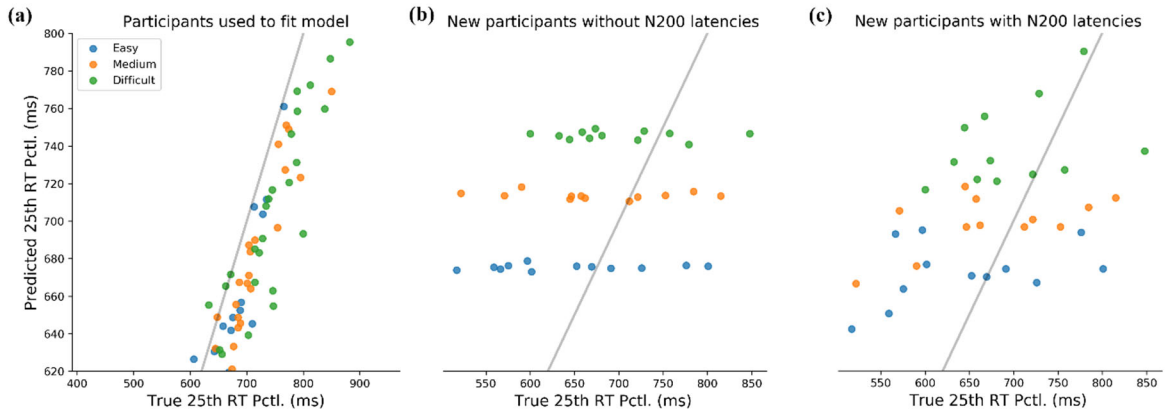
Abstract: Multiple brain areas have been shown to be involved in evidence accumulation during decision making tasks with data from electrophysiological recordings and inactivation studies in animals, including brains areas involved in both perceptual and motor processing. However the whole-brain computational process of decision making remains unclear. We show how simultaneous computational modeling of human behavior and macro-level recordings using human scalp EEG can reveal an individual's cognitive process of evidence accumulation, with measurable cognitive components that include attention, figure-ground segregation, perceptual categorization, motor preparation, and motor execution. In particular, we find that these cognitive components can be measured in a preregistered confirmatory study of 48 human participants. We show how hierarchical Bayesian methods can be used to understand and predict individuals' behavior and EEG potentials during decision making. For example, Hierarchical Neural Drift-Diffusion Models (HNDDMs) with embedded early visual event-related potentials (ERPs; in particular N200 latencies) can not only explain early (e.g. 25th percentile) response times (RTs) well (see Fig. 1a), but also predict, to a certain extent, early response times of completely new participants (see Fig. 1c) when only their ERPs are observed. Prediction of new participants' early RTs are greatly improved by N200 latencies because N200 latencies are confirmed to have a close relationship to non-decision times across experimental conditions (e.g. "Easy", "Medium" and "Difficult" in Fig. 1), replicating our previous study that suggests N200 latencies are a marker of figure-ground segregation and track the onset of evidence accumulation. The results of our work to date suggest a future of studying evidence accumulation using model-based cognitive neuroscience.



Disclosures: **M.D. Nunez:** None. **J. Vandekerckhove:** None. **R. Srinivasan:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.13

Topic: H.03. Decision Making

Title: A single neural circuit that captures divisive normalization, working memory, and winner-take-all choice

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Abstract: Purpose: Circuit models of decision-making utilize two motifs to capture decision-related neural activities. One motif dynamically captures divisively normalized value representation, including value adaptation (Louie et al., 2014; LoFaro et al., 2014; Zimmerman et al., 2018; Padoa-Schioppa, 2009; Cohen & Padoa-Schioppa, 2019). The other motif captures the dynamics of a form of working memory and winner-take-all (WTA) competition (Wang, 2002; Wong & Wang, 2006). However, both motifs are restricted in their explanatory power. Results from fixed-duration tasks exhibit a transition from representation to competition which appears to be under the top-down guidance of action signals (Roitman & Shadlen, 2002; Shadlen & Newsome, 2001). It remains unknown how a single circuit model can capture all of these features. Here we propose a hybrid model that unifies value normalization, working memory, and WTA competition, and test the model in capturing neural and behavioral data across different tasks. Methods: The hybrid model implements recurrent excitation and global inhibition drawn from existing models and incorporates a new element of local disinhibition. The model was implemented as a dynamical system capturing firing rates response to bottom-up signals of stimuli and top-down signals for action execution mediated through local disinhibition. We tested this model in capturing neural dynamics and behavior in a range of empirical paradigms: (1) a classic reaction-time task (Roitman & Shadlen, 2002), (2) fixed-duration tasks (Roitman & Shadlen, 2002; Louie et al., 2011), and (3) a multiple alternative choice task (Churchland et al., 2008). Results: We show that the hybrid model captures the dynamic of WTA competition in the reaction-time task, the phase transition in fixed-duration tasks, and the quantitative effects under multiple inputs. In addition, the model fits well to empirical distributions of reaction time and choice accuracy from the reaction-time task. Finally, the predicted effect of GABAergic manipulation distinguishes models with and without local disinhibition, providing neural and behavioral predictions for pharmacologic/optogenetic experiments. Conclusions: We find that a biologically-plausible decision circuit with recurrent excitation, global inhibition, and local disinhibition easily adapts to the neural dynamics from different paradigms. This model precisely captures empirical psychometric and chronometric data. These results suggest that the specific computations of divisive normalization and WTA competition, under the switch control of local disinhibition, is a core circuit motif in decision-making.

Disclosures: B. Shen: None. **K. Louie:** None. **P.W. Glimcher:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.14

Topic: H.03. Decision Making

Title: Discovering the correlation between personality determinants & risky choices

Authors: *Z.-Y. YAN[1], P. GLIMCHER[2];
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Abstract: A variety of personality determinants and emotional states have been linked to the individual differences of the risk preferences. However, most variables reported in the literature were investigated independently and tested without repeated measurements. In order to understand the interaction between these traits and states and risk preferences, we performed longitudinal examinations of emotional states and personality traits from the following domains: attitude toward uncertainty, impulsivity and the level of psychological distresses. All subjects were recruited in the US on CraigsList. We recruited a 50 subject "discovery cohort" and another 50 subjects as the "replication cohort". In the discovery cohort, our goal was to identify the magnitude of the measured traits and states and risk preferences. We employed a mobile smartphone-based experimental platform (Linkt) for gathering daily data from our participants. Each participant was tested with multiple instruments presented over a two-month window. Subjects completed instruments for about 5 minutes per day (one or two tasks and/or questionnaires). We gathered the following instruments at least once every week: a risky choice task (Levy et al. 2010), a delay discounting task (Kable & Glimcher, 2007), the self-report positive and negative affect (Kahneman et al. 2004). Over the course of the study, we gathered a total of 13 personality inventories. We found that our data collection system yielded a high level of compliance, 49 out of 53 subjects finished all instruments. Here we present data showing how the personality traits are correlated with willingness to take risks and its relationship with the parameters estimated with EU (Expected Utility) and ESVT (Expected Subjective Value Theory) models (Glimcher & Tymula, 2016). We found that the ratio of choosing risky option over safe option is positively correlated with the psychological distress as well as the willingness to take financial risk. Parameter governing reward expectation (M) in ESVT model is positively correlated with the willingness to take health and safety risk. M is also positively correlated with the self-reported expected benefit of the financial risk and negatively correlated with the self-reported perceived health and safety risk. Our findings demonstrate that our data collection system is effective in capturing the relationship between the variables. For the next step, we will be testing these effects with our replication cohort.

Disclosures: **P. Glimcher:** Other; PWG is an officer and stockholder in Datacubed Health..

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.15

Topic: H.03. Decision Making

Support: NARSAD Young Investigator Award to CMR

Title: Decision-making among individuals reporting a COVID-19 infection: Time, probability, and ambiguity preferences

Authors: *A. MELLIS[1], C. M. RAIO[2], A. VINA-ALBARRACIN[1], P. W. GLIMCHER[3], O. OLUFEKO[1];
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Abstract: Overview: The COVID-19 pandemic imposes a universal threat to individuals' well-being, with individual risks of infection varying based on medical, social, and economic determinants of health. One question that has yet to examined is whether individuals' decision-making may also determine, or be influenced by, infection risk. Based on the previous literature linking economic choice patterns to health-related and clinical outcomes, here we directly test whether preferences for risk and ambiguity, as well as impulsive choice, differ in individuals as a function of COVID-19 infection.
Methods: Between April 8 and July 22, 2020, a total of 232 participants consented to participate in "Our Covid Story" (www.datacubed.com/ourcovidstory). Participants were recruited via Facebook advertising and e-mail listservs, focused on the New York City area. Participation involved downloading a mobile application for administration of tasks and surveys, and completing 5-10 minutes of a rotating battery of assessments. These assessments included a complete demographic battery, self-report instruments, and gamified decision-making tasks, which included the 27-item Monetary Choice Questionnaire to assess temporal discount rate, and an abbreviated (35-item) version of the Levy task which distinguishes between preferences in risk and ambiguity.
Results: Overall, 6% of individuals reported a likely or confirmed case of COVID-19. To assess decision-making, we calculated the proportion of immediate choices in the Monetary Choice Questionnaire to determine degree of impulsive choice; similarly, we calculated percent of lottery choices in the risk and ambiguity trials of the Levy task. In cross-sectional analyses, we observed that individuals who reported a positive history of COVID-19 made fewer risky lottery choices ($t=-2.81$, $p = 0.01$) and made fewer ambiguous lottery choices ($t = -2.22$, $p = 0.04$), suggesting lower risk and ambiguity tolerance in participants reporting COVID-19 infections. We additionally observed a small difference in the proportion of impulsive choices between these groups, with those reporting a history of COVID infection showing higher levels of impulsivity, although this group difference did not reach statistical significance ($t=1.23$, $p = 0.26$) in our initial sample.
Discussion: Our longitudinal data collection is ongoing, to determine whether differences in

decision-making between those who report a history of COVID and those who do not are cause or consequence of the disease. Our preliminary data support the application of econometric approaches to understanding decision-making in COVID.

Disclosures: **A. Mellis:** None. **C.M. Raio:** None. **P.W. Glimcher:** A. Employment/Salary (full or part-time):; Datacubed Health. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Datacubed Health. **A. Vina-Albarracin:** None. **O. Olufeko:** None.

Digital Abstract Session

P306. Decision Making and Computational Models

Program #/Poster #: P306.16

Topic: H.03. Decision Making

Support: R01MH119511

Title: Information seeking on the horizons task does not predict anxious symptomatology

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Abstract: The explore-exploit dilemma, or the tradeoff between choosing a known reward (exploitation) versus a lesser-known option which might be better (exploration), is a ubiquitous problem in decision making. Excessive information seeking, or exploratory behavior to minimize the uncertainty of unknown options, is a feature of anxiety disorders (Kobori & Salkovskis, 2013). The horizons task (Wilson et al. 2014) is a popular task for measuring exploratory and information-seeking behavior, and has recently been used to identify over-exploitation in psychotic patients (Waltz et al. 2020). The horizons task has not, however, been evaluated as a tool for measuring information seeking symptoms in anxious individuals. We recruited 100 participants from Amazon Mechanical Turk to complete an online version of the horizons task and to complete several self-report symptom measures. Anxiety symptoms were measured with the Penn State Worry Questionnaire (PSWQ), and beliefs and attitudes related to information seeking were measured with the Intolerance of Uncertainty scale (IUS) and the Need for Closure scale (NCS). Information seeking behavior on the horizons task was measured per participant using hierarchical Bayesian modeling. We then correlated each participant's model parameter with their respective total scores on the self-report scales.
We confirmed that behavior on the online version of the horizons task is similar to previously published studies in which participants were tested in person. Contrary to our hypotheses, however, we found no evidence of a relationship between information seeking behavior on the task and anxiety symptoms (condition 1: $r = -0.091$, $p = 0.431$; condition 2: $r = -0.036$, $p = 0.756$) or the information seeking scales (IUS: condition 1: $r = -0.060$, $p=0.602$; condition 2: $r = 0.050$, $p = 0.663$; NCS: condition 1: $r = -0.051$, $p = 0.655$; condition 2: $r = 0.056$, $p = 0.629$). Our results suggest behavior on the horizons task is not predictive of self-report beliefs and

attitudes towards anxious information seeking. We suspect this may reflect design features of the task that reduce the instrumental utility of information seeking behaviors. We conclude by proposing several modifications to the task that may improve its utility as a measure of information seeking behavior in anxiety.

Disclosures: I. Zaller: None. **S. Zorowitz:** None. **Y. Niv:** None.

Digital Abstract Session

P307. Orbitofrontal Cortex: Cellular Mechanisms of Decision-Making

Program #/Poster #: P307.01

Topic: H.03. Decision Making

Support: NIDA K08 DA039351
Pew Biomedical Scholars Program
Brain and Behavior Research Foundation NARSAD Young Investigator Award

Title: Decision making in the context of multi-attribute options

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Abstract: When making a decision, there may not be an obvious best choice. Often decisions are made between options that have multiple features, or attributes, that must be considered and integrated before deciding. For example, when deciding between cars to purchase, one might factor the price, miles per gallon, and acceleration into one's selection. The OFC plays an important role in representing associations between stimuli and values, and also may represent the overall option value or that of the stimulus attributes. However, it remains unknown whether options are necessarily evaluated on the basis only of an integrated value, as suboptimal decision-making effects like the attraction effect point to the possibility of within-attribute comparison. In order to investigate how multi-attribute options are represented in neural activity, we trained two rhesus macaques on a multi-attribute decision making task, in which two simultaneously-presented options were represented by stimuli reflecting the sweetness of that option's sucrose reward, and the probability of receiving that reward. These composite stimuli represented information about the attributes of the options with separate bars that either increased *or* decreased with increasing attribute value, allowing us to investigate both free-viewing gaze behavior and changes in choice behavior due to perturbations in attribute presentation. We found that when shared attributes were congruous with each other across option (e.g., both reward bars increased in size with increasing sweetness), choice behavior improved from baseline. However, when shared attributes were not congruous with each other across option (e.g., reward bar A increased in size with increasing sweetness, while reward bar B decreased), choice of the better option became suboptimal, implying a role for within-attribute comparison in the choice process. We will record from OFC using acute electrodes and multi-contact linear probes to determine how complex stimuli are represented in neural code and how

regions related to oculomotor processing and visual attention might contribute to the evaluation of multi-attribute options.

Disclosures: A. Perkins: None. **E.L. Rich:** None.

Digital Abstract Session

P307. Orbitofrontal Cortex: Cellular Mechanisms of Decision-Making

Program #/Poster #: P307.02

Topic: H.03. Decision Making

Support: NSF DGE 1752814

Title: Nonhuman primates use hidden state inference during reversal learning

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Abstract: Understanding the world requires making inferences about the hidden causes, or belief states, generating our observations. During value-based decision making, converging evidence from rodent, human, and computational models suggests that the orbitofrontal cortex (OFC) represents these hidden belief states by contextualizing relevant information. The hippocampus (HC) is also implicated in this process by representing a cognitive map, a structural organization of relational information, of the current task environment. Given their strong anatomical and functional connections, OFC and HC likely work together to drive inferential learning and decision-making behavior. However, the neural mechanisms of these processes remain unknown. Here, we trained two male monkeys (*Macaca mulatta*, 6.5 years old, 10.5 and 10.1 kg) on a probabilistic reversal learning task where state inference is required for optimal performance. We analyzed the effect of reward history and task state on subsequent choice behavior, and observed that subjects inferred hidden states to rapidly flip choice preferences at reversal points, even for unexperienced contingencies. This pattern of behavior evolved over time. Early in training, choices were largely influenced by reward history alone. After several training sessions, subjects were able to detect and adapt to state changes, informing decisions on trials where reward contingencies had not yet been directly experienced. These results suggest that subjects acquired a working knowledge of task structure; a cognitive map. Currently, we are simultaneously recording neuronal activity from OFC and HC to investigate how this cognitive map is represented and exploited for behavioral inference.

Disclosures: C.F. Ford: None. **J.D. Wallis:** None.

Digital Abstract Session

P307. Orbitofrontal Cortex: Cellular Mechanisms of Decision-Making

Program #/Poster #: P307.03

Topic: H.03. Decision Making

Support: 1 R56 MH119150-01
F31 MH122121-01A1

Title: Circular reasoning: circHomer1 is a molecular modulator of cortical synchrony during visual reversal learning via Homer1b synaptic localization.

Authors: *A. ZIMMERMAN, A. HAFEZ, N. MELLIOS, J. BRIGMAN;
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Abstract: Circular RNAs (circRNAs) are an emerging class of long non-coding RNAs with complex regulatory potential. Some have been shown to impact gene expression and function by sponging microRNAs or RNA binding proteins, while others affect transcription and alternative splicing. However, the vast majority of circRNAs have not been characterized and little is known about their function in the brain despite their abundance in cortical tissue. Recent work has focused on circRNAs as biomarkers for disease and therapeutics due to their increased stability over linear RNA since their biogenesis through backsplicing renders them resistant to exonuclease-mediated RNA degradation. CircRNAs have recently been shown to accumulate in the brain with aging and to be differentially regulated in neurodegenerative and psychiatric disorders including Alzheimer's Disease (AD) and schizophrenia (SCZ). While plasticity-related protein-coding genes have been associated with cognitive dysfunction in these disorders, little is known about the potential for circRNAs in regulating synaptic activity and cognitive ability. A recent paper identified *circHomer1* as a candidate molecule in AD in its correlation with duration and severity of illness. The linear transcript of *Homer1* produces multiple proteins involved in synaptic function and has been implicated in psychiatric disorders including SCZ and major depression. We have previously shown that *circHomer1* is reduced in postmortem orbitofrontal cortex (OFC) samples from patients with SCZ and bipolar disorder (BD) and have shown that reduction of *circHomer1* in rodent OFC is sufficient to produce an OFC-dependent behavioral deficit on a touchscreen visual reversal task. Further, expression level of *circHomer1* is inversely correlated with reversal behavior in a trial type-dependent manner. Our current work demonstrates *circHomer1* is capable of controlling other molecular correlates of behavior including the synaptic localization of *Homer1* linear isoforms, *Homer1a* and *b*. We use long-term *in vivo* electrophysiological recordings from bilateral depth microelectrodes during visual discrimination and reversal learning in twenty adult male C57BL/6J mice to examine changes in excitatory neuronal activity that underlie OFC dysfunction following loss of *circHomer1*. Our results indicate changes in OFC local field activity during impaired reversal stages that closely correlate with those seen in human patients with SCZ. This work is the first to demonstrate a psychiatric-related circRNA is capable of modulating synaptic regulatory processes involved in learning as well as neuronal activity underlying cognitive impairment.

Disclosures: **A. Zimmerman:** None. **A. Hafez:** None. **N. Mellios:** None. **J. Brigman:** None.

Digital Abstract Session

P307. Orbitofrontal Cortex: Cellular Mechanisms of Decision-Making

Program #/Poster #: P307.04

Topic: H.03. Decision Making

Support: NIH Grant R01-DA032758
NIH Grant R01-MH104494
McDonnell Center for Systems Neuroscience pre-doctoral fellowship

Title: Economic decisions under simultaneous or sequential offers rely on the same neural circuit

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Abstract: Economic choices involve assigning values to each option; a decision is then made by comparing values. In a series of studies, monkeys chose between two juices offered in variable amounts. Analyses of spiking activity in orbitofrontal cortex (OFC) revealed that different groups of neurons encode the value of individual options (*offer value*), the binary choice outcome (*chosen juice*) and the *chosen value*. These variables capture both the input and the output of the choice process, suggesting that these groups of cells constitute the building blocks of a decision circuit. A series of experimental and theoretical results support this hypothesis (Padoa-Schioppa and Conen, 2017). Importantly, current notions emerge almost exclusively from studies in which two offers were presented simultaneously. Yet, in most daily circumstances, options available for choice appear or are examined in sequence. Thus an open question is whether choices under sequential or simultaneous offers take place in the same neural circuit. Thus in a recent study we let monkeys choose between different juices offered sequentially. (Ballesta and Padoa-Schioppa, 2019). An analysis of OFC responses across time windows revealed the presence of different cell groups seemingly analogous to those previously found under simultaneous offers. In particular, different groups of neurons encoded individual offer values, the juice identity or the chosen value, with positive or negative slope. The present study was designed to assess whether the cell groups identified in the two choice modalities - under simultaneous or sequential offers - may be identified with each other. Two monkeys chose between different juices offered in variable amounts, and trials with the two task modalities were pseudo-randomly interleaved. We recorded the activity of 1526 from central OFC. Trials from the two choice modalities were analyzed separately, and each neuron was classified in relation to each task using the procedures developed in previous studies. The results obtained for the population were then combined in a 9x9 contingency table (4 variables x 2 signs + untuned cells), which we examined using analyses for categorical data. The correspondence between the two classifications was remarkable. Coincident classification was captured by diagonal entries. We found that 8 of 9 diagonal entries were significantly above chance ($p<0.001$; Fisher's exact test); conversely 70/72 off-diagonal entries were at or significantly below chance ($p<0.001$;

Fisher's exact test). These results indicate that economic decisions under sequential or simultaneous offers rely on the same neural circuit.

Disclosures: W. Shi: None. **C. Padoa-Schioppa:** None. **S. Ballesta:** None.

Digital Abstract Session

P307. Orbitofrontal Cortex: Cellular Mechanisms of Decision-Making

Program #/Poster #: P307.05

Topic: H.03. Decision Making

Title: The medial orbitofrontal cortex modulates of evoked spiking in nucleus accumbens in an age-dependent manner

Authors: *M. K. LOH, J. A. ROSENKRANZ;
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Abstract: Appropriate decision-making is evolutionarily important for survival, but is immature in adolescents, who characteristically are risk-takers and reward-centric. The circuits that underlie age-dependent differences in decision-making are not well understood but can further our understanding of high-risk behaviors. The nucleus accumbens (NAc) integrates excitatory inputs from both cortical and limbic structures, which are communicated to downstream motor regions. As inputs from cortical and limbic structures carry goal- and reward-related information, the summation of these signals at the level of the NAc likely combines information about rewards and risks to direct decision-making. Prior experiments suggest that medial orbitofrontal cortex (MO) to NAc inputs are key in integrating risk-based information, as MO-NAc interactions permit risk assessment behaviors. Along these lines, adolescents do not exhibit risk assessment behaviors as frequently as adults, suggesting that MO to NAc inputs may drive age-dependent risk-assessment differences. Here, we used single-unit electrophysiology to identify how MO stimulation modifies reward-seeking pathways, specifically the basolateral amygdala (BLA)-NAc circuit, in both adult and adolescent rats. We hypothesized that MO stimulation diminishes BLA-NAc interactions and that the effect is more robust in adults than in adolescents. We delivered single pulses at increasing interstimulus intervals or frequency-varying trains to the MO and recorded MO's effect on BLA-evoked NAc spike latency and probability. While single-pulse MO stimulation did not affect the strength of BLA-NAc interactions, MO stimulation reduced BLA-evoked NAc spike latencies in both ages. Further, MO train stimulation produced frequency-dependent effect on BLA-evoked NAc spike probability in adults. MO train stimulation delivered at 10 Hz facilitated BLA-evoked NAc spike probability but diminished the interactions at 40 and 60 Hz. These opposing effects by various MO train stimulation frequencies may reflect biases in optimal decision-making, where a subject is inclined towards large-reward/high risk or small-reward/low-risk choices based on previous outcomes and overall long-term benefit. Unlike adults, the adolescent MO failed to produce the bimodal effects on BLA-NAc interactions; MO train stimulation diminished BLA-NAc spiking at all tested frequencies. These results may reflect adolescent suboptimal decision-making. While the adult MO can

regulate the strength of reward-related circuits, the adolescent MO is unable to produce the same bimodal effect to perhaps appropriately guide decision-making.

Disclosures: **M.K. Loh:** None. **J.A. Rosenkranz:** None.

Digital Abstract Session

P307. Orbitofrontal Cortex: Cellular Mechanisms of Decision-Making

Program #/Poster #: P307.06

Topic: H.03. Decision Making

Support: NIAAA Grant R00AA021780
NIAAA Grant R01AA026077
NSF Grant DGE-1650112
NIAAA Grant F31AA027439
Whitehall Foundation Inc

Title: Subjective Experience and Recruitment of Orbitofrontal Cortex Cell Types during Decision-making

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Abstract: Subjective experience is inherently necessary for adaptive decision-making. However, how subjective experience affects decision-making computations and their neural correlates is not clear. One candidate region is the orbitofrontal cortex (OFC) as it is implicated in decision-making based on prior subjective experience, but little is known about how these computations are instantiated by specific populations in OFC or what influence prior subjective experience may have on that activity. Here, we used an action differentiation task in which mice learn to hold down a lever-press for a minimum duration for food. Our task is self-paced and uncued, such that prior self-initiated actions and their associated consequences provide crucial feedback for future successful performance. Using a linear mixed-effects (LME) modeling approach, we found that current lever press duration depends on prior subjective experience, including a recent and a longer history of past press durations, time passed, and reward checking. Using a genetically engineered caspase-3 Cre-dependent virus to lesion OFC projection neurons in vivo, we found that OFC lesions disrupt the contribution of prior subjective experience to the current lever press. To examine OFC activity during decision-making based on subjective experience, we used virally mediated, genetically encoded fluorescent calcium indicators as proxies for neuronal activity. Activity correlates during action differentiation contrasted between genetically identified OFC populations; for example, excitatory projections decreased, and inhibitory interneurons increased, their calcium activity as animals held down the lever. To investigate whether OFC activity reflects or relies on prior subjective experience, we used LMEs to predict calcium activity across decision-making epochs. Preliminary analyses revealed a predictive relationship between prior subjective experience and population activity correlates in excitatory

projection neurons. Overall, our results suggest that OFC populations have a distinct role in executing decision-making actions that use prior subjective experience.

Disclosures: C. Cazares: None. **D.C. Schreiner:** None. **C.M. Gremel:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.01

Topic: H.03. Decision Making

Support: CIHR project grant (PJT-162312)

Title: Sex differences in preference for uncertainty following chronic chemogenetic excitation of dopamine neurons in the substantia nigra

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Abstract: Parkinson's disease (PD) is characterized by a loss of dopamine neurons in the substantia nigra (SNc) that project to the dorsal striatum, resulting in the hallmark motor symptoms of PD. Dopamine replacement therapies (DRTs) are able to treat these motor symptoms, but they also cause impulse control disorders (ICDs) and gambling disorder in a significant minority of patients. The "overdose" hypothesis suggests that DRTs replenish the lost dopamine in the deteriorated dorsal striatum, producing an improvement in motor symptoms. Meanwhile, the spared mesolimbic reward system is inundated with dopamine, resulting in the development of ICDs or gambling disorder. However, recent studies suggest that the nigrostriatal, rather than the mesolimbic, pathway is involved in the development of gambling disorder. We therefore chronically activated the nigrostriatal pathway by using designer receptors exclusively activated by designer drugs (DREADDS) and measured preference for uncertainty with the rodent Betting Task (rBT), which we have previously shown to be a valid approximation for gambling disorder. The rBT begins with the wager size at play being indicated by the number of holes illuminated at the back of the chamber, corresponding to the number of sugar pellets that can be won. Once the rat nose-pokes in each illuminated hole to extinguish the light, two levers extend, allowing the rat to select either the uncertain lever that delivers double the wager size or nothing at 50:50 odds or the safe lever that guarantees the delivery of the wager size at play. In our first experiment, the excitatory adeno-associated virus AAV-hSyn-DIO-hM3D(Gq)-mCherry was infused into the SNc of 32 female transgenic Long Evans rats (16 TH:cre positive, 16 TH:cre negative). Once trained on the rBT, the rats were administered 1 mg/kg of clozapine N-oxide via intraperitoneal injection twice daily, once 30 minutes prior to rBT testing and again at least 7 hours after the first injection to reflect the chronic administration of DRTs. After 4 weeks, activation of the dopamine neurons in the SNc increased preference for uncertainty on the rBT in wager-sensitive female rats, mimicking the effect of the DRT

ropinirole on the rBT. Following the same procedure, we repeated this experiment with 32 male rats (16 TH:cre positive, 16 TH:cre negative), and there was no significant effect on decision making after 4 weeks of chronically activating the SNc. The findings from these studies suggest that the nigrostriatal pathway may contribute to the development of both iatrogenic and idiopathic gambling disorder in females. The activation of this pathway may also underlie the development of DRT-induced ICDs as well.

Disclosures: **B. Russell:** None. **M. Tremblay:** None. **M. Silveira:** None. **K. Hrelja:** None. **T. Hynes:** None. **C. Winstanley:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.02

Topic: H.03. Decision Making

Support: NIH Grant R01MH095953
NIH Grant R01MH101207
NIH Grant R01MH110404
NIH Grant R01NS108740

Title: Distinct temporal difference error signals in dopamine axons in three regions of the striatum in a decision-making task

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Abstract: It has been postulated that dopamine neurons send reward prediction error (RPE) signals to their target areas to learn reward predictions and to reinforce actions. However, how different brain areas, which have distinct functions, learn from dopamine signals is not fully understood. Here we compared dopamine axon Ca^{2+} activities in the ventral, dorsomedial and dorsolateral parts of the striatum (VS, DMS, and DLS, respectively), while mice performed in a decision task. Specifically, mice were trained in an odor discrimination task with odor mixtures while the reward amounts were biased between the left and right reward ports (BIG side, big or medium rewards; SMALL side, medium or small reward; one of the two sizes was chosen randomly each trial). We found that dopamine axon activities in all three areas were modulated by the amount of received reward as well as the expected reward size both at the onset of cue (big reward predicting cue vs. small reward predicting cue, $p=5.0\times10^{-6}$, t-test, n=22) and reward (big vs. medium water with expectation big, $p=1.2\times10^{-11}$; and medium vs. small water with expectation small, $p=3.8\times10^{-9}$; and medium water with expectation big vs. expectation small, $p=3.9\times10^{-4}$, t-test, n=22), consistent with RPE. However, the overall activity level of reward responses was different between the three regions (p=0.002, ANOVA, n=9, 7, and 6 for VS,

DMS, and DLS). Specifically, reward responses in DLS was positively shifted (VS vs. DLS, p=0.005, t-test, n=9 and 6, respectively); the axonal activity was not inhibited even when the outcome was worse than expected although it was still modulated by reward amounts and expectation. The lack of inhibitory signals in DLS suggests diminished weakening of reward prediction or reinforced actions. On the other hand, reward responses in DMS were shifted negatively (DMS vs. DLS, p=0.003, t-test, n=7, 6, respectively), resulting in large negative dopamine signals even when the outcome was only slightly worse than expected. This suggests a relatively greater sensitivity of DMS to negative RPEs, and a greater requirement to re-learn reward prediction or rewarding actions. These biased dopamine RPE responses provide specific constraints on the behaviors learned through dopamine-mediated reinforcement in these striatal regions.

Disclosures: I. Tsutsui-Kimura: None. **H. Matsumoto:** None. **K. Akiti:** None. **M. Yamada:** None. **N. Uchida:** None. **M. Watabe-Uchida:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.03

Topic: H.03. Decision Making

Support: ERC-2015-CoG-683209_PRIORS
MINECO SAF2015-70324-R
MINECO IJCI-2016-29358
AGAUR 2017-BP-00305

Title: Neuronal responses track transition evidence in the rat dorsomedial striatum

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Abstract: To make an adequate perceptual decision, animals need to evaluate not only the current sensory information but also past experiences; indeed, rats performing a perceptual categorization task develop different history choice biases such as the win-stay, the lose-switch, and the transition bias -a tendency to repeat or alternate the previous response (Hermoso-Mendizabal et al., 2020). Previous reports have suggested ventro- and dorso-lateral striatum may be involved in lose-switch responses (Skelin et al., 2014; Thapa et al., 2018), while dorsomedial striatum (DMS) has been shown to encode reward prediction error (Roesch et al., 2009; Stalnaker et al., 2012) and being related with win-stay dynamics (Sakamoto and Okaichi 2001). However, little is known about the possibility that DMS encodes more complex rules such as the transition bias. To investigate if DMS is able to keep track of the sequence of transitions, we first built a theoretical model with a linear-exponential filtering of past transitions. We used this model to obtain an objective measure of the transition evidence (zT), which then will be

correlated with neuronal activity. Next, we recorded from 840 neurons in the DMS of 5 rats while they were performing an auditory two-alternative forced-choice (2AFC) categorization task. As the probability of having the reward on the same side as the previous trial was adjusted to generate serial correlations, the resulting repetitive and alternating blocks could guide the responses of the rats according to the variable zT. We found that most DMS neurons carried task relevant information such as previous choices and outcomes. Moreover, roughly 20% of the neurons recorded in DMS were encoding for the variable zT, thus keeping track of the transition bias. Preliminary data suggests that the activity of these zT neurons -in combination with that of choice neurons- is correlated with animals' choices on a trial-by-trial basis. Finally, to determine if DMS has a causal role encoding the transition bias, we performed bilateral lesions with ibotenic acid to permanently inactivate DMS (n=6). Such inactivation significantly diminished the transition bias, suggesting a key role of DMS in the generation and maintenance of complex expectation biases. Our results suggest that DMS has the capability to encode complex transitions that go beyond the classical model-free reward prediction errors neurons, which only allow for the generation of first-order biases such as the win-stay. Our experiments suggest that DMS has a crucial role in the computation and generation of expectation biases and the potential capability of coding for abstract sequential rules.

Disclosures: D. Duque: None. **A. Hermoso-Mendizabal:** None. **A. Hyafil:** None. **L. Bektic:** None. **M. Molano-Mazon:** None. **J. de la Rocha:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.04

Topic: H.03. Decision Making

Support: UNM Grand Challenge

Title: Neural correlates of explore-exploit decision-making in alcohol use disorder

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Abstract: One crucial component of pathological decision-making in addiction is the explore-exploit tradeoff, wherein individuals choose between exploring options with uncertain outcomes or exploiting options with familiar outcomes. The multi-armed bandit task captures this tradeoff and can dynamically model behavior in response to changing stimulus probabilities. A small number of studies have assayed explore-exploit processes as a potential marker of pathological decision processes in individuals with addiction, but few studies have used drug cues and none have measured task-related neural activity. The present study aims to elucidate neural correlates of disordered decision-making in AUD through EEG recording during bandit task performance.

Individuals with AUD (n = 28) and healthy age and gender-matched controls (n = 16 , data collection ongoing) completed a three-armed bandit task with images of alcoholic beverages and non-alcoholic beverages. The task presents three stimuli for participants to choose from that have particular probabilities of yielding a reward (a display of a green '+1' or a red '~'). A novel stimulus replaces one of these stimuli every 5 to 9 trials with a new reward probability. When individuals continue to choose particular stimuli despite the introduction of new stimuli, they're using an exploit strategy. When individuals choose the novel option in order to earn potentially greater reward, they're using an explore strategy. The BONUS value is a computationally derived parameter which reflects trial-to-trial changes in explore behavior and preliminary results show that it is augmented for alcohol cues in AUD when compared to controls, $t(17)=-2.514$, $p=0.022$. Preliminary time-frequency analysis shows that theta band activity in the frontal cortex scales with surveyed AUD severity when contrasting novel and best choices for alcohol cues, $F(1,16)=4.39$, $p=0.052$. Once data collection of controls has finished, separate multiple linear regression analyses will be used to assess the relationship between frontal theta activity, the P3a component, and computational predictors of explore-exploit behavior between AUD and healthy controls. Both neural responses could reflect an increased recruitment of frontal cortex while making dynamic decisions in response to alcohol imagery. These findings could contribute to the development of biomarkers for AUD that are sensitive to treatment-induced change. Moreover, measurement of EEG response in the present study will lead to a better understanding of the brain dynamics that underlie fundamental cognitive processes and how they're altered in substance use pathology.

Disclosures: E.M. Campbell: None. **V.D. Costa:** None. **J.F. Cavanagh:** None. **G. Singh:** None. **J. Hogeveen:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.05

Topic: H.03. Decision Making

Support: ERC-2015-CoG-683209_PRIORS
SAF2015-70324-R

Title: A causal role for the dorsal striatum in task-adaptive expectation-based choice biases

Authors: C. SINDREU, Y. FUENTEALBA, R. MARIN, L. JIMENEZ, D. DUQUE, ***J. DE LA ROCHA**;
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Abstract: Learned expectations can influence perceptual decisions, but the underlying neural mechanisms have yet to be specified. To promote the use of expectations in adult male rats, we used a two-alternative auditory discrimination task with serial correlations in the stimulus sequence in the form of repeating and alternating trial blocks. Recent work showed that rats

leverage on these correlations to estimate the probability that the previous rewarded side is repeated and bias their choices accordingly, particularly when stimulus evidence is equivocal. A generative model that summed up the stimulus evidence, and the outcome-dependent running estimate of the first- and second-order statistics of the sequence was able to accurately predict such flexible behavior. One brain region that may support the repeating bias is the associative striatum (i.e. dorso-medial division; DMS), as it encodes the value of the different options. However, interventions with high-temporal (millisecond) resolution and a trial-by-trial estimate of the different biases are both required to disentangle to roles of the DMS in expectation-guided choices. Thus, we virally expressed the chloride-conducting opsin stGtACR2 in striatal projection neurons, and implanted fiberoptic cannulae to inhibit their somata. Unilateral illumination during the whole trial generated a strong ipsilateral bias, confirming the role of the DMS in choice selection and the effectiveness of the method. We then hypothesized that silencing the DMS during the inter-trial interval (ITI), i.e. just before stimulus onset, would ablate the repetition bias. We obtained a real-time estimate of the repeating bias and used it to perform closed-loop DMS inhibition while rats performed the task. Bilateral photostimulation occurred in half the trials with a high bias (15% of total trials). Light-on trials showed reduced repetition bias compared with light-off trials, as evidenced by a marked decrease in the separation of the psychometric curves obtained in each of the blocks; no such effect was observed in controls. Moreover, the impact of ITI inhibition on performance depended on the congruency of the trial with the stimulus sequence. We conclude that the dorsal striatum is important to maintain expectation priors that inform perceptual decisions.

Disclosures: **C. Sindreu:** None. **Y. Fuentealba:** None. **R. Marin:** None. **L. Jimenez:** None. **D. Duque:** None. **J. de la Rocha:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.06

Topic: H.03. Decision Making

Support: CONACyT, Grant PN2463
UNAM, Grants IN205417, IN214520
CONACyT fellowships 614528, 858896

Title: Orbitofrontal-accumbens pathway mediates choice behavior guided by an aversive taste memory

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Abstract: The ability to choose what to ingest based on previous experiences is critical for survival. Although much is known about the brain mechanisms that underlie associations with

aversive taste stimuli, little is known about how these stored associations guide choice behavior. By testing aversive memory retrieval during choice versus no-choice conditioned taste aversion (CTA), we previously showed that activity in the lateral orbitofrontal cortex (lOFC) is recruited and necessary during choice behavior guided by an aversive taste memory (Ramirez-Lugo et al., 2016). But which brain structure(s) use(s) such lOFC-mediated choice information to select the appropriate action to execute? The striatum, a brain structure involved in the execution of selected actions, receives strong projections from OFC. Here, we combined immunohistochemical and pharmacological tools with taste-guided tasks to investigate the role of the striatum and its functional connectivity with OFC during choice behavior. c-Fos immunolabeling revealed that ventral, but not dorsal, striatum activity increased in choice CTA compared to no-choice CTA. Interestingly, within the ventral striatum, we identified specific coordinates in the rostro-caudal axis of the nucleus accumbens (NAc) that were highly recruited during choice CTA, compared to no-choice CTA. We used these coordinates to target pharmacological inactivations to determine whether core and shell regions of the NAc were necessary for choice behavior guided by an aversive memory. We found that core, but not shell, inactivations affected choice CTA, as compared to no-choice CTA. Consistent with the notion of opposing roles of NAc along the rostro-caudal axis in motivated behaviors, we found that rostral core inactivations impaired choice CTA, whereas caudal core inactivations facilitated it, as compared to no-choice CTA. Finally, using a pharmacological disconnection strategy, we found that contralateral, but not ipsilateral, inactivations of lOFC and rostral (but not caudal) core impaired choice CTA in contrast to no-choice CTA. Together, our findings reveal that connectivity between lOFC and the rostral region of the NAc core is crucial for animals to make the appropriate decision guided by a previous aversive taste experience.

Disclosures: **A. Peñas-Rincón:** None. **L. Ramírez-Lugo:** None. **A. Minguer-Uribe:** None. **F. Sotres-Bayón:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.07

Topic: H.03. Decision Making

Title: Reward resonance in amygdala-based circuits

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Abstract: The ability to learn from trial and error feedback is dependent on the basolateral amygdala (BLA), ventral striatum (VS), and orbitofrontal cortex (OFC), but how information is communicated between populations of neurons in these interconnected regions is not well understood. Empirical and theoretical work has demonstrated that interactions between low and high frequency oscillations in local field potentials (LFPs), known as cross frequency coupling, may serve as a mechanism to facilitate information transfer between interconnected neuronal

networks by linking system wide inputs and outputs. In particular, coupling between the phase of theta oscillations and the power of gamma oscillations, a phenomenon known as phase-amplitude coupling (PAC), has been linked to a variety of biological functions. Here, we recorded LFPs from the BLA, VS, and OFC while monkeys performed a three-armed bandit task to determine whether PAC within and between these structures is modulated as a function of learning. Specifically, we examined how PAC values were modulated as a function of reward prediction errors (RPEs), a key reinforcement learning signal. In the BLA and VS, large portions of channels showed RPE-related modulation of LFP activity in the theta (5-9 Hz) and gamma (42-55 Hz) bands. Theta encoding in both structures peaked around 1250ms post-outcome (BLA: 97/295 channels, 33%, $p < .001$; VS: 86/291, 30%, $p < .001$). Gamma encoding in both structures peaked around 1100ms post-outcome (BLA: 83/295 channels, 28%, $p < .001$; VS: 63/291, 22%, $p < .001$). No clear peak in theta and gamma encoding in the OFC was present. We next looked at the relationship between the phase of theta oscillations and the power of gamma oscillations between pairs of electrodes that were lowered into the *same* brain region. We found that local PAC values were more robustly modulated by RPE in the BLA (121/295, 41%, $p < .001$) than in the VS (84/291, 29%; BLA > VS, $Z = 3.06$, $p = .0022$) or OFC (19/128, 15%; BLA > OFC, $Z = 5.23$, $p < .001$). We also found that interregional PAC was modulated as a function of RPE in the BLA_{gamma} <-> VS_{theta} (253/666, 38%) and BLA_{gamma} <-> OFC_{theta} (71/191, 37%) pathways. Both local and interregional modulation of PAC by RPE occurred long after the outcome of the trial had been delivered (~800-1100ms post-outcome). Lastly, we found that PAC of gamma power and theta phase was largest when the monkeys' experienced large negative prediction errors and smallest when they experienced positive prediction errors. In conclusion, we found that learning-related information was correlated with changes in local and interregional coupling of fast and slow oscillations in the BLA, VS, and OFC.

Disclosures: J.K. Morrow: None. **V.D. Costa:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.08

Topic: H.03. Decision Making

Support: NIH/NIMH grant R00 MH101234
R01 MH116008

Title: Diverse oscillatory dynamics predicted by network models of layer-specific premotor- and amygdala-targeting pyramidal neurons in the anterior cingulate cortex

Authors: *S. IBAÑEZ[1], W. CHANG[1], T. GUILLAMON-VIVANCOS[2], A. KAPITONAVA[1], J. LUEBKE[1], M. MEDALLA[1];
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Abstract: The diverse temporal and oscillatory dynamics within anterior cingulate (ACC) cortical motor and limbic networks are linked to a wide range activity and behavioral states that function to coordinate and integrate affective and motor signals for flexible, goal-directed behavior. The biophysical and connectional properties of ACC pyramidal neurons that project to motor planning and emotional processing centers, such as the dorsal premotor cortex (PMd) and the amygdala (AMY), are fundamental building blocks of these network interactions. Here, we used a pyramidal-interneuron network model to simulate how single-cell properties can affect oscillatory synchrony in 4 networks consisting of distinct pools of AMY-targeting and PMd-targeting ACC pyramidal (E) neurons in layers 3 (L3) and 5 (L5). Each network included 2 functionally-distinct pools of perisomatic-targeting inhibitory interneurons -- parvalbumin (PV) and cholecystokinin (CCK). We varied the intrinsic membrane and excitatory/inhibitory synaptic properties of pyramidal neurons for each network, based on our empirical data derived from combined tract-tracing and *in vitro* whole-cell patch clamp recording experiments in rhesus monkeys. In all networks, the interplay between inhibitory and excitatory synaptic strength determines the synchrony and frequency of oscillations. The greater the conductance of excitatory recurrent connections, the greater inhibition needed to synchronize activity. Further, the greater the strength of PV to E inhibitory synapses, the more the network synchronizes to fast oscillations driven by high external input currents. Compared to the other networks, the L5 AMY-targeting network, with relatively greater CCK to E inhibition, exhibited oscillations more synchronized and resilient to noise at low input currents and frequencies in the theta-alpha range, consistent with the role of these frequencies in limbic affective and memory processing. This L5 AMY-targeting network can elicit high frequency gamma synchrony only if excitatory connections and CCK inputs to pyramidal neurons are suppressed, elucidating an important role of neuromodulation in shifting cortico-limbic oscillatory states. In contrast, the other ACC networks -with a greater PV to E synapses - are more synchronized at high beta-gamma frequencies, which is implicated in sensorimotor cortico-cortical processing. Our findings predict how single-cell and circuit-specific biophysical synaptic properties confer signaling diversity in ACC networks that control emotional and motor processing and have important implications for understanding mechanisms of flexible goal-directed behavior.

Disclosures: S. Ibañez: None. **W. Chang:** None. **T. Guillamon-Vivancos:** None. **A. Kapitonava:** None. **J. Luebke:** None. **M. Medalla:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.09

Topic: H.03. Decision Making

Support: F32MH115550
MH057440

Title: The medial septum enhances strategy switching when cognitive demand is high

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Abstract: Cognitive flexibility is the act of adjusting a current behavior or ongoing strategy (cognitive plan to achieve a goal) to account for changing environmental contingencies. Strategies that result in stable reward acquisition, and are repeated over time, eventually become outcome-insensitive. Because a decrease in outcome sensitivity will necessarily lead to a decrease in trial-by-trial flexibility, switching strategies becomes increasingly more difficult and requires more cognitive effort as repetitions increase. Furthermore, deficits in cognitive flexibility are significant in disorders that feature maladaptive habits and compulsions, such as addiction, OCD. Therefore, identifying the circuitry and mechanism that drives the increased cognitive effort required to adjust a well-learned behavior or strategy would both increase our understanding of cognitive flexibility circuitry and reveal novel drug targets to treat several psychiatric illnesses.
Previous studies have hinted at a role for the medial septum (MS), a sub region of the basal forebrain, in cognitive flexibility; however, the extent of this role is unclear. Because the basal forebrain is known to increase cognitive effort under demanding circumstances for other higher order cognitions, such as attention, we hypothesized that the MS may play a similar role in cognitive flexibility. To test this hypothesis, we activated the MS of male and female Sprague-Dawley rats with designer receptors exclusively activated by designer drugs (DREADDs) and measured their performance on an operant-based strategy switching task, following 1, 10, or 15 days of discrimination training. Rats that were tested following 1 day of training were similar across all groups and both sexes, demonstrating no effect of MS activation under these conditions. Following 10 days of training, however, MS activation significantly improved strategy switching, but only in female rats. In female rats, MS activation significantly reduced both the number of trials and number of errors to perform the strategy switch. Following 15 days of training, MS activation improved performance in both male and female rats, demonstrating both a significant reduction in trials and errors to perform the strategy switch. The difference in male and female performance may be explained by learning rate during training as female rats reached an average of >92% correct performance in significantly fewer days than males. As such, our data suggests that MS activation improves strategy switching performance as a function of level of training, suggesting that MS activation increases cognitive effort for strategy switching when cognitive demand is high.

Disclosures: D.M. Bortz: None. **A.A. Grace:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AAG also received funds from the following organizations: Lundbeck, Pfizer, Otsuka, Lilly, Roche, Asubio, Abbott, Autofony, Janssen, Alkermes, Newron..

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.10

Topic: H.03. Decision Making

Support: NIMH ZIA MH002928-01

Title: Distributed representation of value and state in reinforcement learning

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Abstract: The amygdala (AMY), ventral striatum (VS), orbitofrontal cortex (OFC), and lateral prefrontal cortex (LPFC) are key brain regions in the neural circuitry that underlies reinforcement learning. Previous studies have shown that these areas all play roles in reinforcement learning. However, responses across these areas to the same task factors from the same task have not been compared previously. To compare how task factors are encoded across these regions, we recorded neural activity from 5 rhesus macaques as they performed a three-armed bandit reinforcement learning task. During the task, novel choice options with unknown values periodically replaced familiar options, and the monkeys had to explore them to choose the options with the highest value. We found that neurons recorded across these regions showed the highest response to visual stimulus and reward information. Spatial information, on the other hand, was only encoded in the LPFC regions. The values of choices were encoded across all regions, although less robustly than the other factors. We also found these regions have different coding properties for the same task factors. At the single-unit level, individual LPFC neurons tended to encode more factors simultaneously, but the effect sizes for each factor were smaller than the AMY, VS, and OFC neurons. However, at the population level, the LPFC regions carried more information than the other regions. Our results revealed the distributed representation of task factors and differences in coding properties among these brain regions relevant to reinforcement learning.

Disclosures: H. Tang: None. **V.D. Costa:** None. **R. Bartolo:** None. **B.B. Averbeck:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.11

Topic: H.03. Decision Making

Support: R00MH111926
R01MH125571
DP2MH126376
5T90DA043219-04

Title: Using neuromodulatory dynamics to arbitrate between distinct behavioral strategies

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Abstract: A fundamental component of modern decision theories is “reference dependence," in which people evaluate outcomes as gains or losses relative to an internal reference point that likely reflects their expectations. When the reference point is high, an identical reward is viewed less favorably than when the reference point is low. We developed a novel task to study reference dependence in rats, using high-throughput behavioral training. In this task, water-restricted rats are offered a water reward to be delivered after a variable and unpredictable delay. They can either choose to wait or opt out at any time to start a new trial. On a proportion of trials, reward is withheld. The wait time on these trials provides an analog readout of the rat’s subjective value of that offer. By adapting rats to blocks of either the highest or lowest rewards, we can manipulate rats’ reference points. We find that rats wait significantly less time for mid-range offers in high blocks compared to low blocks, which is consistent with reference-dependent valuation. We developed several behavioral models that instantiate different strategies for computing the reference point, including one that uses model-free reinforcement learning, and another that is model-based in that it assumes knowledge of the latent block structure of the task. The models draw from optimal foraging theories, and estimate the trial-by-trial opportunity cost, or the cost of missing out on other potential offers by continuing to wait, in order to determine the optimal wait time on each trial. The models allow us to estimate latent cognitive variables driving behavior, including the reward prediction error (RPE), or the difference between expected and experienced rewards. In reinforcement learning, the RPE acts as a teaching signal; in our task, it is used to update the reference point, or the rat’s estimate of the opportunity cost of time. We used fiber photometry with fluorescent dopamine (DA) sensors in rats performing the task, and found that DA release in the nucleus accumbens core encodes the RPE several seconds after the cue that indicates the rat can receive its reward. Because our behavioral models made distinct predictions about the trial-by-trial RPE, we used these in vivo measurements of DA release to determine which strategy was most consistent with each individual rats’ neurobiological data. Here, we report rats’ strategies for computing an economic reference point, using measurements of neuromodulation to arbitrate between competing behavioral models. We view this as a promising avenue for performing model comparison of behavior in complex tasks.

Disclosures: A. Mah: None. **Y. Li:** None. **C. Constantinople:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.12

Topic: H.03. Decision Making

Support: 1R21MH117687
1R01MH102394

Title: The effect of Re suppression on Vicarious Trial-and-Error events

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Abstract: During decision making tasks, rats sometimes pause and look around their environment, as if they are contemplating a future choice. These behaviors are commonly referred to as vicarious trial-and-error (VTE) events and are hypothesized to be a behavioral manifestation of deliberation (Redish, 2016). Recent work suggests that medial prefrontal cortex (mPFC) ensemble activity may guide dorsal hippocampal (dHPC) representations of future information during VTE's (Hasz and Redish, 2020). In line with these results, it has recently been hypothesized that mPFC-dHPC interactions may directly inform VTE behavior (Redish, 2016). The nucleus reuniens (Re) is critical for mPFC and dHPC-dependent tasks, as well as mPFC-dHPC communication (Ito et al., 2015; Layfield et al., 2015; Hallock et al., 2016; Maisson et al., 2018). It is therefore possible that the Re is also implicated in deliberation, which would be reflected in VTE behavior. To this end, we analyzed VTE events in a previously-published dataset from our lab (Hallock et al., 2016). In this experiment, rats were trained to perform a spatial working memory T-maze task that required them to alternate between left and right trajectories at a choice-point, with each traversal being separated by 30 seconds. Male rats (n = 6) were implanted with cannulae targeting the Re and/or rhomboid nuclei (Rh). Each rat underwent 3 manipulations on separate sessions: a baseline day (no manipulation), a saline infusion day (manipulation control), and a muscimol infusion day (neuronal suppression). Per day, rats completed a set of baseline trials, underwent a manipulation, then performed a second set of trials 30 minutes later. Using IdPhi to identify VTE's (code from David Redish), we find that inactivating Re/Rh results in variable effects on VTE expression across rats, with 4/6 animals exhibiting a significant increase in IdPhi scores. By looking across trials, we further show that Re/Rh inactivation coincides with an increased chance of observing deliberative-like behaviors ($D = .44$, $p = 6.1e\text{-}09$, 2-sample KS-test). Thus, Re/Rh functionality is not critical for the behavioral manifestation of VTEs. Interestingly, however, we observed a negative correlation between the proportion of VTE's per session and choice accuracy ($R = -.58$, $p < .001$) that was entirely driven by muscimol sessions. Thus, Re/Rh inactivation resulted in poor choice accuracy and an increase in deliberative-like behavior. Moreover, we found that rats exhibiting perseverative dominant strategies during Re/Rh inactivation also exhibited consistent VTE's. These results may implicate the Re/Rh nuclei in the physiological 'content' associated with VTE events.

Disclosures: J.J. Stout: None. **H.L. Hallock:** None. **A.L. Griffin:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.13

Topic: H.03. Decision Making

Support: NIH/NNDS R01 Grant NS107599
Whitehall Foundation Grant 2017-05-71

Title: Optimal prestimulus cortical state predicts response outcome in a selective detection task

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Abstract: In the field of sensory detection, most studies focus on sensorimotor processing after a stimulus arrives (post-stimulus). However, the prestimulus neural activity can strongly alter stimulus encoding and behavioral outcomes. We sought to determine the impacts of prestimulus activity throughout neocortex on stimulus detection, for both target and distractor stimuli. We trained mice in a selective whisker detection task, in which they learned to respond to target stimuli in one whisker field and ignore distractor stimuli in the contralateral whisker field. During expert task performance, we used widefield Ca^{2+} imaging to assess prestimulus and post-stimulus neural activity broadly across frontal and parietal cortices. We find, indeed, that prestimulus activity strongly predicts trial outcome, with lower activity preceding response trials (hits and false alarms). Interestingly, the activity predictive of trial outcome does not localize to whisker regions but globally distributes throughout dorsal neocortex. The global activity is not reflected in whisker movement energy and therefore may reflect internal changes in task engagement. Using principal component analysis, we demonstrate that response trials are associated with a distinct and less variable prestimulus neural state. We interpret these findings as supporting an optimal prestimulus neural state for task performance that presents globally and affects responses to both target and distractor stimuli.

Disclosures: K. Marrero: None. **K. Aruljothi:** None. **C. Gao:** None. **B. Zareian:** None. **Z. Zhang:** None. **D.B. Salkoff:** None. **E. Zagha:** None.

Digital Abstract Session

P308. Neural Mechanisms of Reward Learning and Decision Making

Program #/Poster #: P308.14

Topic: H.03. Decision Making

Support: NIH Grant R01-MH107620
NIH Grant R01-NS089521
NIH grant R01 - NS108410
Alice and Joseph Brooks Fund Fellowship
Leonard and Isabelle Goldenson Postdoctoral Fellowship
Uehara Foundation Research Fellowship
NARSAD Young Investigator Grant

Title: Mixed representations in a visual-parietal-retrosplenial network for flexible navigation decisions

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Abstract: The survival of many animals requires the ability to navigate to goal locations by making decisions based on sensory inputs and past experience. These navigation decisions arise from the flexibility to respond to the same sensory input with distinct motor outputs depending on recent memories. However, navigation decisions have often been studied in tasks that require a stereotyped motor output in response to a given sensory stimulus, precluding investigation into the flexibility of decision-making. Here, we studied which brain areas are essential for flexible navigation decisions and how their neural activity endows such flexibility.
We trained mice to perform a delayed match-to-sample task in a virtual reality T-maze. As a mouse ran through the maze, it sequentially observed two cues separated by a short delay (1-2 s). The mouse was required to combine the short-term memory of the first cue and visual information of the second cue to choose an appropriate turn direction at the T-intersection. For a given visual stimulus in the second cue, the mouse responded flexibly with a different turn direction based on the memory of the first cue.
We used an optogenetic screen to identify areas that are involved in flexible navigation decisions. We bilaterally inhibited different sites across the dorsal cortical surface by optogenetically activating inhibitory neurons in VGAT-ChR2 mice. Inhibition of V1, posterior parietal cortex, or retrosplenial cortex (RSC) induced a large decrease in task performance, suggesting that these areas were critical for mice to perform the task.
Two-photon calcium imaging revealed distributed representations of task-related events across areas. Notably, a large number of individual neurons were selectively active for a particular combination of the first cue's memory and the second cue's sensory signal. Such mixed representations encoded information about a correct choice direction in a format useful for guiding navigation, and appeared critical for accurate decision-making because they were prominent on correct trials but weak on error trials. The analysis of activity in populations of neurons revealed that these mixed selectivity neurons efficiently encoded a correct choice direction, compared to populations of neurons selective only to the first or second cue. Although mixed selectivity neurons were found across all areas studied, they existed most densely in RSC, and had a strong correlation with the mouse's choices. Together, we propose a mechanism for flexible navigation decisions based on mixing memory and visual signals primarily in RSC neurons to guide choices, within a visual-parietal-retrosplenial network.

Disclosures: S. Kira: None. **H. Safaai:** None. **A. Morcos:** None. **G. Pica:** None. **S. Panzeri:** None. **C.D. Harvey:** None.

Digital Abstract Session

P309. Lateral Prefrontal Cortex: Adversity Effect On Cognitive Control

Program #/Poster #: P309.01

Topic: H.03. Decision Making

Support: NIMH R21 MH097583 (Teitelman and Childress, MPI)
Penn SON Faculty Pilot Grant Program (Teitelman)
University of Pennsylvania, Provost's Research Facilities Development Fund (Teitelman/Childress)
T32DA028874-10 (Childress
Drexel BIOMED Faculty Dev Award (Ayaz)

Title: Ineffective affective processing: neural correlates of cognitive control in young urban women with a history of sexual violence

Authors: L. SINKO[1], P. REGIER[2], A. M. TEITELMAN[3], J. ELKIND[3], S. ARYAL[3], A. CURTIN[4], H. AYAZ[4,5,6,7], A. R. CHILDRESS[2];
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Abstract: MOTIVATION / PROBLEM STATEMENT: Women's experiences of sexual violence (SV) are not only often psychologically and physically traumatizing, but may also have lasting effects on brain functions, including cognitive control relating to the inhibition and processing of emotion. Despite this, few studies have explored underlying neural correlates of SV's impact on cognitive control in women. The purpose of this study was to understand cognitive control differences between women who have a history of SV vs. those who do not using functional near-infrared spectroscopy (fNIRS), a portable neuroimaging technology used to characterize inhibitory control at the level of the brain. We hypothesized that prior SV might be linked to poor inhibition, and to lower recruitment of cognitive control circuitry.
METHODS / APPROACH: Thirty women (ages 21-30) were recruited from an urban setting in the northeastern United States. Surveys assessed demographic and mental health characteristics including SV history (i.e. childhood and adult SV). All participants underwent an affect-congruent Go-NoGo task, using positively-valenced (baby animal) pictures as "Go" stimuli , and negatively-valenced (spiders, scorpions) pictures as NoGo stimuli, while prefrontal activity was monitored by 16 optodes via fNIRS. An ANOVA tested for main effects of the condition (Go vs. No-Go), group (SV vs. no prior SV), and potential interactions.
RESULTS: Fifteen of 30 women reported a history of childhood (n=6) and/or adult (n=12) SV. Those with SV histories reported higher levels of depression ($p<0.01$), anxiety ($p<0.01$), and posttraumatic stress ($p<0.01$) symptoms, as well as increased impulsivity ($p=0.02$). Behavioral performance (errors of commission) did not differ between the groups, however, fNIRS data revealed a significant (group x condition) interaction in optodes 13 ($p=0.001$) and 16 ($p = 0.008$). Women with a history of SV had significantly different cognitive control responses compared to their peers, with lower recruitment in the right dorsolateral prefrontal cortex (e.g., optodes 13, 16) during the "NoGo" condition, but heightened recruitment during the "Go" condition.
CONCLUSION / IMPLICATIONS: These results suggest altered prefrontal cortical activity during cognitive processing in women with a history of SV, showing hypoactivity in regions

important to response inhibition and hyperactivity to the positive/approach stimuli. These findings highlight a significant link between SV and critical brain functions, demonstrating a strong translational promise for innovative assessment and prevention of untoward effects (impaired affective processing) among women with SV.

Disclosures: L. Sinko: None. **P. Regier:** None. **A.M. Teitelman:** None. **J. Elkind:** None. **S. Aryal:** None. **A. Curtin:** None. **H. Ayaz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); fNIR Devices LLC. manufactures the optical brain-imaging instrument and licensed IP and know-how from Drexel University. Dr. Ayaz was involved in the technology development and thus offered a minor sh. **A.R. Childress:** None.

Digital Abstract Session

P310. Medial Prefrontal Cortex: Networks and Cognition

Program #/Poster #: P310.01

Topic: H.03. Decision Making

Support: NARSAD
NIDDK

Title: Investigating obesity-linked cortico-accumbal plasticity mechanisms underlying enhanced hedonic feeding

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Abstract: Obesity is a leading cause of preventable death in this country. However, despite widespread knowledge of the prevalence and health disparities linked to chronic obesity, effective treatments remain elusive - most people who lose weight will re-gain it. Though prior research has often explored metabolic or energy adaptations to explain weight re-gain, this does not address the proven contribution of hedonic feeding mechanisms to weight re-gain after obesity. Human and rodent studies show that weight loss after obesity causes increased motivation to consume palatable foods, and that activity in brain areas involved in food motivation - namely the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc) - is altered with obesity. Yet the neural mechanism underlying enhanced motivation for palatable food following weight loss is unknown. Using in vivo fiber photometry recordings on a novel assay measuring motivation for palatable foods, our data shows that a subpopulation of NAc neuronal activity is enhanced following obesity and subsequent weight loss. We hypothesized this reflected increased input from mPFC to NAc, and used in vivo electrophysiology and optogenetic stimulation to reveal that long-term synaptic depression (LTD) was occluded in obese mice. This reveals a mechanism likely underlying sustained NAc activity during and after obesity and potentially leading to obesity-linked persistent increases in hedonic feeding. These

experiments may contribute to obesity treatments, potentially helping to hone current initiatives such as deep brain stimulation in the NAc.

Disclosures: B.A. Matikainen-Ankney: None. **A.V. Kravitz:** None.

Digital Abstract Session

P310. Medial Prefrontal Cortex: Networks and Cognition

Program #/Poster #: P310.02

Topic: H.03. Decision Making

Support: NSERC (RGPIN-2018-04295)

Title: Optogenetic and pharmacological investigation of prelimbic cortex involvement in strategy set-shifting

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Abstract: Behavioral flexibility is an adaptive behavior entailing altering actions in response to environmental changes. Strategy set-shifting is a form of flexibility where animals learn to use one rule to receive reward (e.g. select the lever denoted by a cue light) but after a switch in reward contingencies, a different strategy must be used (e.g always select the left/right lever for reward). Pharmacological inactivation studies have shown that set-shifting is facilitated in part by the prelimbic (PL) subregion of the medial prefrontal cortex (mPFC). Additionally, neurophysiological findings have shown that mPFC activity is correlated with different task events. However, it is unclear whether the PL is important for performing more than 1 rule shift and how phasic PL activity during discrete periods of the choice sequence contributes to flexibility. Here we used pharmacological inactivations to determine how PL is involved in multiple shifts between cue and response rules and optogenetic inhibition to address whether PL activity during cue presentation is important for set-shifting. In one study, rats were trained to learn an initial discrimination and then perform 3 rule-shifts within a session. PL inactivation early in training (~8 days) increased perseverative and non-perseverative errors made during rule shifts. Inactivation of PL after extended training (~30 days) resulted in perseveration of the previous rule. These results suggest PL is necessary to keep track of changing contingencies and remember which rules have already been performed in a given day. In contrast, PL inactivation did not impair shifting in rats trained for ~8 days to perform a single shift each day, suggesting that PL is not necessary when rats perform an easier task of 1 rule-shift per day. Finally, we used optogenetic suppression of neural activity in the PL to probe how phasic firing of PL neurons prior to choice influences the ability to perform 3 within-session set-shifts. During testing, PL neural activity was inhibited 3-5s prior to each choice trial. Compared to baseline, inhibition of pre-choice PL activity impaired performance when shifting from the response to cue rule but not from the cue to response rule, suggesting that PL activity prior to choice is important for flexibility when it involves a shift to a more attentionally demanding rule. Together, these

findings indicate that the PL does not play a role in mediating a single shift in well-trained animals but does play a role when task difficulty is increased, and rats must perform multiple shifts within a session. Furthermore, PL activity prior to action selection facilitates shifts under conditions where attentional demands are high.

Disclosures: V.M. Lo: None. **D.A. Bercovici:** None. **S.B. Floresco:** None.

Digital Abstract Session

P310. Medial Prefrontal Cortex: Networks and Cognition

Program #/Poster #: P310.03

Topic: H.03. Decision Making

Support: NIH Grant K99MH101234
NIH Grant R00MH101234
NIH Grant R01 MH116008

Title: Lamina-specific biophysical and structural properties of amygdala and premotor targeting pyramidal neurons in monkey anterior cingulate cortex

Authors: *M. MEDALLA[1], W. CHANG[1], S. IBAÑEZ[1], T. GUILLAMON-VIVANCOS[2], M. NITTMANN[3], A. KAPITONAVA[1], S. E. BUSCH[4], T. L. MOORE[1], D. L. ROSENE[1], J. I. LUEBKE[1];
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Abstract: Within the frontal executive network, the anterior cingulate cortex (ACC) is a strategic hub for synthesizing and coordinating motivational and motor signals to guide flexible, goal-directed behavior. The dynamics of this cortical processing likely depend on the signaling properties of lamina-specific pyramidal neurons in the ACC that send coordinated output to dorsal premotor cortex (PMd) for motor planning and to emotional processing centers in the amygdala (AMY). We combined tract-tracing with *in vitro* whole-cell patch clamp recording in rhesus monkeys to assess the detailed biophysical and structural properties of neurons targeting AMY or PMd in ACC layers 3 (L3) and 5 (L5). Results demonstrate significant differences in the biophysical properties of ACC pyramidal neurons that are lamina and pathway specific (Two-way ANOVA with post-hoc comparisons, $p < 0.05$). Hence, pyramidal neurons targeting AMY in L5 are significantly more excitable, exhibiting higher input resistance and action potential (AP) firing rates, then those in L3. Further, pathway diversity was found specifically for L5 pyramidal neurons, with L5 AMY-targeting neurons exhibiting significantly less firing frequency adaptation, more complex apical dendritic tufts, and greater densities of dendritic spines and non-parvalbumin perisomatic inhibitory inputs than PMd-targeting neurons. Simulations using a pyramidal-interneuron network model predict that these differences in AMY versus PMd targeting neurons at the single-cell level contribute to distinct network temporal and

oscillatory dynamics. Compared to other simulated networks, the network model comprised of L5 AMY-targeting neurons is more robustly synchronized to slow theta/alpha frequencies, supporting the important role of these frequencies in affective and memory processing in limbic structures. In contrast, network models of L3 neurons and L5 neurons targeting PMd are more tuned to beta/gamma synchrony, which is implicated in perceptual motor decision making and cortico-cortical processing. Our data demonstrate the relevance of laminar location as strong predictors of cortical pathway functional diversity. The lamina-specific properties of ACC projection neurons directed to AMY and PMd enable diverse control of emotional and motor processing and provide the building blocks to understand the networks mediating motivational and affective aspects of flexible decision-making.

Disclosures: **M. Medalla:** None. **W. Chang:** None. **S. Ibañez:** None. **T. Guillamon-Vivancos:** None. **M. Nittmann:** None. **A. Kapitonava:** None. **T.L. Moore:** None. **D.L. Rosene:** None. **J.I. Luebke:** None. **S.E. Busch:** None.

Digital Abstract Session

P310. Medial Prefrontal Cortex: Networks and Cognition

Program #/Poster #: P310.04

Topic: H.03. Decision Making

Support: NIH/NIMH R00 MH101234
R01 MH116008

Title: Distribution of inhibitory synapses on amygdala targeting projection neurons in dorsal and ventral monkey anterior cingulate cortex

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Abstract: The anterior cingulate cortex (ACC) sends robust output to the amygdala (AMY) forming a key cortico-limbic pathway critical for regulating emotions and stress. The dorsal (area 24) and ventral (area 25) subdivisions of ACC are thought to have distinct roles in affective processing, likely conferred by the properties of lamina-specific AMY-targeting pyramidal neurons from these areas. Unique to the primate is the strong bilaminar origin patten of these ACC projections to the amygdala, with AMY-targeting pyramidal neurons located in both layers 3 (L3) and 5 (L5). Laminar location influences the extent and nature of excitatory and inhibitory inputs that these projection neurons receive, which, in turn, shapes signal integration and firing activity. The current study focuses on comparing the distribution of perisomatic and proximal dendritic inhibitory inputs onto AMY-targeting projection neurons in L3 and L5 of ACC areas 24 (A24) and 25 (A25) in rhesus monkey (*Macaca mulatta*). Using retrograde neuronal tract-tracing and high-resolution confocal imaging, tracer-labeled cell bodies and dendrites were reconstructed in 3D, and appositions with inhibitory nerve terminals immuno-labeled with

vesicular-GABAergic transporter (VGAT) were quantified. We found that the density and neurochemical nature of VGAT+ inhibitory inputs on AMY-targeting pyramidal neurons within each layer differed between the two ACC areas (Two-way ANOVA with post-hoc comparisons, $p < 0.05$). Both L3 and L5 AMY-targeting neurons in the dorsal A24 receive significantly more inhibitory inputs on somata and proximal dendritic compartments compared to those in A25. This regional difference was mainly due to a significantly lower density of inhibitory inputs from parvalbumin-expressing neurons that mediate strong and rapid inhibition on AMY-targeting neurons in ventral A25. Thus, compared to dorsal ACC A24, the projection neurons from the ventral A25 directed to the amygdala are subject to less inhibition. These findings may help explain the specific susceptibility of the ventral subgenual ACC to pathologic hyperexcitability, excitatory: inhibitory imbalance, and excitotoxicity associated with affective disorders such as depression.

Disclosures: A. Kapitonava: None. **M. Medalla:** None.

Digital Abstract Session

P310. Medial Prefrontal Cortex: Networks and Cognition

Program #/Poster #: P310.05

Topic: H.03. Decision Making

Title: An fmri study on the encoding of political fake news

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Abstract: The concept of “fake news” has gained increasing attention in the past few years, but much is still unknown about the neural processes underlying the encoding of false news stories in the political context. We used functional magnetic resonance imaging (fMRI) to investigate the effect of congruency, when participants believed a political news statement was “real” or “fake” and were then shown a news label that was either congruent (i.e., matched) or incongruent with their beliefs. In this pilot study, human participants (N=14; mean age: 25.14; 57.14% female) were shown negative news statements in the form of a Twitter ‘tweet’ from two hypothetical political opponents in the scanner. Participants were asked to initially rate the news as probably real or fake, and then shown whether the news was in fact “real” or “fake”. They were asked to remember the news label given for an immediate memory task. After the scan session, participants completed a set of questionnaires to assess their political orientation, attitudes, and beliefs, and were then debriefed. Preliminary results suggest that participants recruit different areas of the brain depending on whether the news label was congruent with their initial judgement of the news statement compared to when the label and judgement were incongruent. This can be broken down even further into conditions when the news was judged as real and labeled as real, compared to when it was judged as fake and labeled as fake. Activation in these congruency conditions were found in areas such as the posterior cingulate, precuneus, supramarginal gyrus, and superior medial frontal cortex. These areas have been shown to be

implicated in cognitive processes such as decision confidence, emotional responses, and memory retrieval. Understanding how people process political fake news can lead to more insight on political cognition and decision-making. Bridging this gap between psychology, neuroscience, and political science can better help understand issues that are important to society but subject to partisanship.

Disclosures: B.L. Gonzalez: None. **Y. Chen:** None. **T. Canli:** None.

Digital Abstract Session

P310. Medial Prefrontal Cortex: Networks and Cognition

Program #/Poster #: P310.06

Topic: H.03. Decision Making

Support: F32MH115416
R01MH108358

Title: Dorsomedial frontal cortex participates in both evidence accumulation and trial history-based updating during perceptual decision-making

Authors: *T. Z. LUO[1], T. D. HANKS[2], D. GUPTA[1], A. G. BONDY[1], C. D. BRODY[1]; [1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]Univ. of California, Davis, Davis, CA

Abstract: When making a decision based on noisy sensory stimuli, integration over time mitigates the impact of noise and is therefore a core component of many types of decisions. In trial-based perceptual tasks, the decision process is learned and continually updated through the evaluation of actions in past trials and their outcomes. The neural mechanisms underlying evidence accumulation and the updating of the decision process by trial history remain poorly understood. It is unknown whether the same brain region that mediates evidence integration in expert observers is also involved in updating the process of choice formation. To identify a candidate brain region, we first examined whether changes in history-dependent updating can be detected after the pharmacological inactivation of five separate sensorimotor brain regions in rats accumulating noisy auditory evidence. Only the perturbation of dorsomedial frontal cortex (dmFC) resulted in both an impairment in behavioral sensitivity to the sensory stimulus and also a change in trial history-dependent updating. Chronic lesion of dmFC similarly changed both behavioral sensitivity and history-dependent updating. Three separate experiments indicate that dmFC is causally involved in the gradual accumulation of evidence. Recordings from Neuropixels probes indicate that the encoding of accumulated sensory evidence is more robust and emerges more rapidly in neural populations in dmFC than in four other anterior brain regions. Optogenetic inactivation of dmFC specifically during trial epochs when evidence was accumulated impaired behavioral sensitivity. Lastly, either optogenetic or chronic inactivation of dmFC resulted in an impaired representation of sensory evidence in anterior brain regions interconnected with dmFC. These results indicate the rat dorsomedial frontal cortex is a shared

node in the circuits that mediate the gradual accumulation of evidence and history-dependent updating and suggests that activity in dmFC encodes action values that are used for both choice formation and modification of subsequent decisions.

Disclosures: **T.Z. Luo:** None. **T.D. Hanks:** None. **A.G. Bondy:** None. **D. Gupta:** None. **C.D. Brody:** None.

Digital Abstract Session

P310. Medial Prefrontal Cortex: Networks and Cognition

Program #/Poster #: P310.07

Topic: H.03. Decision Making

Support: NIH MH080318
NIH MH112688

Title: Certainty and uncertainty of the future changes planning and sunk costs

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Abstract: Many foraging experiments have found that subjects are suboptimal in foraging tasks, waiting out delays longer than they should given the reward structure of the environment. Additionally, theories of decision-making suggest that actions arise from interactions between multiple decision-making systems and that these systems should depend on the availability of information about the future. To explore suboptimal behavior on foraging tasks and how varying the amount of future information changed behavior, we ran rats (4M, 4F) on two matching neuroeconomic foraging tasks, Known Delay (KD) and Randomized Delay (RD), with the only difference between them being the certainty of the cost of future opportunities. On these tasks, rats foraged for food by encountering reward sites that provided the opportunity of reward after delay. On KD, delays remained constant at each individual reward site throughout the session; on RD, delays were random (1-30s) and were revealed on each encounter. We found that when rats were given knowledge of the future, they were able to plan their actions. The behavior of rats on the task providing known outcomes indicated that they understood the future offers before encountering them. Rats on both tasks exhibited suboptimal behavior, and when we explored hypotheses to explain these suboptimalities, we found that the rats more heavily accounted for pre-reward foraging times than post-reward lingering or traveling times. Additionally, we found that rats on both tasks showed a sensitivity to sunk costs; however, sunk cost susceptibility was diminished in the task enabling planning (KD). These results suggest that while future certainty reduced decision-making errors, more complex decision-making processes unaffected by future certainty were involved and likely produced these decision-making errors within subjects on these foraging tasks. These results led us to a hypothesis that there was an inverse relationship between planning and sunk costs. To test this hypothesis, we re-analyzed data in rats from a similar foraging task with medial prefrontal disruption (using h4MDi DREADDS) and found that

mPFC disruption, which has been previously shown to decrease planning, led to increased sunk cost behavior.

Disclosures: **A.A. Duin:** None. **L. Aman:** None. **B. Schmidt:** None. **A.D. Redish:** None.

Digital Abstract Session

P311. Network Activity

Program #/Poster #: P311.02

Topic: H.04. Executive Functions

Title: Cognitive implications of brain structural connectivity alterations between nodes of the Default Mode Network related to cannabis use and HIV infection

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Abstract: The default mode network (DMN) is a large-scale functional brain network composed of brain regions that show greater activity in the absence of explicit task demands relative to when engaged in a task. Emerging evidence suggests that human immunodeficiency virus (HIV) infection and chronic cannabis use are both separately linked with alterations in DMN functional connectivity. People living with HIV (PLWH) infection often report using cannabis to alleviate HIV-related symptoms (e.g., pain, nausea, loss of appetite). Of the 38 million PLWH, nearly half demonstrate neurocognitive impairments highlighting a need to clarify the brain-based manifestations of such impairments. Using diffusion weighted imaging (DWI) data, we characterized the structural connectivity profiles of DMN regions among 100 participants stratified by HIV serostatus (+ vs. -) and cannabis use (+ vs. -). A deterministic fiber tractography algorithm was used to track cingulum tracts (CT), the fronto-occipital fasciculus (FOF), and the genu of the corpus callosum (CC). A general linear model was then used to compare the effects of both HIV and cannabis on tract length (mm) and tract volume (mm^3) averaged across all tracts of interest. A second general linear model analysis was used to characterize the effect of HIV and cannabis on cognitive control in the Error Awareness Task (EAT), a response-inhibition task in which participants make errors of which they are either aware or unaware. We detected a significant HIV x cannabis interaction when considering error awareness in the EAT, ($F(5,94)=1.5$, $p<.05$). However, we did not detect a significant interaction when considering the average tract length of all tracts ($F(3,96)=0.7$, $p=0.8$) or the average volume of all tracts ($F(3,96)=0.9$, $p=0.4$). These outcomes indicate that chronic cannabis use by PLWH was linked with a reduced ability to self-detect performance errors. A compromised ability to self-monitor one's errors may lead to the continuation of sub-optimal behaviors in the real-world (e.g., HIV-medication management). The null structural connectivity results suggest that consideration of individual tract analysis (as opposed to the average of multiple) is warranted.

Disclosures: **P.M. Viera Perez:** None. **J.S. Flannery:** None. **R. Gonzlez:** None. **A.R. Laird:** None. **M.T. Sutherland:** None.

Digital Abstract Session

P311. Network Activity

Program #/Poster #: P311.05

Topic: H.04. Executive Functions

Support: KAKENHI 17H01758

Title: Cognitive fatigue induces changes in resting-state functional connectivity

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Abstract: Sustained cognitive demands cause a decline of cognitive resources accompanied by subjective feeling of mental exhaustion, which is called "cognitive fatigue (CF)" (Pattyn, 2018). The purpose of this study is to elucidate the CF-related changes in brain state. The current study employs functional MRI to evaluate resting-state functional connectivity between brain regions to investigate changes in brain networks under CF. Eighteen young adults (22.8 ± 1.8 years of age, all right-handed) participated in the study which includes two days of lab visits, separated by more than a week. On each visit, the participants conducted two sessions of MRI data acquisition separated by either cognitive task sessions for fatigue loading (CF condition) or passive environmental video viewing sessions as control. The order of CF and control conditions was randomized between participants. The fatigue loading in the CF condition consisted of 4 sessions of 30-minutes continuous simple mental arithmetic task with 5 minutes of interval. MRI scans were conducted before and after the fatigue loading/control sessions, which include T1 structural scans and 12-minutes resting-state fMRI (rsfMRI) scans (TR=2,000 ms, TE=35 ms, FA=90 deg, 31 slices with 3.7 mm thickness) using 3 T scanner (Philips Ingenia 3.0T). CONN software (Whitfield-Gabrieli, 2012) was used to analyze the effective connectivity between brain regions. This study was approved by the internal review board of AIST. The flicker perception threshold (FPT) after the fatigue loading session in the CF condition decreased significantly compared to the FPT measured before the session ($p < 0.05$) while FPT did not changed significantly in the control condition. Results of the connectivity analysis of the rsfMRI data showed that (a) significant increase in effective connectivity between the posterior cingulate cortex (PCC), which is a part of the default mode network (DMN), and the bilateral inferior occipital gyrus (IOG), and (b) decrease in connectivity between the frontal eye field (FEF), one of the nodes included in the dorsal attention network (DAN), and the anterior cingulate cortex (ACC), in the CF condition compared to the control condition ($p<0.05$; FDR corrected). The current results indicate the changes in resting-state brain network after the CF loading which might reflect the difficulty in allocating attentional resources to specific task performance under CF (Esterman, 2013).

Disclosures: **S. Iwaki:** None. **T. Fujiwara:** None.

Digital Abstract Session

P311. Network Activity

Program #/Poster #: P311.06

Topic: H.04. Executive Functions

Support: NIAAA Grant P60AA010760
NIAAA Grant U01AA013510

Title: Resting state functional connectivity networks associated with cognitive flexibility in rhesus monkey

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Abstract: Cognitive flexibility is an essential component of behavioral control related to variety of psychiatric disorders. In rhesus monkeys, cognitive flexibility shows individual differences in rates of task learning when assessed with a set-shifting procedure. This study used resting state functional connectivity (rsfcMRI) to identify brain regions associated with cognitive flexibility in monkeys. We hypothesized that the functional connectivity of brain regions involved in executive control network in human subjects will be predictive of learning a set-shifting task in rhesus monkeys. Rhesus monkeys (N=35, 18 females) were housed individually with computer-controlled touch-screens imbedded in their cage wall. The set-shifting procedure employed serial discriminations between two 2D stimuli (geometric shape and color) and included shape- or color-based discrimination and their reversals. Animals were given 30 sessions to assess rate of task acquisition, which resulted in distinguishing two groups with high (n=17) and low (n=18) learners using *k*-mean clustering. Animals with low learning rates had a higher perseverative response and short latencies to respond during task performance. rsfcMRI data were obtained in anaesthetized monkeys (1% isoflurane) prior to cognitive testing and resting state networks (RSNs) were identified using independent component analysis. The MRI data revealed 4 RSNs showing visual similarity to known RSNs identified in human and nonhuman primates. A dual regression analysis based on the high and low group-dependent differences was preformed using the 4 RSNs. The two groups of learners exhibited differences in functional connectivity related to one of the RSNs termed the Prefrontal-Orbitofrontal Network (PON), due to its overlap with brain regions included in the human executive control and default mode network. Functional connectivity of putamen, caudate, orbitofrontal and ventrolateral prefrontal cortices with the PON were stronger in the group of monkeys that exhibited high rates of learning. Higher levels of coactivation of caudate and putamen with the PON were predictive of low perseverative responding. Higher levels of coactivation of ventrolateral and ventromedial prefrontal cortex with the PON were predictive of greater response inhibition. Thus, connectivity within the PON that is involved in executive control over human cognition is also associated with the rhesus monkey's ability to learn a task that requires high levels of cognitive flexibility.

Disclosures: **T. Shnitko:** None. **C. Kroenke:** None. **K. Grant:** None.

Digital Abstract Session

P311. Network Activity

Program #/Poster #: P311.07

Topic: H.04. Executive Functions

Title: Clustering of excitatory neurons and slow synaptic dynamics lead to longer autocorrelation timescales and slow ignitions in spiking networks

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Abstract: Spontaneous voluntary actions are canonically preceded by anticipatory signals that "ramp up" before the time of action, such as the canonical EEG Readiness Potential (RP), which begins around 0.5-1.5 seconds before movement (e.g. Libet et al., 1983). These anticipatory buildups also emerge when backwards-averaging an autocorrelated stochastic process (e.g. drift-diffusion) aligned to a threshold-crossing (Schurger et al., 2012). However, anticipatory ramping in single-neuron firing rates begins even earlier, up to 4 seconds before movement (Fried et al., 2011), even though the autocorrelation timescales of single neurons are only on the order of hundreds of milliseconds (e.g. Cavanagh et al., 2016)—far too short to explain such early ramp ups, if the underlying dynamics are those of a stochastic accumulator. In an exploratory study of a network of leaky integrate-and-fire neurons, we demonstrate 1) that clustering groups of excitatory neurons in a balanced network leads to slow fluctuations (Litwin-Kumar & Doiron, 2012), spontaneous ignitions (rate models: Moutard et al., 2015), and increased autocorrelation timescales in a critical range of clustering (in-between homogenous and WTA); 2) aligning to upswings in a cluster's firing rates results in exponential ramping in cluster firing rates, as well as a counter-intuitive negative ramping of post-synaptic potentials on excitatory neurons owing to overall increased inhibition; and 3) the dynamics and critical clustering range differ markedly for fast and slow synapses, with more gradual ramping present in networks with slower synapses. These results suggest the pre-movement buildups seen in voluntary action and possibly other spontaneous cognitive events (e.g. Broday-Dvir & Malach, 2020) are reliant on slow synaptic signaling (NMDA, GABA-B) and fluctuations in a clustered network architecture.

Disclosures: **J.C. Gavenas:** None. **U. Rutishauser:** None. **A. Schurger:** None. **U. Maoz:** None.

Digital Abstract Session

P312. Subcortical-Cortical Interactions: Arousal Systems

Program #/Poster #: P312.01

Topic: H.04. Executive Functions

Support: Tiny Blue Dot Foundation

Title: Diverse cortical responses to claustrum perturbation

Authors: *E. G. MCBRIDE, S. R. GANDHI, J. R. KUYAT, A. ARKHIPOV, S. R. OLSEN, C. KOCH;
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Abstract: The claustrum is a small and thin structure between cortex and basal ganglia whose function remains elusive. It primarily receives inputs from cortical regions and projects back to most, in particular midline and frontal structures. Hypotheses of claustrum function include cortical coordination, salience detection, allocation of selective attention, and consciousness. A recent study demonstrated a feedforward inhibitory effect of claustrum on neurons in the medial prefrontal cortex of mice (Jackson et al. 2018), but it is unknown to what extent this generalizes across claustrum's many projection targets in the cortex.

To characterize the effect of claustrum activity on its projection targets across cortical areas, we employed the transgenic line Gnb4-IRES2-CreERT2;Ai32 to largely limit expression of opsin to the claustrum. Previous work at the Allen Institute characterized the Gnb4 driver line (Wang et al. 2017) and demonstrated single claustrum Gnb4+ neurons send axonal projections to a median of 21 cortical areas (Wang et al. 2019). In these mice, we implanted an optic fiber cannula above the claustrum and recorded with multiple Neuropixels probes across many cortical areas during optogenetic stimulation with both short (5ms) and long (500ms) pulses. Six female and four male mice aged 5-10 months were used for these experiments, though sex differences were not directly assessed.

We found, both within and across cortical areas, a diversity of responses including both inhibition and excitation following optical perturbation of claustrum. 500ms pulses elicited robust, largely excitatory responses, and the proportion of neurons recruited differed across cortical areas. In anterior cingulate cortex (ACA), more fast-spiking (FS; putative inhibitory) neurons were recruited than regular-spiking (RS; putative excitatory) neurons, which is consistent with the idea of a feedforward inhibition mechanism. However, in retrosplenial cortex (RSP), roughly equal proportions of RS and FS neurons were recruited, despite the fact that many Gnb4+ claustrum neurons project to both ACA and RSP. Single 5ms pulses elicited a mix of excitation and inhibition, though inhibition was not as potent or widespread as in the previous study (Jackson et al. 2018). The difference could be explained by a different method for opsin expression or the Neuropixels probes detecting and differentiating more neurons. Additionally, in some neurons we observe trial-trial variation of both the magnitude and the sign of responses to claustrum perturbation. Our findings thus far suggest that the effect of claustrum on its cortical projection targets is more complex than previously thought.

Disclosures: **E.G. McBride:** None. **S.R. Gandhi:** None. **J.R. Kuyat:** None. **A. Arkhipov:** None. **S.R. Olsen:** None. **C. Koch:** None.

Digital Abstract Session

P312. Subcortical-Cortical Interactions: Arousal Systems

Program #/Poster #: P312.02

Topic: H.04. Executive Functions

Support: Tiny Blue Dot Foundation

Title: Detecting consciousness in mice using perturbational complexity

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Abstract: Currently, the clinical evaluation of consciousness relies on a patient's ability to interact with the surrounding environment. However, under certain circumstances, such as in locked-in syndrome, during dissociative anesthesia, or after severe brain injury, a person can be conscious but disconnected from the external environment. In these situations, the current clinical practice fails to reliably access the level of consciousness of a patient.
To overcome this limitation Casali et al. (Casali, et al. 2013) developed a procedure that discriminated between the presence and absence of consciousness in human subjects. They used transcranial magnetic stimulation to evoke neural responses, measured using electroencephalography (EEG), and quantified the spatio-temporal complexity of the responses using a novel scalar measure called perturbational complexity index (PCI). They showed that a single threshold, PCI*, across subjects, discriminated with 100% specificity and 100% sensitivity conscious from unconscious states in a variety of subjects - wakefulness, sleep, anesthesia, neurological patients, etc. Although very promising, these results do not address the mechanisms underlying the differential complexity in different states of consciousness.
We aim to extend the technique to mice so we can use invasive tools to reveal the circuits and mechanisms underlying the change in PCI as it relates to presence or absence of consciousness. We record EEG-like activity using a flexible 30-electrode surface array on the skull and multiple Neuropixels (NPX) probes in cortical and subcortical areas. While the animal is awake and head-fixed on a wheel, we use a bipolar electrode in the secondary motor cortex to deliver a single, biphasic electrical pulse (100 trials) to evoke cortical responses recorded simultaneously by the EEG and NPX electrodes. The same procedure is repeated after inducing an anesthetized state.
During the awake state, we observe robust evoked responses in the EEG signals across many cortical regions. These responses are also reflected in the neural responses recorded on NPX probes up to 4 mm away from the stimulation site. During the anesthetized state, the responses in the EEG signals lack the spatial diversity seen in the awake responses, as expected based on the previous work. Our preliminary results show that we can discriminate between conscious and unconscious states by calculating PCI-st (Comolatti, et al. 2019) on the EEG evoked responses. With this work, we aim to investigate whether the complexity

differences are purely a cortico-cortical phenomenon or rely on cortical and subcortical, in particular, cortico-thalamic interactions.

Disclosures: **L.D. Claar:** None. **I. Rembado:** None. **S.R. Gandhi:** None. **J.R. Kuyat:** None. **A. Arkhipov:** None. **S.R. Olsen:** None. **C. Koch:** None.

Digital Abstract Session

P313. Studies of Executive Function and Inhibitory Control in Human Subjects

Program #/Poster #: P313.01

Topic: H.04. Executive Functions

Support: NIH Grant-1R01HD086011-01A1
NICHD Diversity Supplement-PA-18-586

Title: The role of maternal education on executive functions and attention system networks for school-age children

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Abstract: Reading difficulties (RD, or dyslexia) affect 5-15% of school-age children with deficits in phonological processing, fluency, and executive functions (EFs). Children with RD have decreased abilities in EFs such as working memory, inhibition, and self-monitoring compared to typically developing readers (TRs). Although RD can be genetically inherited, many children have RD due to environmental variables with a lack of exposure to literacy materials and parental involvement during emergent literacy. Maternal education is a construct of socioeconomic status (SES) which has been shown to be a strong predictor of language development, reading acquisition, and educational outcomes for their children compared to household income and occupation. The goal of the current study was to relate maternal education to EFs and reading in children with RD compared to TRs using behavioral and neurobiological resting state fMRI data. The results show that higher maternal education is related to better EFs for children with RD and TRs. Additionally, higher maternal education is associated with decreased connectivity between attention system networks and regions related to visual word recognition and planning behaviors for children with RD greater than TRs (*P<0.05, FDR-corrected) . Our results suggest maternal education may have different roles in EFs for children with varying reading profiles. We conclude that higher maternal education may be associated with differences in the synchronization of attention system networks for better abilities related to reading for children with RD compared to TR's.

Disclosures: **P.B. Greenwood:** None. **E. Scott:** None. **J. Dudley:** None. **J. Hutton:** None. **M. DiFrancesco:** None. **R. Farah:** None. **T. Horowitz-Kraus:** None.

Digital Abstract Session

P313. Studies of Executive Function and Inhibitory Control in Human Subjects

Program #/Poster #: P313.02

Topic: H.04. Executive Functions

Support: NIH Intramural Grant Z1A AA 000130
NIH Intramural Grant Z1A AA 000466

Title: Latent factor analysis of the neurocognitive assessments of executive function in the Addictions Neuroclinical Assessment battery.

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Abstract: RATIONALE: The Addictions Neuroclinical Assessment (ANA) is a clinical framework that encompasses three neurofunctional domains, executive function, incentive salience, and negative emotionality, that are hypothesized to underlie the etiology and heterogeneity of alcohol use disorder (AUD). The ANA battery consists of self-reports and neurocognitive assessments. The present report focuses on the characterization of the executive function domain of the ANA battery. **METHODS:** Participants (n = 251; 43% female) completed a series of questionnaires and neurocognitive behavioral tasks assessing the executive function domain of the ANA. Behavioral tasks consisted of the Digit Span Task - Backwards (DST-B), Continuous Performance Task (CPT), Paced Auditory Serial Addition Test (PASAT), Stop Signal Reaction Time Task (SSRT), Manikin Mental Rotation Task (MAN), Approach Avoidance Task (AAT), and the Trail Making Task (TMT). A three-factor model of Executive Function (Updating, Shifting, Inhibition) was used to fit the data using confirmatory factor analysis. Latent factors were then used to evaluate for associations with demographic variables, drinking-related outcomes, and the self-reported impulsivity measures (UPPS-P). **RESULTS:** The three-factor model provided excellent fit to the data (CFI = .99, TLI = .99, RMSEA = .01). Indicators of the Updating factor consisted of performance on the DST-B, CPT, and PASAT, Shifting was indicated by performance on the MAN, AAT, and TMT, and Inhibition was indicated performance on the SSRT. Age and race were associated with all three latent factors (p's < 0.005). Sex was associated with Inhibition (p = 0.04), but not Updating or Shifting. Current AUD diagnosis was negatively associated with Updating and Shifting (p's < 0.005), but not Inhibition. Updating and Inhibition, but not Shifting, were associated with number of heavy drinking days (p's < 0.05). Finally, self-reported sensation seeking was associated with all three latent factors (p's < 0.014). Positive urgency was associated with Updating and Shifting (p's < 0.002), but not Inhibition. Negative urgency, premeditation, and perseverance was not significantly associated with any of the latent factors. **CONCLUSIONS:** Latent factor structure of the neurocognitive tasks in the ANA battery is consistent with a three-factor model of

Executive Function. Associations between the three factors and demographic variables, alcohol-related outcomes, and self-reported impulsivity were found. Together with the Incentive Salience and Negative Emotionality domains, the ANA battery can provide a framework for an improved understanding of the neurobiology of addiction.

Disclosures: T. Gunawan: None. **K. Parida:** None. **G.F. Koob:** None. **D. Goldman:** None. **E.L. Vogt:** None. **B. Manor:** None. **M.L. Schwandt:** None. **L.E. Kwako:** None. **T. Vinson:** None. **Y. Horneffer:** None. **D.T. George:** None. **N. Diazgranados:** None. **V.A. Ramchandani:** None.

Digital Abstract Session

P313. Studies of Executive Function and Inhibitory Control in Human Subjects

Program #/Poster #: P313.03

Topic: H.04. Executive Functions

Title: Related Neural Networks Underlie Suppression of Emotion, Memory, Motor Processes as Identified by Data-Driven Analysis

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Abstract: Goal-directed behavior benefits from self-regulation of cognitive and affective processes, such as emotional reactivity, memory retrieval, and prepotent motor response. Dysfunction in self-regulation is a common characteristic of many psychiatric disorders, such as PTSD and ADHD. This study sought to determine whether common neural regions are involved in the regulation of emotion, motor, and memory, and if a data-driven approach using independent components analysis (ICA) would successfully identify intrinsic connectivity networks (ICNs) that contribute to inhibitory regulation. Eighteen participants underwent neuroimaging while completing an emotion regulation (ER) task, a memory suppression (think/no-think; TNT) task, and a motor inhibition (stop signal; SS) task. In the ER task, participants were presented with negatively valenced pictures and instructed to either feel or inhibit their emotions. In the TNT task, participants received training to remember face-picture (cue-target) pairs. Participants were then presented with only the cue and told to either recall the picture or try to block its retrieval. In the SS task, participants were presented with either a stop- or go-signal and instructed to press a button only at the go-signal - not before the go-signal or at the stop-signal. ICA (CONN; MATLAB) was conducted on the neuroimaging data. Corresponding components were selected across task based on interrelated patterns of activation. Subsequently, ICNs were correlated with behavioral variables. ICA indicated a common medial prefrontal network, striatal network, and frontoparietal executive control network, as well as down-regulation in task-specific ROIs. These results illustrate that overlapping ICNs were exhibited across three distinct inhibitory regulation tasks as successfully identified through a data-driven approach (ICA).

Disclosures: **K.J. Hunt:** None. **L. Knight:** None. **B. Depue:** None.

Digital Abstract Session

P313. Studies of Executive Function and Inhibitory Control in Human Subjects

Program #/Poster #: P313.04

Topic: H.04. Executive Functions

Title: The effect of transcranial direct current stimulation of the pre-supplementary motor area on the imitation-inhibition task depends on the autism spectrum traits

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Abstract: Adequate inhibition of automatic imitative tendencies is crucial in our daily lives. Many studies have investigated the neural mechanism of control over automatic imitation and suggested two brain networks as the neural basis of imitation control; one is the theory-of-mind (ToM) network, and the other is that of multiple-demand (MD) network. However, evidence supporting the MD network theory, with intervention to modulate network activity, is scarce. In addition, an individual difference in the engagement of the two networks has been little considered, which is one possible reason that the previous findings about the neural basis of imitation control were not consistent. Thus, we investigated the engagement of the MD network in imitation inhibition using transcranial direct current stimulation (tDCS). Twenty-four right-handed human subjects (20 men, mean age 21.44 ± 1.25 years) participated in the study. We employed a single-blind and within-subject study design. Each participant underwent all three conditions (anodal, cathodal, and sham stimulation) on different days in a randomized order, counterbalanced across participation. Transcranial direct current stimulation was applied to the pre-supplementary motor area (pre-SMA), which is a node of the MD network, at 2mA, for 15 min. Following the stimulation, participants performed the imitation-inhibition task, which required them to lift either their index or middle finger in response to a number cue presented on the screen. We analyzed only the trials where the number cue was “1” and the index required to be lifted, because of the apparatus’s accuracy of measurement. As a result, tDCS on the pre-SMA did not significantly affect the performance of the imitation-inhibition task. However, Pearson’s correlation analysis revealed a significant negative correlation between the Autism-Spectrum Quotient (AQ) score, which is a measure of autism spectrum traits, and reaction time (RT) on incongruent trials, which requires the inhibition of the automatic imitative tendency under the anodal condition ($r = -0.49$, $n = 24$, $p = 0.01$). The AQ score significantly correlated neither with RT on the other trials under the anodal condition nor with RT on incongruent trials under the other conditions. Assuming that people with higher autism spectrum traits tend to engage the pre-SMA in the imitation-inhibition task, the anodal tDCS in this study could enhance the ability to inhibit automatic imitation of the participants with such traits. Therefore, our findings suggest that the brain regions involved in the imitation-inhibition task differ depending on autism spectrum traits.

Disclosures: **N. Matsuda:** None. **M.O. Abe:** None.

Digital Abstract Session

P313. Studies of Executive Function and Inhibitory Control in Human Subjects

Program #/Poster #: P313.05

Topic: H.04. Executive Functions

Support: NIH grant T32GM108540
NIH grant R01NS102201
NIH grant R01NS117753
Carver College of Medicine/Iowa Neuroscience Institute Research Programs of Excellence Awards

Title: Cortico-subcortical beta burst dynamics underlying action stopping in humans

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Abstract: Beta band activity in the neural field potential in both subcortical and cortical motor regions indexes net-inhibition of the motor system. This beta-band activity is typically studied as an average of many trials. However, recent work has demonstrated that motoric beta is in fact not a sustained but a transient, burst-like signal. During movement initiation and cancellation in tasks like the stop-signal task, these beta bursts reveal fine-grained dynamics that are lost when averaged over trials. During movement initiation, decreases in beta burst rates are observed over contralateral motor cortex. During movement cancellation, increases in bursting are observable over frontocentral regions, which are followed shortly by a surge in bursts over motor cortex (a purported signature of rapid re-instantiation of inhibition after a signal to cancel an action; Wessel 2020, *J Neurosci*). Furthermore, beta bursts are also known to exist in key subcortical motor regions such as the subthalamic nucleus (STN) and thalamus, but how these bursts relate to inhibitory processes and motor output is unclear. Here, we investigated the role of subcortical beta bursts during stopping, namely: a) whether bursts in the subcortex relate to movement cancellation, and b) how beta bursts in the subcortex influence burst rates, and thereby motor output, in the motor cortex. To address these aims, we collected a unique sample of data from surgical patients undergoing deep brain stimulator (DBS) implantation in the STN (N = 9), the ventral intermediate nucleus (VIM, N = 11) of the thalamus, or in both STN and VIM (N = 1). Patients completed an auditory stop-signal task peri-operatively while we recorded from bilateral DBS leads and subgaleal strips over primary motor cortices (M1). In the STN, we observed an increase in beta bursts within 100ms of the stop signal, which suggests beta bursts in the STN indeed relate to rapid deployment of motor inhibition during stopping. Moreover, our results seem to indicate this early deployment of inhibition in the STN produces downstream effects in M1. On average, bursts in STN following the stop signal were more likely to be followed within 25ms by a burst in M1. On the other hand, thalamic bursts were observed 25ms *after* M1 bursts

in stop trials, suggesting that the earliest stages of inhibition might not rely on the thalamus. Finally, we observed earlier beta bursting in STN than in VIM following stop signals in the overall sample and in a rare patient with implants in both regions, which supports existing network models of stopping and the theory that inhibitory control is ultimately accomplished via inhibition of motoric signaling in recurrent thalamocortical pathways.

Disclosures: D.A. Diesburg: None. **J.D.W. Greenlee:** None. **J.R. Wessel:** None.

Digital Abstract Session

P313. Studies of Executive Function and Inhibitory Control in Human Subjects

Program #/Poster #: P313.06

Topic: H.04. Executive Functions

Support: JSPS KAKENHI (Grant Number 18K07348 to T.O., 19K07807 to A.O.)
Takeda Science Foundation (to S.K.)

Title: Precision mapping of the right inferior frontal cortex of human brains and its function for response inhibition at the single-subject level

Authors: *A. SUDA[1,2], T. OSADA[1], A. OGAWA[1], M. TANAKA[1], K. KAMAGATA[3], S. AOKI[3], N. HATTORI[2], S. KONISHI[1];
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Abstract: The right inferior frontal cortex (IFC) is critical to response inhibition. The right IFC referred in the human studies of response inhibition is located in the posterior part of the inferior frontal gyrus and the surrounding regions, and consists of multiple areas that implement distinct functions. Recent studies using resting-state functional connectivity have parcellated the cerebral cortex and revealed across-subject variability of parcel-based cerebrocortical networks. However, how the right IFC of individual brains is functionally organized and what functional properties the IFC parcels possess regarding response inhibition remain elusive. In the present functional MRI study, precision functional mapping of individual human brains was adopted to the parcels in the right IFC to evaluate their functional properties related to response inhibition. Twenty right-handed subjects (10 men and 10 women, age: 26.6 ± 9.2 years (mean ± SD)) participated in the experiments. Based on group data of resting-state fMRI, six parcels were identified in the right IFC (vpIFC, dpIFC, IFJ, mIFC, vPCS, dPCS). Parcels in the right IFC of individual subjects were classified into the six “modules” or subsets of parcels, which were defined by the spatial pattern of the group-level parcel-cortex functional connectivity. The functional properties of each module were then evaluated in terms of the brain activation and its correlation with stop signal reaction time (SSRT) during the performance of the stop-signal task at the single-subject level. Each module revealed unique characteristics of brain activity and its correlation to behavior related to response inhibition. The brain activation was prominent in the parcels near the anterior bank of the precentral sulcus, the vpIFC module ($t(19) = 4.0$, $p < 0.001$,

one sample t-test), dpIFC module ($t(19) = 5.6$, $p < 0.001$, one sample t-test), IFJ module ($t(19) = 7.2$, $p < 0.001$, one sample t-test), and dPCS module ($t(19) = 7.8$, $p < 0.001$, one sample t-test). On the other hand, the correlation between the brain activity and SSRT was significant in the vpIFC module ($r = -0.53$, $p = 0.02$), mIFC module ($r = -0.49$, $p = 0.04$), and vPCS module ($r = -0.69$, $p = 0.004$). These results provide updated functional features of the IFC and demonstrate the importance of individual-focused approaches in studying response inhibition in the right IFC.

Disclosures: **A. Suda:** None. **T. Osada:** None. **A. Ogawa:** None. **M. Tanaka:** None. **K. Kamagata:** None. **S. Aoki:** None. **N. Hattori:** None. **S. Konishi:** None.

Digital Abstract Session

P313. Studies of Executive Function and Inhibitory Control in Human Subjects

Program #/Poster #: P313.07

Topic: H.04. Executive Functions

Support: NSERC

Title: The executive demand of response suppression is related to a preparatory hemodynamic response: Evidence from fTCD

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Abstract: Executive function represents a constellation of higher order processes composed of three core components: inhibitory control, working memory and cognitive flexibility. Antisaccades are goal directed eye movements mirror-symmetrical to a presented target that engage all three components of executive function. Notably, antisaccades result in longer and more variable reaction times (RTs) than their prosaccade (i.e., saccade to target location) counterparts. These deficits in antisaccade planning and control are related to the executive demands of withholding a prepotent response (i.e., response suppression) and inverting a target's coordinates (i.e., vector inversion). Additionally, previous literature has demonstrated that antisaccades produce a larger hemodynamic response when compared to their prosaccade counterparts. It is however, unclear whether response suppression and/or vector inversion contribute to this hemodynamic effect. Here, Experiment 1 required participants (N=22) complete blocks of stimulus-driven (SD) prosaccades and antisaccades. In Experiment 2, the same participants performed blocks of SD and minimally delayed (MD) prosaccades. MD prosaccades require participants look toward a target only after target offset, and therefore require the same response suppression demands as those present during antisaccades. However, MD prosaccades are performed independent of the executive demand of vector inversion. During Experiments 1 and 2, blood flow velocity in the middle cerebral artery was measured via functional transcranial Doppler ultrasound (fTCD) in order to provide an estimate of cerebral

blood flow. Experiment 1 demonstrated that RTs for antisaccades were reliably longer than those of SD prosaccades ($p<0.001$); however, there was no reliable difference between their respective hemodynamic responses ($p=0.59$). Experiment 2 showed that MD prosaccades produced longer RTs ($p<0.001$), and a reliably larger hemodynamic response ($p=0.01$) than SD prosaccades. Accordingly, our results provide evidence that response suppression, independent of the executive demand of vector inversion, is related to a reliably larger hemodynamic response when compared to a non-executive task.

Disclosures: B. Tari: None. **M. Shirzad:** None. **N.A. Badcock:** None. **G.R. Belfry:** None. **K. Shoemaker:** None. **M. Heath:** None.

Digital Abstract Session

P313. Studies of Executive Function and Inhibitory Control in Human Subjects

Program #/Poster #: P313.08

Topic: H.03. Decision Making

Title: A potential neural signature of impaired self-control pertinent to excessive asset trading: Low functional connectivity between dorsolateral and ventromedial prefrontal cortex

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Abstract: Objective: Neuroeconomic studies have begun to examine heuristics (i.e., simple rules that guide behavior while minimizing effort) and biases (i.e., systematic behavioral deviations from rational-choice models [Mobbs et al., 2018]). Haracz (2020) proposed that inadequate deliberation could bias traders toward simply using asset-price changes as guides, or heuristics, to popular or unpopular assets, thereby letting investors fall under the spell of a bubble or crash bias, respectively. These biases may promote trend-following investment strategies that yield price bubbles and crashes. Ogawa et al. (2014) used functional magnetic resonance imaging (fMRI) to study neural mechanisms of a bias to buy stocks (i.e., a bubble bias) while subjects traded in lab markets with stock-price bubbles. In the studied lab-market conditions, a bubble bias was found only during the bubble period of exposure to historical 2003-2008 Lehman Brothers stock-price changes. However, Ogawa et al. (2014) failed to discern evidence from their data suggesting that the bubble bias was associated with a neural signature of impaired self-control. Therefore, their findings are presently re-examined.Methods: In Figs. 2C and 2D of Ogawa et al. (2014), fMRI functional-connectivity data were examined to determine whether a bubble bias was associated with the previously reported neural signature of decreased self-control (Hare et al., 2014; Maier et al., 2015).Results: Among all lab-market conditions (Ogawa et al., 2014), low dorsolateral prefrontal cortex (DLPFC)-ventromedial PFC (VMPFC) connectivity was specific to the bubble period of Lehman Brothers stock prices. Among all conditions, the finding of a bubble bias was specific to the bubble period of Lehman Brothers stock prices.Conclusions: The low DLPFC-VMPFC connectivity associated with a bubble bias (Ogawa et al., 2014) resembles findings of low DLPFC-VMPFC connectivity linked to impaired

self-control when subjects were exposed to a food reward (Maier et al., 2015). Low DLPFC-VMPFC connectivity also was found when subjects failed to resist readily available monetary rewards in an intertemporal choice task (Hare et al., 2014). Therefore, subjects with a bubble bias show connectivity changes that may represent the neural signature of impaired self-control. Neuroimaging studies should determine whether less-profitable investors who trade stocks excessively (Barber and Odean, 2000, 2001, 2002) may show similarly decreased DLPFC-VMPFC connectivity.These results encourage further research on neural mechanisms underlying the bubble bias and traders' self-control problems that may promote asset-price bubbles.

Disclosures: J.L. Haracz: None.

Digital Abstract Session

P313. Studies of Executive Function and Inhibitory Control in Human Subjects

Program #/Poster #: P313.09

Topic: F.04. Stress and the Brain

Support: U.S. Air Force AFOSR Grant FA9550-17-1-0399

Title: Cognitive fatigue protein and microRNA biomarkers in saliva neuronal exosomes

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Abstract: Cognitive fatigue (CF) is an important factor in the efficiency and safety of performance-dependent tasks. It is postulated that the degree of CF experienced by an individual may be reflected by biomarkers in biofluids, such as saliva. Neuronal innervation of the salivary gland provides a mechanism through which neuron-derived exosomes are secreted into saliva, while harboring signals (proteins, lipids, mRNA, miRNA, DNA) that are indicative of changes in the cellular and molecular landscape of the central nervous system. The overall objective of this study was to elucidate protein and microRNA (miR) biomarkers of CF in salivary neuronal exosomes. Subjects were medical residents who underwent a cognitively-fatiguing 12-hour work shift. Before and after the work shift, CF was measured by the POMS questionnaire and saliva samples were obtained. Total exosomes and neuronal exosomes were isolated from saliva using magnetic beads coupled to antibodies against CD9+CD63+CD81 (markers for exosomes), or CD56 (NCAM, a neuronal surface marker), respectively. Total protein and miR were purified and subjected to proteomic (using mass spectrometry) or miR-omic (using Nanostring panel of 800 curated miRs) analysis. We identified many proteins and miRs that change (increase or decrease) significantly with CF in neuronal exosomes. Several of these proteins, AMY1A,

CSTB, and 14-3-3 protein zeta/delta, have been shown to be discordant between monozygotic twins discordant for chronic fatigue syndrome (Ceriga 2013). Many miRs change (increase or decrease) significantly with CF, including miR29a-3p, miR519, miR24-3p, and miR1296-3p. Additionally, several miRs known to regulate the expression of genes encoding three proteins were changed with CF (in the opposite direction to the direction that the protein changed with CF), namely the protein gene/miR pairs *PGK1*/miR3185, *PIGR*/miR642a-5p, and *YWHAZ*(14-3-3 protein zeta/delta)/miR134-3p. Bioinformatics identified several protein interaction networks and molecular pathways associated with the CF-changing proteins. These results identify potential biomarkers of CF in saliva exosomes and provide intracellular signaling (protein-protein and miR-gene/protein) pathways that may underly the effects of CF on the homeostatic physiology of neurons that innervate the salivary gland.

Disclosures: B. Teter: None. **C. Zhu:** None. **W. Cohn:** None. **J. Campagna:** None. **T. Bilousova:** None. **P. Spilman:** None. **G. Rong:** None. **F. Li :** None. **A. Avidan:** None. **D. Elashoff:** None. **J. Whitelegge:** None. **G. Cole:** None. **D. Wong:** None. **P. Bradshaw:** None. **V. John:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.01

Topic: H.05. Working Memory

Title: Episodic Memory Training Induces Functional Plasticity in PFC - Hippocampal Neural Circuitry

Authors: *J. M. KANE, O. COOK, K. HUNT, B. DEPUE;
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Abstract: Episodic Memory Training Induces Functional Plasticity in PFC – Hippocampal Neural Circuitry Kane, J., Cook, O., Hunt, K., Naaz, F. & Depue, B.University of Louisville Training programs for cognition are becoming more ubiquitous in mainstream society. However, there are relatively few studies exploring whether specific task related training leads to functional plasticity in the brain. Furthermore, understanding which neural regions show changes across training highlights important areas underlying the putative neural networks under investigation. We explored this question using fMRI before and after episodic memory training. Eighteen undergraduate students were recruited in a 5-day training study. Participants were scanned on day 1 and day 5. The episodic memory task and training consisted of mnemonic practice of cue-target paired associates (scenes and objects) or a single scene presented in isolation. A cued recall task (approximately 30 minutes later) required participants to either recall the name of the object when presented with the scene, or indicate if the scene was presented alone. Behaviorally, a significant increase in recall accuracy on day 5 compared to day 1 was found (Day 1= 67%; Day 5=75%). Functional analyses revealed greater activation in left ventral lateral prefrontal cortex (VLPFC), bilateral hippocampus (HC), bilateral inferior parietal

sulcus (IPS), and ventral visual processing stream (VVPS) during the task (Paired > Single) on day 5 compared to day 1. Using accuracy as a regressor in functional analyses revealed positive relationships with the same regions mentioned above, but also included the dorsal attentional network. The results indicate that training leads to increased activation of the neural regions associated with encoding/retrieval, attention, and visual processing.

Disclosures: J.M. Kane: None. **O. Cook:** None. **K. Hunt:** None. **B. Depue:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.02

Topic: H.05. Working Memory

Support: Lycaki-Young Funds (State of Michigan)
DMC Foundation
Ethel and James Flinn Foundation
Mark Cohen Neuroscience Endowment
National Institutes of Mental Health (MH111177)

Title: The recovery of directional relationships between brain nodes in schizophrenia by dynamic graphical models

Authors: *T. ATTISHA, S. BAAJOUR, M. GAPPY, A. CHOWDURY, J. STANLEY, V. A. DIWADKAR;
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Abstract: Schizophrenia (SCZ) is characterized by cognitive impairments relating to associative memory and learning and to brain networks that sub serve these processes. Here we co-opted a recently proposed method, Dynamic Graphical Models (DGM) (Schwab et al., 2018) to estimate directional network interactions induced during an associative learning paradigm (Stanley et al., 2017). DGMs can recover instantaneous directed "parent-child" relationships between nodes in a network (Smith et al., 2011) and unlike methods like granger causality, are less vulnerable to variations in hemodynamic lag. Here we applied DGM to a) estimate parent-child relationships between brain network nodes induced during a learning paradigm and b) summarized these relationships using graph theoretic measures.
fMRI data were collected for 79 subjects (46 SCZ; $18 \leq Age \leq 50$). The paradigm oscillated between Encoding, Passive Consolidation and Cued Retrieval Epochs (27 s), and participants were required to learn nine object - location association pairs. A conjunction analysis (across conditions and groups) was used to identify a commonly activated substrate of twelve bilateral nodes in hetero- and uni-modal cortex (Frontal-Hippocampal: hippocampus, DLPFC, dACC; Uni-Modal: SPC, ITG and FG). Time series for each participant and condition were averaged and submitted for DGM using publicly available code (in *R*)(Schwab et al., 2018). The directional relationships recovered using DGM were summarized using graph theoretic analyses

(Rubinov and Sporns, 2010).
DGM recovered a common set of directional relations in both HC and SCZ, most notably across hemispheric pairs (e.g., $dACC_L \leftarrow \rightarrow dACC_R$). High levels of complementarity (relationships in one group, but not the other) were observed between nodes in hetero-modal and unimodal regions. When the large swathes of binarized relationships across the network were summarized using graph theoretic measures, the following trend emerged. During Encoding, SCZ were characterized by lower outdegree centrality of the HPC, and the ITG, and lower indegree centrality of the dPFC and ITG. During Retrieval, SCZ were characterized by lower out-degree centrality of the dACC, but greater out-degree centrality of the dPFC.
A disruption of parent-child relationships within and across frontal and hippocampal networks, render our results consistent with previously hypothesized deficits in schizophrenia (Stephan et al., 2006) and complement recent investigations using granger causality (Baajour et al., 2020). These analyses reinforce the notion that the flow of information in brain networks is disrupted in schizophrenia, particularly during learning and memory.

Disclosures: T. Attisha: None. **S. Baajour:** None. **M. Gappy:** None. **A. Chowdury:** None. **J. Stanley:** None. **V.A. Diwadkar:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.03

Topic: H.05. Working Memory

Support: NSF2020844

Title: Multiple memory systems contribute to one-shot reinforcement learning

Authors: *A. R. ZOU[1], A. G. E. COLLINS[1,2];
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Abstract: Human learning recruits multiple cognitive processes like reinforcement learning (RL), episodic memory (EM), and working memory (WM). How can we disentangle their contributions? In the context of learning reward-driven stimulus-action associations, RL represents values of action choices relevant for future reward-driven decisions, EM stores information for long-term retrieval, and WM serves as temporary, capacity limited storage. However, what information is gated in WM's limited store remains unclear. To clarify WM's role and disentangle it from RL and EM, we designed a new one-shot reinforcement learning task. We manipulated the information seen before and after encoding to identify the relevant features of a stimulus-action association (e.g. feedback received, delay between stimulus presentations) for WM input and output gating. We used a test phase to parse out EM and RL contributions from WM's. In 16 training phase blocks, subjects used one-shot reward to learn new stimulus-action associations. In each independent block of 8 trials, they pressed one of two keys for each of 4 images shown sequentially, and received a pre-determined pseudo-randomized

positive (+1) or negative feedback (0) for each choice. The 4 images were shown again in a different order, and subjects had to press the correct key for the image based on the feedback received during the first presentation; they received no feedback on these trials (5-8). After the training phase, we assessed long-term retention in a test phase where all 64 stimuli were shown in randomized order; subjects were asked to press the correct key learned previously, and received no feedback. We analyzed 81 human adult subjects (48 female) who used the optimal choice policy during training. We found signatures of both RL and EM in test phase choices, where performance was dependent on training phase presentation order (primacy effect) interacting with feedback, indicating storage of both stimulus-choice and stimulus-choice-outcome associations. Trials 5-8 showed evidence of additional WM contributions in training, with high but delay-dependent performance. We found that feedback affected WM gating: subjects performed better for trials where they received +1, even though with only two keys, 0 provided the same correct key information. Furthermore, WM retrieval in training impacted RL/EM-dependent performance in the test phase. Our results show that our new protocol successfully parses out three memory systems contributing to reward-based learning. This opens the possibility to better understand each system's exact contributions, and particularly, what information WM prioritizes during learning.

Disclosures: A.R. Zou: None. **A.G.E. Collins:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.04

Topic: H.05. Working Memory

Support: Western Strategies Support for CIHR Success

Title: The effects of resistance exercise on cognition and hippocampal volume in older adults at-risk for diabetes

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Abstract: Background: Older adults with type 2 diabetes experience cognitive and brain decline beyond normative aging, including deficits in memory and executive function, and reduced hippocampal volume. Studies show that those at-risk for type 2 diabetes (based on high body mass index (BMI) and blood glucose levels) already experience some neurocognitive deficits. One lifestyle intervention that may improve these deficits is exercise. Six months of aerobic training has been found to improve cognitive and brain function in overweight and prediabetic older adults, however whether resistance training (RT) can produce comparable results in this population is unknown. **Methods:** We conducted a 26-week, thrice-weekly randomized controlled trial of RT in older adults at-risk for type 2 diabetes to assess the effects of RT on cognitive function and hippocampal volume. Participants (n = 24, mean age 68.7 ± 5.7

years; 50% female) were older adults with BMI ≥ 25 and/or fasting blood glucose in the prediabetic range (6.0-6.9 mmol/L). Participants were randomized into a progressive RT (weight training) group or a balance and tone (BAT) control group consisting of stretching and balance exercises. All participants were blinded to group allocation. At pre- and post-intervention (0 and 26 weeks, respectively), we measured cognitive performance via standardized neuropsychological tests and hippocampal volume via T1 weighted structural images from a 3T MRI scanner. **Results:** Compared to BAT training, 26 weeks of RT led to significant improvements in task switching and item memory. Both groups improved in conflict resolution and short-term verbal memory, but showed a decrease in hippocampal volume overtime. **Conclusions:** Findings from our exploratory study suggest that RT may improve specific cognitive functions in older adults at-risk for type 2 diabetes, however more research is needed on whether RT leads to structural brain changes. Unexpected improvements with BAT training suggests that stretching and balance exercises may also improve cognitive function in this population of older adults. This study was funded through Western Strategies Support for CIHR Success, and the authors have no competing interests to declare.

Disclosures: J. Furlano: None. **L. Nagamatsu:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.05

Topic: H.05. Working Memory

Support: AHA #16BGIA27250047

Title: Functional cortical activation changes and working memory loss in postmenopausal women with Type II Diabetes

Authors: *S. L. GORNIAK[1], A. E. HERNANDEZ[2], S. A. MONTERO HERNANDEZ[3], L. POLLONINI[3];
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Abstract: The overall aim of this project was to evaluate the relationship between deficits in working memory and functional cortical activation in postmenopausal females with Type II Diabetes (DM). Our recent studies have shown that adults with DM experience undiagnosed mild cognitive impairment, specifically in the domain of recall/working memory. Currently, no specific consistent structural deficits have been identified as a cause of such deficits in persons with DM. The reported behavioral deficits may be exacerbated in older adult females, who are at the highest risk of cardiovascular decline as DM is considered a cardiovascular disease. Twenty-one (21) community-dwelling DM patients (age = 65 ± 6 years, A1c = 7.5 ± 1.1) and twenty-one (21) age- and sex-matched healthy controls (age = 66 ± 6 years, A1c = 5.4 ± 0.3) were recruited and evaluated in this cross-sectional study. Working memory performance (via N-back) was evaluated while study participants donned cortical functional near-infrared spectroscopy devices

(fNIRS) in one testing session. Bilateral cortical regions of interest (ROIs) included: PFC, SMA, M1, S1, and Brodmann area 40. Health state, metabolic (e.g. glycated hemoglobin values, A_{1c}), and menopausal status data were collected. Deficits in working memory response accuracy ($p < 0.001$) and response time ($p < 0.05$) were found in the DM group as compared to controls. DM differences in HbO ($p < 0.05$) and HbR ($p < 0.05$) measures were found across all ROIs, such that the DM group exhibited significantly larger HbO and HbR values across all tasks. Increased HbO and HbR responses in the DM group were not associated with worsened health state measures (e.g. lipidemia, increased adiposity, etc). These data are the first to indicate functional cortical activation changes during cognitive tasks in postmenopausal females with DM, particularly in the performance of working memory tasks. These data indicate increased cortical activation across the entire cortex during memory tasks in persons with DM while simultaneously exhibiting symptoms of memory loss, suggesting neuronal dedifferentiation in postmenopausal women with DM.

Disclosures: **S.L. Gorniak:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; American Heart Association. **A.E. Hernandez:** None. **S.A. Montero Hernandez:** None. **L. Pollonini:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.06

Topic: H.05. Working Memory

Support: the Fonds de recherche du Québec en Santé (FRQS)
the Center de Recherche en Neuropsychologie et Cognition (CERNEC) at Université de Montréal

Title: Contribution of descending modulation to load dependant reduction of pain by working memory

Authors: *Z. DELDAR[1], I. BLANCHETTE[2], M. PICHE[3];
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Abstract: Contribution of descending modulation to load dependant reduction of pain by working memory

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Abstract Working memory (WM) engagement produces pain inhibition. However, it remains

unclear whether higher WM load increases this effect. The aim of this study was to investigate the interaction between WM load and pain inhibition by WM and examine the contribution of descending inhibition. Thirty-eight healthy volunteers were assigned to one of two n-back groups for which WM load was different (2-back or 3-back). The experimental protocol comprised five counterbalanced conditions (0-back, n-back, pain, 0-back with pain, and n-back with pain). Pain and the nociceptive flexion reflex (NFR) were evoked by transcutaneous electrical stimulation of the sural nerve. Pain was significantly different between conditions, but not between n-back groups. Both the 0-back and n-back tasks reduced pain compared with pain alone, but the n-back task produced stronger pain inhibition compared with the 0-back task. NFR amplitude was significantly different between conditions but not between n-back groups. NFR was inhibited by the 0-back and n-back tasks, with no difference between the two tasks. These findings indicate that pain inhibition by WM is increased by WM load, but only to a certain point. NFR inhibition by WM suggests that inhibition of pain by WM depends, at least in part, on descending inhibition.
Keywords: cognitive load, cognitive pain inhibition, nociceptive flexion reflex, attention, cognition.
Acknowledgments: This work was supported by a grant from the Natural Science and Engineering Research Council of Canada (MP: 06559). The contribution of MP was supported by the Fonds de recherche du Québec en Santé (FRQS). The contribution of Zoha Deldar was supported by the Department of Anatomy and the Center de Recherche en Neuropsychologie et Cognition (CERNEC) at Université de Montréal.
Conflicts of interest: The authors declare no competing interests and no relationship that may lead to any conflict of interest.

Disclosures: Z. Deldar: None. **I. Blanchette:** None. **M. Piche:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.07

Topic: H.05. Working Memory

Support: IN217918 from DGAPA-UNAM to AERC
IN215218, from DGAPA-UNAM to OPG
IA205218 from DGAPA-UNAM to MMD,
ISSSTE-010.2015 to JAGB

Title: The rs2180619 polymorphism of CB1 receptor is associated with Working Memory difficulty but not with maintenance or manipulation processes

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Abstract: Working memory (WM) is the ability to maintain (Mt) and manipulate (Mp) information in goal-directed behavior. WM performance is associated with the rs2180619 of CNR1 (the gene which encodes for cannabinoid receptor 1): GG genotype had lower accuracy (vs. AA genotype) as task difficulty increases; we hypothesized this effect could be due to the Mt or Mp processes. This study evaluated if the accuracy in Mt and Mp is associated with rs2180619 genotypes. Eighty-six healthy young volunteers responded to a delay-match to sample task with two conditions (Mt vs. Mp) and two types of trials (target and non-target). For the percentage of correct responses (%CR), no evidence of a genotype effect were found. However, for the type of trial: the %CR was significantly higher for the target than for non-target, suggesting this latter type were more difficult to discriminate than targets. For reaction times (RT), AG and GG genotypes, but not AA, had longer RT for non-target than for target trials. These results indicate that the effect in WM previously observed might not be related to a particular process in WM (i.e., Mt or Mp), but with the task demand, in which AG and GG genotypes are more prone to decrease the accuracy as difficulty rises.

Disclosures: T.V. Roman-Lopez: None. **U. Caballero-Sanchez:** None. **C. Sánchez-Gachuz:** None. **A. Franco-Rodriguez:** None. **J.A. Gonzalez-Barrios:** None. **S. Romero-Hidalgo:** None. **M. Mendez Diaz:** None. **O. Prospero-Garcia:** None. **A.E. Ruiz-Contreras:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.08

Topic: H.05. Working Memory

Support: Mrs Anne Uren and the Michael Uren Foundation
BBSRC BB/R022437/1

Title: Forced alternation in an air-lifted T maze for assessing spatial working memory in head-fixed mice

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Abstract: Head-fixation of awake mice provides for behavioral testing and neural recording *in vivo*. While mouse navigation and choice-based discrimination tasks under head-fixation have been demonstrated, forced alternation tasks (widely used for assessing short-term spatial working memory in rodents) using a T maze have not been demonstrated in head-fixed animals. Here we present the assessment of spatial working memory used a novel modified T maze based on an

air-lifted platform in head-fixed mice. Methods: We implanted mice (C57BL/6J, male, 2 months, N=5) with metal head plates. Animals were under recovery at least one week after head plate implantation surgeries. For this reinforced T maze task, water restriction was used to motivate maze traversal and learning motivation. After habituation to head-fixation in the apparatus (Mobile HomeCage Large, Neurotar Ltd) and the air-lifted T maze cage with phosphorescent visual cues and tactile cues, mice were trained for 45 min sessions twice daily for 11-16 consecutive days. Animals were transferred to the start arm for the first trial running and they were given one reward (4 µl 1% sucrose water) after either a right or left choice. They then ran back to the start arm, pushing through a one-way door. After the first trial choice, rewards were only given when animals entered the goal arm opposite to the arm visited on the previous trial. Using a magnetic position tracking system, we recorded rewards, movements and locations with real-time velocity; data were analyzed using MATLAB. Results: Head-fixed mice performed habituated to navigation behaviour after training day 6 and significantly improved alternation behaviour from training day 10. With further extension of training they were able to reach a 70% correct alternation score, a typical criterion for freely-moving rodents in traditional T maze. We found that the number of left and right turn trials were not significantly different, indicating the lack of direction preference by head-fixed mice in this T maze, as desired. We also found that the altered velocity patterns in the choice point, start and stem arm can predict the next trial decision. Our results suggest that the forced alternation T maze task in head-fixed mice may be useful for assessing spatial working memory. Our novel behavioral paradigm for spatial working memory shows promise for combination with longitudinal cellular resolution imaging *in vivo.*

Disclosures: **Y. Li:** None. **J. Song:** None. **M.A. Go:** None. **S.R. Schultz:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.09

Topic: H.05. Working Memory

Support: DID-M is supported by Conacyt-México PhD fellowship 629957
PAPIIT, UNAM grant number IN224019

Title: Oxidative stress generated by sub chronic hyperglycemia impairs spatial memory in CD1 mice

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Abstract: Oxidative stress generated by sub-chronic hyperglycemia impairs spatial memory in CD-1mice

AbstractDiabetes mellitus (DM) is a metabolic disorder that induces serious micro and macrovascular consequences, affecting several organs and tissues. Increasing evidence suggests that cognitive dysfunction is an important comorbidity of DM, that correlate with abnormal structural and functional brain magnetic resonance imaging in diabetic patients. Oxidative stress has been pointed out as one of the neurodegenerative processes implicated in cognitive loss caused by DM, since the CNS is characterized by an elevated production of free radicals (FR), due of the high consumption of O2 and glucose, making it vulnerable to the harmful effects of reactive oxygen species. Several mechanisms of neuronal damage induced by hyperglycemia like redox imbalance, mitochondrial dysfunction, brain insulin resistance, β-amyloid accumulation, as well as neuronal apoptosis has been described. There is a wide variation in the results reported in this field, mainly because of methodological differences between studies (e.g., DM model employed, the time lapse between hyperglycemia induction and behavioral evaluation, and the learning paradigms used). The aim of this study was to evaluate the effects of sub chronic hyperglycemia induced by systemic STZ administration on spatial and recognition memory, and to analyze its correlation with oxidative stress markers. Fifteen days after STZ-induced hyperglycemia, mice were evaluated for learning, short-term and long-term memory capabilities in three explicit memory tasks: 1) eight-arm radial maze, 2) buried food location test, or 3) novel object recognition task. Lipoperoxidation and antioxidant enzymes activity of superoxide dismutase (SOD) and catalase (CAT) were measured in brain regions primarily related to explicit memory. The results showed spatial memory deficits and short-term recognition memory impairment in diabetic mice. Moreover, an increased free radicals' level as well as a decreased enzymatic activity of both SOD and CAT in diabetic animal's hippocampus was observed. We conclude that hyperglycemia-induced free radicals increase, probably due to antioxidant enzymatic activity decline in key brain regions, contributed to the cognitive impairment observed in diabetic mice, however, biochemical and molecular studies are needed to understand the pathophysiological mechanisms underlaying these findings.

Disclosures: D.I. Del Moral: None. **C. Mendoza:** None. **T. Molina-Jiménez:** None. **C.J. Juárez-Portilla:** None. **T. Meza- Menchaca:** None. **M. Flores-Muñoz:** None. **O. López-Franco:** None. **A. Cortés- Sol:** None. **R.C. Zepeda:** None. **G. Roldán Roldán:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.10

Topic: H.05. Working Memory

Support: NIH/NIAAA 5R01AA027269-02

Title: Investigating spatial working memory deficits in a rodent model of Fetal Alcohol Spectrum Disorder

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Abstract: Fetal Alcohol Spectrum Disorder (FASD) is estimated to affect 1-5% of live births in the United States. FASD patients may show cognitive impairments such as working memory deficits. Alcohol exposure (AE) during the 3rd trimester of human pregnancies results in underdevelopment of key brain regions critical for working memory, specifically medial prefrontal cortex (mPFC) and hippocampus (HC). We have shown that there is damage to the thalamic nucleus reuniens (Re) in the rodent model of 3rd trimester AE. Re functions as a key intermediary node in the mPFC - HC circuit. Our lab has also demonstrated that Re is critical for mPFC - HC oscillatory synchrony and spatial working memory (SWM). From these findings, we hypothesized that AE during the brain growth spurt (BGS) in rats would result in SWM deficits that would be more pronounced with higher SWM demand. We compared choice accuracy between AE and sham intubated (SI) groups on a SWM-dependent delayed alternation (DA) task with different delay lengths in both male and female rats. The AE pups were given 5.25 g/kg/day ethanol in milk formula via intragastric intubations on postnatal days (PD) 4-9. The SI group received the same intubation as the AE but without any liquid. The handling and pre- training began on PD90 followed by a continuous alternation (CA) T-maze task. Upon reaching a choice accuracy criterion of 80% for two consecutive days on CA, the rats underwent 6 DA sessions, consisting of 12 each of 10-second (s), 30 s, and 60 s delay trials in a pseudorandom sequence. There were no significant differences in performance of the CA task between AE and SI groups for both sexes, which suggest the ability to learn tasks with a low SWM demand is not impaired by AE. Choice accuracy on the DA task was assessed using a mixed factor 2(sex) x 2(postnatal treatment) x 3 (delay) x 6 (day) ANOVA, with subsequent posthoc tests as appropriate. We find a trending sex x delay x postnatal treatment interaction ($F(1.63, 40.85)=1.71$, $p =.198$), which is driven by the fact that the AE group performed uniformly poorly on all delays, whereas SI group showed the expected delay-dependent decrease in choice accuracy. Interestingly, this trending pattern was observed in males ($n(AE) = 7$, $n(SI) =11$, $F(1.38, 22)=1.80$, $p =.194$), but not female groups ($n(AE) = 6$, $n(SI) =5$, $F(2, 18)=.43$, $p=.660$). These preliminary findings support the hypothesis that AE during the BGS leads to SWM deficits with increasing SWM demand, and our data suggest that males and females show different vulnerability. This study will lead to a better understanding of how damage to HC-Re-mPFC circuitry resulting from early AE impacts cognitive function later in life.

Disclosures: S. Kim: None. **A.Y. Klintsova:** None. **A.L. Griffin:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.11

Topic: H.05. Working Memory

Title: The Role of the MCH system in Regulation of the Length of Primary Neuronal Cilia

Authors: *W. S. ALHASSEN, B. ROBBINS, H. NGUYEN, T. MYINT, A. ALACHKAR; Univ. of California Irvine, Irvine, CA

Abstract: The MCH system has been implicated as a regulator of energy homeostasis and food intake, sleep, stress, mood, aggression, reward and cognition. MCH works by interacting with a G protein-coupled receptor, MCHR1, which has widespread distributions in the brain, particularly in the frontal cortex, amygdala, nucleus accumbens, septum and hippocampus providing an anatomical basis for an MCH role in the modulation of social, emotional, and cognitive functions. MCHR1 localizes to primary neuronal cilia, small microtubule backbones that protrude from the plasma membrane of almost every cell including neurons. Cilia act as cells' antennas and play crucial roles in cell signaling through detecting and transducing external stimuli, and regulating the proper cell differentiation and migration. Specifically, 77 cilia genes have been associated with neurological deficits. We asked whether the MCHR1 localization on cilia membranes is the basis of the uniqueness of these receptors in the ciliary signaling, through converging different sensory signals in the extracellular environment, and transmitting these signals into the cell. This is supported by the fact that the activation of MCHR1 causes shortening of the cilia length in *in vitro* experiments through cytoskeleton-related regulation. We aim to establish study the effect of the activation and inhibition of MCH system on cilia length in the brain. This study is one of the first to show *in vivo* manipulation of the MCH system regulates the length of primary neuronal cilia. This was done via pharmacological techniques in mice, including intracerebroventricular infusions of the MCH peptide and intraperitoneal injection of an MCHR1 antagonist, and MCHR1 knockout and MCH conditional knockout. We found that ex vivo MCH exposure shortens cilia length. We also found that cilia length is increased in MCH and MCHR1 deficit mice in the brain, including the prefrontal cortex, striatum, nucleus accumbens, and hippocampus. Infusions of MCH via i.c.v displayed a decrease in cilia length while administering an MCHR1 antagonist resulted in an increase in cilia length. In this study we were able to uncover the role between the MCH system in regulation of the length of primary neuronal cilia in mice and an *ex vivo* setting and concluding that the activation of MCH reduces cilia length while inhibition increases cilia length.

Disclosures: W.S. Alhassen: None. **B. Robbins:** None. **H. Nguyen:** None. **T. Myint:** None. **A. Alachkar:** None.

Digital Abstract Session

P314. Behavioral Studies: of Mice and Men

Program #/Poster #: P314.12

Topic: H.05. Working Memory

Support: PIE 16 00014
SAF 2015 70324 R
RYC 2011 08755

BFU 2012 33413

Title: History biases and working memory mechanisms alternate but do not interact in a rodent 2AFC task

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Abstract: Working memory (WM) is an essential brain function, which is central for cognition and severely impaired in multiple brain disorders. To investigate the underlying mechanisms limiting WM we developed a two-alternative delayed response task in which head-fixed mice are presented with an acoustic stimulus coming from either a left or a right side speaker. Animals had to remember the prospective response during a variable delay (duration D=0, 1, 3 and 10s) after which, they had to lick the corresponding side port (Fig. 1a).To understand how the length of the delay period affected the mice accuracy, we fitted a Generalized Linear Model that quantified the impact of the different factors on choice (Fig. 1b). The fitted model captured the forgetting process that took place during the delay as a reduction of stimulus impact on choice as D increased. Mice also showed (1) an idiosyncratic fixed bias showing a preference for one choice that was affected by the delay and (2) a delay-independent history bias favoring the previously rewarded side and opting away the previously unrewarded side. To recapitulate these two biases and their dependence (or lack of) on memory, we propose a two state hidden Markov Model in which animals switch between a WM and a reinforcement learning module (RL) (Fig. 1c). The WM module is a double well potential where the information about the planned response is degraded during the delay. Since the double well is asymmetric, this introduces a modulation of the side bias with the delay. The RL one is a logistic function that only depends on previous response and outcomes and is thus unaffected by the delay. Also, we observed an increase in the weights of history regressors with trial index within a session and a reduction of stimulus importance. This suggests that the transitions to the RL module are more frequent towards the end of the session. Lastly, we performed pharmacological manipulations where we inhibited NMDA receptors using NR2B antagonists. These animals showed a decrease of stimulus relevance that was taken over by a more prevalent presence of reinforcement learning strategies.

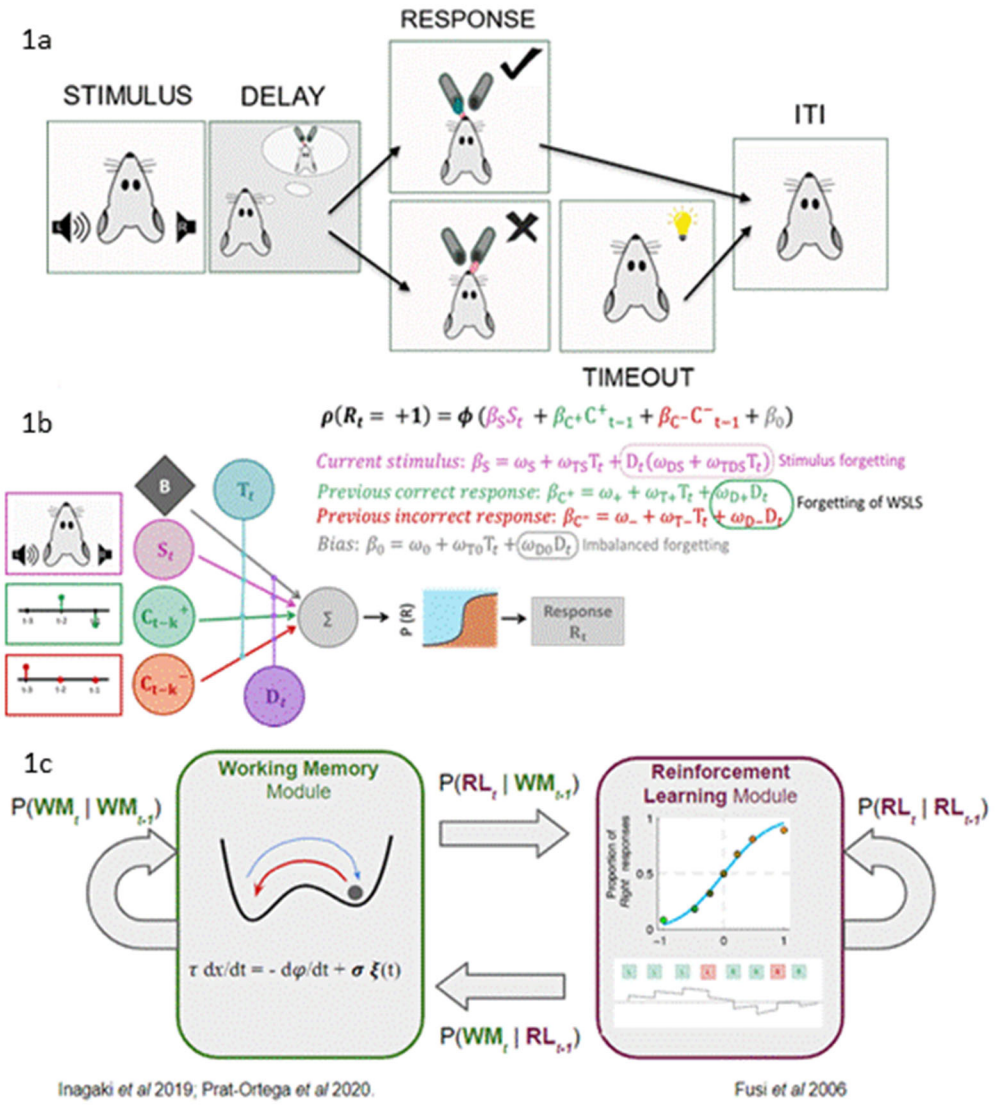

Figure 1. a. Schematic of the working memory two-alternative forced choice task. **b.** GLM model composed by 4 modules: current stimulus, previous correct and incorrect response and bias. **c.** Hidden Markov model proposed to explain observed behavior.

Disclosures: T. Ona-Jodar: None. **G. Prat-Ortega:** None. **A. Compte:** None. **C. Li:** None. **J. de la Rocha:** None. **J. Dalmau:** None.

Digital Abstract Session

P315. Working Memory: Prefrontal Mechanisms

Program #/Poster #: P315.01

Topic: H.05. Working Memory

Support: R01 NS105471
Children's Miracle Network Research Grant (2017-2018 #10)
Brain & Behavior Research Foundation Young Investigator Award (19469)

Title: Superior effect of a dopamine D_1 agonist compared with methylphenidate on prefrontal cortical modulated working memory

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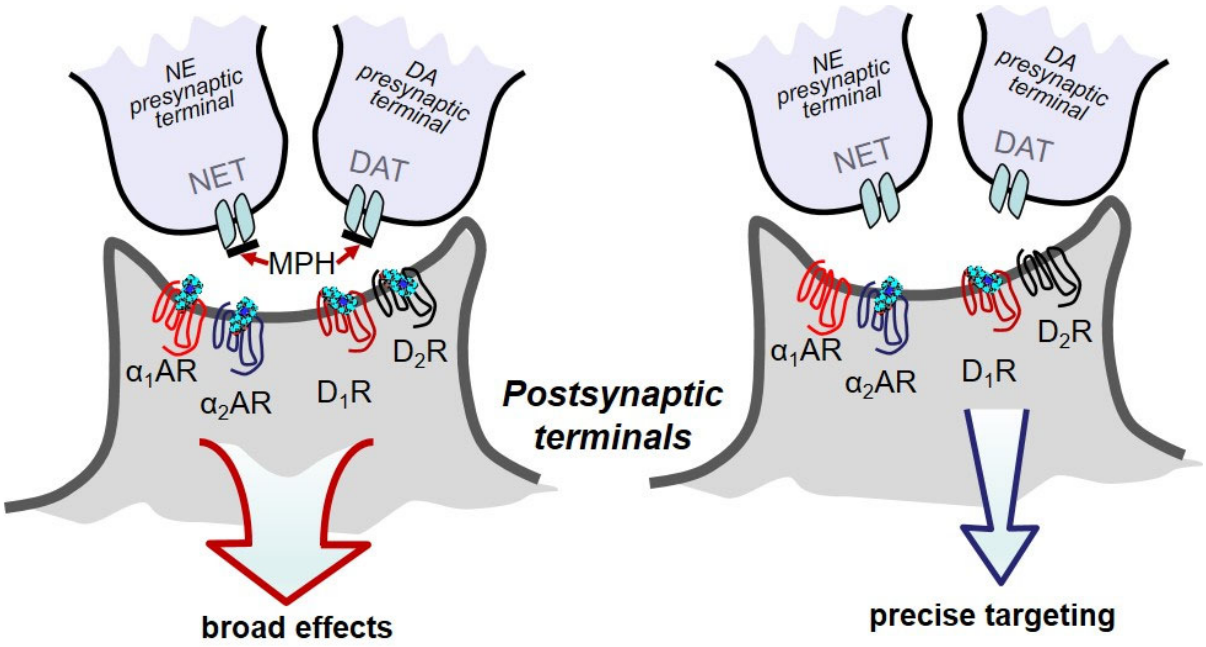
Abstract: Methylphenidate (MPH) is used widely to treat symptoms of attention-deficit/hyperactivity disorder (ADHD), but like other stimulants, has significant side effects. This study utilized a rodent model of spatial working memory (sWM) to compare the effects of MPH with a novel dopamine $D_{1/5}$ receptor (D_1R) agonist, 2-methyldihydrexidine (2MDHX). Administration of MPH caused a complex result in which 50% of the rats had improved sWM, whereas the other half showed impairment. Both improvement and impairment on sWM induced by MPH were eliminated when the D_1 antagonist SCH39266 was administered directly into the prefrontal cortex (PFC). The MPH-induced improvement in sWM was correlated negatively with sWM capacity, indicating rats with lower sWM capacity tended to improve more. Compared to MPH, 2MDHX showed greater improvement in sWM without significant impairment in any subject. These behavioral effects were reflected in firing rates of PFC neurons leading to alterations in both single neuron sensitivity to correct or error behavioral responses and in the power of local field potentials. Overall, 2MDHX was superior to MPH in decreasing the strength of neural sensitivity prospectively and increasing it retrospectively, and also in decreasing the oscillation power at alpha and beta bands. These results suggest that a $D_{1/5}$ agonist is more effective than MPH in regulating WM-related behavior and neural activities in the PFC, supporting the hypothesis that $D_{1/5}$ agonists would be superior to MPH or other stimulants as a treatment for ADHD.



Disclosures: **Y. Yang:** None. **R.B. Mailman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); RBM has interests in issued and pending patents related to dopamine D1 receptor mechanisms that constitute a conflict of interest for which there is oversight by the Penn State College of Medicine..

Digital Abstract Session

P315. Working Memory: Prefrontal Mechanisms

Program #/Poster #: P315.02

Topic: H.05. Working Memory

Support: NIH T32-AG049688
NIH R21-NS096936
NIH RF1-AG063837
NIH RF1-MH117040
NIH R01-MH110822

Title: Pathway-specific chemogenetic neuromodulation enhances working memory in rhesus monkeys

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Abstract: Acetylcholine plays a critical role in promoting neuronal plasticity and shaping synaptic connections throughout the brain, largely by projections from the basal forebrain

cholinergic system. Deficits in cholinergic neurotransmission impair cognitive performance and stimulation of cholinergic receptors in prefrontal cortex enhances task-related firing in working memory tasks. Although systemic pro-cholinergic drugs and electrical deep brain stimulation of the basal forebrain have shown to improve memory performance in nonhuman primates and humans, it has yet to be determined whether circuit-specific activation of a cholinergic neuromodulatory system can affect memory performance. Using a dual-viral intersectional approach, we transduced excitatory hM3D-Gq-coupled DREADDs (designer receptors exclusively activated by designer drugs) into the basal forebrain to reversibly activate projections from the nucleus basalis of Meynert to the dorsolateral prefrontal cortex. We tested whether circuit activation could overcome cognitive deficits caused by the muscarinic antagonist scopolamine (SCOP) in two young male rhesus monkeys. Monkeys were trained on the spatial-delayed response task to assess working memory performance after combined intramuscular injections of either SCOP and vehicle, the DREADD actuator deschloroclozapine (DCZ) and vehicle, or a combination of SCOP and DCZ. Working memory performance after DCZ did not differ from performance after vehicle in either monkey, and working memory was impaired in both monkeys after combined SCOP and vehicle injection. Notably, working memory was significantly improved after SCOP plus DCZ injection compared to performance after SCOP alone, indicating that the activation of the nucleus basalis to dorsolateral prefrontal circuit could offset working memory impairment caused by the cholinergic antagonist. These findings may provide a novel potential neurotherapeutic method for circuit-specific treatment of cognitive impairments seen in aging and disease that result from deficits in cholinergic neuromodulation.

Disclosures: **N.A. Upright:** None. **C. Elorette:** None. **A. Fujimoto:** None. **P.L. Croxson:** None. **B.E. Russ:** None. **P.H. Rudebeck:** None. **M.G. Baxter:** None.

Digital Abstract Session

P315. Working Memory: Prefrontal Mechanisms

Program #/Poster #: P315.03

Topic: H.05. Working Memory

Support: IBS-R002-A1; M.W.J.

Title: Distinct types of cortical projection neurons for working memory and timing

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Abstract: Intratelencephalic (IT) and pyramidal tract (PT) projection neurons, which project primarily to cortical and subcortical structures, respectively, represent two major types of cortical excitatory neurons. Currently, little is known about the division of labor between these different types of cortical projection neurons that allows them to support diverse cortical functions. Here,

we show that IT and PT neurons in the prefrontal cortex are differentially engaged in the maintenance of working memory and tracking the passage of time. We examined the discharge characteristics and inactivation effects of IT and PT neurons in the medial prefrontal cortex of mice performing a delayed response task. We found that IT, but not PT, neurons convey significant working memory-related signals during the delay period. We also found that the inactivation of IT, but not PT, neurons impairs behavioral performance. In contrast, PT neurons conveyed far more temporal information than IT neurons during the delay period. Our results indicate differential contributions of IT and PT neurons to the maintenance of working memory and the tracking of the passage of the delay period. These differences may reflect a more generalized functional segregation between neural networks involving IT and PT neurons. For example, the prefrontal cortex may contribute to high-order cognitive functions by sharing working memory-related signals with other cortical areas (IT neuronal network) and to the temporal organization of behavior by sending temporal information to its downstream subcortical structures (PT neuronal network).

Disclosures: J. Bae: None. **H. Jeong:** None. **C. Bae:** None. **H. Lee:** None. **S. Paik:** None. **M. Jung:** None.

Digital Abstract Session

P315. Working Memory: Prefrontal Mechanisms

Program #/Poster #: P315.04

Topic: H.05. Working Memory

Support: R01MH121480
The Whitehall Foundation
Schneider-Lesser Foundation

Title: Distributed representations in primate DLPFC with strategy use in a self-ordered working memory task

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Abstract: The limited capacity of working memory (WM) can be improved by implementing cognitive strategies. For instance, our previous work showed that incorrect responses decreased when monkeys adopted more routine selection patterns in a spatial self-ordered search task (Chiang and Wallis 2018). At the same time, the spatial tuning in neurons recorded in the dorsolateral prefrontal cortex (DLPFC) decreased with more stereotyped behavior. Here, we assessed how the same WM task is represented at the ensemble level, under these different self-generated behaviors. We re-analyzed data from two monkeys performing the self-ordered WM task with six identical visual targets. Subjects made a saccade to each target, one at a time in any

order, returning their eyes to the center after each selection. Reward was delivered only after the first visit to each target within a trial. When all targets had been visited, the reward contingency was reset and a new trial began. Therefore, monkeys had to use WM to track which targets had been visited and prepare for the next target selection. Blocks of 40 trials with the same target configuration enabled us to quantify selection patterns. We found that two task-relevant variables, target identity and saccade number, could be decoded from ensembles of simultaneously recorded DLPFC neurons. However, decoding performance was similar or better on trial blocks when monkeys used more stereotyped selection strategies, seemingly contradicting single unit results that found less spatial tuning. To investigate this further, we used multiple approaches to determine how much the single-unit information contributed to the neural ensembles under the self-generated behaviors. We found that single units contributed less and the size of optimal neural ensembles for decoding increased with stereotyped strategies, suggestive of a more distributed but less efficient neural code. Lastly, the unique design of resetting target-reward contingency in this self-ordered task allowed us to explore changes in dimensionality of neural ensembles with strategy use in a single trial. We found that the informative dimensionality was significant positively correlated to the WM loads, and negatively correlated to the levels of stereotyped strategy. In sum, our data indicate that a change in the local distribution of WM representations could parsimoniously explain how single unit and ensemble representations are manipulated by behavioral strategies. This model may also shed light on how locally distributed representations may overcome the limited capacity of WM.

Disclosures: F. Chiang: None. **E. Rich:** None. **J.D. Wallis:** None.

Digital Abstract Session

P315. Working Memory: Prefrontal Mechanisms

Program #/Poster #: P315.05

Topic: H.05. Working Memory

Support: none

Title: A spiking neuron model of encoding in working memory without gating

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Abstract: I describe a realistic network of integrate-and-fire neurons, simulating those found in connected neural structures, that addresses questions about how information is “loaded” or encoded into working memory, how salience signals that information be loaded, how the frontal cortex controls retention of salient information, which is stored in the parietal association cortex. This information is learned, such that when encountered again, it is reinstituted. The current model implements striatal-thalamo-cortical interactions resulting in a sustained dynamical state that permits maintenance of information in working memory. The current model achieves greater verisimilitude by virtue of more accurate modeling of neurons comprising the networks. Further,

it demonstrates how these operations may be the “control” portion of information maintenance and processing, while the information itself is represented in other association brain regions.

Disclosures: J.K. Kroger: None.

Digital Abstract Session

P315. Working Memory: Prefrontal Mechanisms

Program #/Poster #: P315.06

Topic: H.05. Working Memory

Support: NIH Grant AG060778

Title: Optogenetic investigation of medial prefrontal cortex contributions to delayed response working memory performance in young and aged rats

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Abstract: Aging is associated with deficits in multiple forms of cognition, including working memory mediated by the prefrontal cortex (PFC). Working memory refers to the maintenance of information over short delays, and studies employing lesion and intracerebral pharmacological approaches have identified a critical role for PFC. Working memory involves multiple phases, however, including acquisition, maintenance, and retrieval of information, and lesion and pharmacological approaches lack the temporal resolution to differentiate the role of PFC across these stages. Moreover, emerging evidence indicates that aging is associated with shifts in the neural substrates supporting specific cognitive functions, such that brain regions critical for a particular aspect of cognition in young adults may be less involved in older subjects. To determine how the PFC is engaged in distinct phases of working memory, and to determine whether its engagement differs across the lifespan, we used an optogenetic approach to selectively activate or inactivate pyramidal neurons in the medial PFC (mPFC) in young adult and aged rats. Young adult (6 mo.) and aged (24 mo.) male Fischer 344 x Brown Norway F1 hybrid rats were surgically implanted with bilateral guide cannulae targeting mPFC, through which AAVs bearing halorhodopsin, channelrhodopsin, or a control construct were delivered and optic fibers were implanted. Rats were subsequently trained in operant chambers in a three-stage delayed response working memory task. On each trial in this task, rats were presented with a sample lever (either left or right), a press on which initiated a delay phase ranging from 0-24 s. After the delay expired, both levers were extended and rats had to choose the same lever pressed during the sample phase. Rats were trained until reaching stable baseline performance, at which point they received laser light of the appropriate wavelength delivered to the mPFC. Aged rats showed impaired task accuracy relative to young under baseline conditions. Inactivation of

mPFC during the sample phase impaired accuracy in both age groups, particularly at longer delays. In contrast (and somewhat surprisingly), mPFC inactivation during the choice phase enhanced accuracy at the longest delay, particularly in aged rats. Experiments with mPFC activation and the control construct are currently ongoing. Considered together, the results suggest a complex role for mPFC in working memory that varies across both age and task phase.

Disclosures: **M. Faraji:** None. **C.M. Hernandez:** None. **A. Wheeler:** None. **K.M. Gonzalez:** None. **C.J. Frazier:** None. **B. Setlow:** None. **J.L. Bizon:** None.

Digital Abstract Session

P316. Working Memory in Humans

Program #/Poster #: P316.01

Topic: H.05. Working Memory

Support: CUNY ASRC Seed Grant (Round 4)
NIH R56MH116007

Title: Eeg connectivity signatures of load-dependent scene memory maintenance

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Abstract: Changes in load-dependent working memory (WM) activity have been studied in animals and humans using simple stimuli, such as shapes, but few studies have examined the neural connectivity changes that support maintenance for complex visual stimuli, such as scenes. We examined this question in an exploratory EEG analysis employing a within-subjects design with a WM load manipulation. Twenty-two participants completed a modified Sternberg WM task with either 5 (high-load) or 2 (low-load) color scene stimuli presented at encoding during 32-channel EEG recording. They were told to remember the scenes during a 6 sec delay period during which phase-scrambled scenes (PS) with similar color and spatial frequencies were presented as a perceptual baseline. Participants viewed the PS but were not required to remember them, so they served as interference. Since sensor-level estimates of connectivity are confounded by volume conduction, a time-frequency analysis (TSA) of delay period data was computed using a source montage (15 brain regions) with phase-locking value (PVL) as the connectivity measure. Performance was better for the low- compared to high-load condition (90.64% correct vs. 78.27%, Wilcoxon Signed Rank Test $p = .002$). Inspection of TSA absolute amplitudes during the delay period showed greater reductions in the alpha and beta across frontal, central, and parietal regions for the high- compared to the low-load condition (5 clusters, $p < 0.05$, permutation testing with a cluster-level multiple comparison correction). There were no significant differences in TSA in these oscillation bands. Analysis of the PVL revealed 3 significant clusters: 1) Increased PVL between left frontal and right temporoparietal regions in the theta and alpha bands for the low- compared to high-load condition, 2) increased PVL

between the right anterior temporal and the left central region in the alpha and lower beta bands, and 3) an increased PVL between the left anterior temporal and left temporoparietal regions in the theta, alpha, and lower beta bands. Low-load scene maintenance was better compared to high-load in terms of performance. Connectivity increased during low-load in frontoparietal regions implicated in top-down control of maintenance and in anterior temporal-temporoparietal regions implicated in semantic and language-based maintenance. The pattern of findings for the low-load could be explained by the availability of attentional resources to filter the PS with fewer stimuli being maintained. Additionally, some subjects may have employed a covert rehearsal strategy that is easier to implement during the delay in the low- compared to high-load condition.

Disclosures: C. Reichert Plaska: None. **B.A. Gomes:** None. **J. Ortega:** None. **T.M. Ellmore:** None.

Digital Abstract Session

P316. Working Memory in Humans

Program #/Poster #: P316.02

Topic: H.05. Working Memory

Support: NIH R56MH116007
CUNY ASRC Seed Grant (Round 4)

Title: Performance in a working memory task is predicted by delay period blink rate and changes in neural activity

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Abstract: Blink rate (BR) is found to be predictive of performance in incentive-based, spatial WM, and IQ based tasks; however few studies have looked at BR across the delay period of a WM paradigm. In the present study, we observed BR at different times during the delay period while participants maintained novel visual stimuli. Nineteen participants completed a modified Sternberg WM task while having their eyes tracked. Seven participants also had 32-channel scalp EEG recorded. EEG artifacts were removed by visual inspection and by using defined topographies. Participants completed three runs of the task, each consisting of 3 novel scenes during encoding, followed by a varied (2, 5, or 9 sec) delay period where participants maintained the scenes in memory. After the delay, a probe was presented and participants had to identify whether it was a new or previously presented image. Task performance was measured by the number of correct trials across all runs. BR was measured by rate of eye closure throughout the entire delay period. The first 2 seconds of all delay periods were used in the analysis and divided into four 500ms periods (0-500ms; 500-1000ms; 1000-1500ms; 1500-2000ms). Time frequency analysis (TFC) was run on EEG data collected during the entire 2-sec delay period. When BR

was examined as a factor of period, BR during the last 500ms of the delay and performance was found to be negatively correlated $r(13) = -.575$, $p = .025$. A polynomial regression model was computed with BR squared as the quadratic term and BR as the linear term. The results revealed BR during the last 500ms and performance had a significant non-linear correlation, $R^2 = .547$, $F(2,12) = 7.251$, $p = .009$. Examining individual TFC for lower performers (high and low BR) revealed an early (first 500ms) period of desynchronous and a later (last 500ms) alpha-theta band activity in the Parietal electrode (Pz), whereas for higher performers there was strong, early synchronous (first 500ms) alpha and lower beta band activity. High BR during later periods (last 500ms) of the delay in a WM task was correlated with low performance. The relationship between BR and performance was also found to be non-linear, where low and high BR during the later period of the delay was predictive of low performance. These results suggest that blinking is predictive of performance with higher and lower BRs leading to lower performance in a WM task. Additionally, results from TFC analysis suggest that early desynchronous alpha activity is related to worse WM performance and earlier synchronous alpha and lower beta is related to better performance.

Disclosures: J. Ortega: None. **C. Reichert Plaska:** None. **B.A. Gomes:** None. **T.M. Ellmore:** None.

Digital Abstract Session

P316. Working Memory in Humans

Program #/Poster #: P316.03

Topic: H.05. Working Memory

Support: NIH R56MH116007
CUNY ASRC Seed Grant (Round 4)

Title: A simultaneous eeg-fmri study of thalamic load-dependent working memory delay period activity

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Abstract: Working memory (WM) is an essential component of executive functions that depend on maintaining task-related information online for brief periods in both the presence and absence of interfering stimuli. Active maintenance occurs during the WM delay period. Previous studies have extensively documented prefrontal and posterior parietal cortex activity during the WM delay period, but the role of subcortical structures, including the thalamus, remains to be fully elucidated.
32-channel EEG data were acquired simultaneously with BOLD-fMRI in 24 participants during a variant of the Sternberg WM paradigm. Participants viewed novel outdoor scenes during an

encoding period followed by Fourier phase-scrambled stimuli with similar color and spatial frequency during a delay period. Scrambled scenes served as a perceptual baseline and interference during delay that participants were instructed to view while maintaining in memory the scenes presented at encoding. Event-related potentials (ERPs) were computed for scene onset at encoding (time window: 0-1000 msec) and for scrambled scenes during the 6 sec delay period (time window: 0-1000 msec). Bilateral dipoles were seeded during source analysis with solutions weighted by the simultaneously-acquired individual fMRI maps.
Each participant completed 50 low- and 50 high-load trials presented in separate runs with order randomized and counterbalanced. A related-samples Wilcoxon Signed Rank test showed a difference in accuracy between low-load (Mean = 90.6% correct, SD = 19.4) and high-load (78.3% correct, SD = 25.9) performance ($p=.002$).
Group analysis of individual dipole source estimates weighted by fMRI activation maps showed greater ERP amplitude in bilateral thalamus during the low- compared to high-load delay condition between 160 msecs and 390 msecs ($p = .003$) after delay period onset.
Group paired t-test difference maps between delay conditions were computed from fMRI data in AFNI. Among other regions, the left thalamus showed higher activation during low- compared to high-load ($t>3.67$, $p<.001$), consistent with the group ERP source results.
We found that thalamic activation was attenuated during high- compared to low-load memory maintenance, suggesting a sensory filtering role for thalamus during consolidation of stimuli in WM. The highest evoked activity occurred during the delay when fewer stimuli were maintained in the presence of interfering perceptual stimuli. The results support the idea that the thalamus plays a role in short-term memory maintenance by regulating processing of interfering stimuli.

Disclosures: **B.A. Gomes:** None. **C. Reichert Plaska:** None. **J. Ortega:** None. **T.M. Ellmore:** None.

Digital Abstract Session

P316. Working Memory in Humans

Program #/Poster #: P316.04

Topic: H.05. Working Memory

Support: University of Minnesota' MnDRIVE initiative
NIH Grant 1RF1MH124909

Title: Brain-state dependent neural mechanisms of transcranial alternating current stimulation in humans

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Abstract: Transcranial alternating current stimulation (TACS) targets electrophysiological activity in the human brain by applying weak time-varying electric currents in a safe, non-invasive manner through the scalp. Medical and cognitive scientists increasingly explore TACS as a possible tool to investigate the causal role of brain oscillations and normalize them in patients with cognitive deficits. However, progress is slowed down by an incomplete understanding of the fundamental mechanisms of TACS. The non-invasive nature of the intervention prevents direct neural recording in humans, while non-invasive human brain activity recordings, like electroencephalography, suffer from large TACS-induced artifacts. On the other hand, translational research in laboratory animals allows for direct invasive exploration; however, it provides little insights into the role of the neural state and cognitive load on the sensitivity of the brain to the external perturbations, such as TACS. Here we aim to answer this question by recording direct human brain activity in surgical epilepsy patients. Patients who met clinical guidelines and provided informed consent were implanted with a combination of intracranial subdural grids, strips, and depth electrodes according to medical needs (100-200 contacts). Intracranial recordings were acquired with a 256-channel amplifier, sampling at 40 kHz with a high dynamic range and 24-bit resolution. The patients performed a classical 2-back verbal working memory task to create the cognitive load condition or remained at rest while being subjected to TACS for 10 min at an average intensity of 1 mA and frequency in either the theta or beta range. The study is conducted in accordance with the Declaration of Helsinki and IRB regulations (University of Minnesota, MN). At this stage, three patients (one female, age range 30-40 y.o.) successfully performed all research procedures and were analyzed. Preliminary analysis using generalized linear mixed effect model with stimulation condition as a fixed effect (theta TACS, beta TACS, or sham) and patient's id as a random effect indicated a significant improvement in memory performance (d-prime metric) during the stimulation (glmm $F = 4.6$, $p = 0.02$). The positive effect emerged specifically in the beta range, known to be involved in working memory processes, which indicate its frequency-specific nature. The present preliminary findings highlight TACS potential in the study of brain oscillations and support continuing research to determine individual brain state-dependent sensitivity to the applied stimulation.

Disclosures: I. Alekseichuk: None. **C. Saiote:** None. **A. Herman:** None. **D. Darrow:** None. **R. McGovern:** None. **M. Park:** None. **A. Opitz:** None.

Digital Abstract Session

P316. Working Memory in Humans

Program #/Poster #: P316.05

Topic: H.05. Working Memory

Support: U01NS103792

Title: Common neural substrate for Working Memory maintenance and Long-Term Memory formation in humans

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Abstract: Long-Term Memory (LTM) and Working memory (WM) interact closely, but the neuronal basis of this interaction between declarative memories and WM remains poorly understood. As part of a BRAIN initiative consortium, we performed single-neuron recordings in the Medial Temporal Lobe (MTL) and the Medial Frontal cortex (MFC) in neurosurgical patients undergoing depth electrode implantation for localization of epileptic seizures. Patients performed a WM task followed by a LTM recognition memory task. During the WM task, we asked subjects to maintain novel images in memory for a few seconds, followed by a probe. Recognition of these images was then subsequently tested in the LTM task. Out of 35 sessions acquired, we analyzed 26 of these sessions; behavioral accuracy was 93% and 72% for the WM and LTM task, respectively. In the MTL, 16.3% of cells were selective for the category of the item(s) held in WM during maintenance. The strength of the category-selective activity during maintenance predicted whether an item was subsequently remembered or forgotten in the LTM recognition task, thereby linking persistent activity during WM maintenance to LTM formation. Further supporting this result, the extent of persistent activity predicted the strength of the response of memory-selective old-new cells during the recognition task. Single-trial decoding showed that it was possible to predict whether a given image will be later remembered based on the activity of category-selective neurons during WM maintenance (accuracy 57.6%, p=0.013). Analysis of a SVM decoder trained to read-out the category currently held in WM revealed that neural activity in trials that were later remembered was located further away from the decoder hyperplane compared to neural activity in trials later forgotten (SVM geometric margin for later remembered trials was 0.43 vs. forgotten 0.1; p=0.0005). Together, these results show a direct link between the activity of neurons in the MTL during WM and the subsequent strength of declarative memories and demonstrate that category-selective neuronal activity acts as a common neural substrate for both WM maintenance and LTM formation.

Disclosures: J. Kaminski: None. **N. Chandravadia:** None. **A.G.P. Schjetnan:** None. **Y. Salimpour:** None. **I. Reucroft:** None. **C. Reed:** None. **J.M. Chung:** None. **S. Kalia:** None. **W. Anderson:** None. **T.A. Valiante:** None. **A.N. Mamelak:** None. **U. Rutishauser:** None.

Digital Abstract Session

P316. Working Memory in Humans

Program #/Poster #: P316.06

Topic: H.05. Working Memory

Support: NIH Grant MH063901

Title: Long-term learning transforms prefrontal cortex selectivity during working memory

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Abstract: The neural representations supporting goal-directed behavior may change with learning. However, uncovering how long-term memory influences working memory (WM) is difficult because of inconsistencies across studies, species, and recording techniques. For instance, non-human primate (NHP) electrophysiology research finds that lateral prefrontal cortex (lPFC) circuitry maintains WM representations, while human neuroimaging suggests that such content is instead stored in sensory cortices. To bridge between these findings, we implemented a unique, longitudinal functional MRI (fMRI) protocol to test the influence of long-term learning on WM processes in each of three human participants. Across three months, each participant was trained on (1) a serial reaction time (SRT) task, wherein complex fractal stimuli were associated with arbitrary response mappings and embedded within probabilistic sequences, and (2) a delayed recognition task that probed WM for trained or novel stimuli. Participants showed a stimulus learning benefit, as they became increasingly faster and more accurate in the WM task for trained, but not novel, fractals. Neurally, long-term learning influenced the activity patterns associated with WM maintenance. Across training, voxelwise WM delay period activity became more distributed throughout lPFC, paralleling a recent finding that spiking activity across NHP lPFC also becomes more distributed with training (Qi et al., 2019). Individual voxels in lPFC also showed increases in stimulus selectivity among the trained fractals. Pattern similarity analyses of WM delay activity demonstrate that across learning, item-level representations emerged within lPFC but not in sensory cortices. Representations of stimulus sequences that were learned over time in the SRT task, but unrelated to the goals of the WM task, also emerged in lPFC during WM. This work helps reconcile disparate findings across species and scales, showing that with learning, human prefrontal areas change their selectivity during WM to develop stimulus-selective properties most commonly found at the neuronal scale in NHP recordings. Further, this dense sampling of changing memory substrates establishes novel evidence for the influence of long-term memory on WM maintenance processes.

Disclosures: J.A. Miller: None. **A. Tambini:** None. **A. Kiyonaga:** None. **M. D'Esposito:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.01

Topic: H.06. Social Cognition

Title: The Default Mode Neural Network Study by rsfMRI in the spidermonkey (Ateles geoffroyi) from an evolutionary perspective

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Abstract: Objective: Characterize the organization and connectivity of the default mode neural network (DMN) in a sample of spider monkeys. **Background:** The DMN consists of an aggregate of dynamic centers and subsystems that play an important role in cognitive processes of “internal mentalization”. Increases its activity during cognitive tasks directed towards objectives and require forms of self-generated thought. We measure the blood oxygenation-dependent (BOLD) contrast signal to register the functional connectivity among different brain regions. Research on the underlying mechanisms, organization and alteration of brain neural networks requires homologous animal models that allow neurophysiological and phylogenetic records. **Desing/Methods:** Whole brain images were obtained from three healthy adult spider monkeys *(Ateles geoffroyi),* followed by resting state sequences. The fMRI data was processed first and then an independent component analysis (ICA) grouped was performed to identify the DMN. The results were compared with those of human studies and the group-level ICA yielded two bilateral and symetrical default-mode networks. **Results:** The group-level ICA yielded two bilateral and symmetrical DMN. Components of the networks were found to be extending into ventral medial prefrontal cortex (*vmPFC*) and posterior cingulate cortex (*PCC*), as well as precuneus and parts of the parietal cortex. The correlated areas suggest that some elements of network may be conserved across primate species. **Conclusions:** The animal studies, although still incipient, are fundamental instruments that have contributed to the understanding of the neural basis underlying the evolution and function of this complex system. The study of the neural networks in vmPFC and PCC, precuneus and parietal cortex, they can provide basic information for the investigation of neurological and psychiatric disorders associated with their cognitive processes. Re-evaluation of these disorders from an evolutionary perspective would generate insights into our comprehension, and open new line of research unveiling the biological mechanisms and thereby prevention and treatments.

Disclosures: Ó.A. Díaz-Pineda: None. **D.A. Platas-Neri:** None. **S.S. Hidalgo-Tobón:** None. **F. Chico-Ponce de León:** None. **B. De Celis Alonso:** None. **J. Muñoz-Delgado:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.02

Topic: H.06. Social Cognition

Support: Ministry of Science and Technology grant 105-2311-B-007-012-MY3
Ministry of Science and Technology 107-2218-E007-033grant

Title: Coordination through inhibition: control of stabilizing and updating circuits in spatial orientation working memory

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Abstract: The Buridan's paradigm is widely used for testing visual spatial orientation and spatial learning of fruit fly. In this study, we firstly explored the mechanism of such fixation performance. Meanwhile, we proved that the wing-shortened fly prefers dark objects instead of salient objects or high-contrast of strips and screen. Then we tested flies' spatial orientation and memory. The results revealed that for the naïve fruit fly, after stimulus exposed for over 60 s, the fly maintained fixation performance for a short time, and such fixation period was positively correlated with the stimulus exposure duration. In the next step, we discussed neural functions in fly ellipsoid body (EB) and protocerebral bridge (PB). The EB is a ring-shaped region contained several sub-ring. In our theoretical model, the C circuit and P circuit play important roles in navigation. The C circuit stabilizes the orientation signal in EB region, while P circuit updates neural activity when rotating. The alternative work of such two circuits maintains neural activity in fly brain. Our results showed that manipulating either of such region produces distinct behavioral deficits, which also consisted with the theoretical model.

Disclosures: R. Han: None. **H. Huang:** None. **T. Wei:** None. **S. Zeng:** None. **C. Lo:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.03

Topic: H.06. Social Cognition

Support: NSERC

Title: Interplay between estrogen & oxytocin receptors on rapid social recognition

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Abstract: Estrogens and oxytocin (OT) play important roles in mediating social recognition (SR). For example, when the genes for the estrogen receptors, OT, or the OT receptor are "knocked out", SR is impaired. From these experiments showing that both estrogens and OT are needed for SR, it was proposed that they could be interacting to mediate it. A model of this

interaction has been proposed, where estrogens bind to the estrogen receptors located in the paraventricular nucleus of the hypothalamus (PVN) to facilitate OT production and/or release. This OT then acts in the medial amygdala (MeA), a region important for SR since it receives direct projections from the olfactory bulbs. The OT will bind to the OT receptor in the MeA and allow the incoming olfactory information to be used to recognize a familiar conspecific, thus mediating SR. We have previously tested this model and found that administering 17b-estradiol (E2) into the PVN can rapidly facilitate SR, and that administering an OT receptor antagonist into the MeA can block the rapid facilitation of SR by E2 in the PVN. These results showed support for the proposed model of an interplay between estrogens and OT in the mediation of SR. Our current experiments are determining which estrogen receptor (ER) is mediating this interplay. Two of the ERs are highly expressed in the PVN: ER beta (ERb) and the G-protein coupled ER (GPER). We have found that the administration of agonists for either ERβ or GPER (DPN and G1 respectively) into the PVN can rapidly facilitate SR. Our current experiment is investigating if the OT receptor antagonist in the MeA can also block the rapid facilitation of SR by the ER agonists in the PVN. Preliminary results have shown that the OT receptor antagonist in the MeA can block the rapid facilitation of SR by the GPER agonist G1 in the PVN, but not the ERb agonist DPN. These results suggest that in the PVN, GPER, but not ERb, is mediating the interplay between estrogens and OT on SR.

Disclosures: **P. Paletta:** None. **G. Dhuga:** None. **E. Watson:** None. **E. Choleris:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.04

Topic: H.06. Social Cognition

Support: NIH Grant MH121009
Conte center Grant 2P50MH100023-06

Title: Autonomic state predicts baseline firing rates and response properties of neurons in the amygdala and somatosensory cortex

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Abstract: Neural responses to environmental stimuli depend not only on the specific features of the stimulus but also on the behavioral state of the organism. With this in mind, we explored the potential role of autonomic state in modulating the neuronal representations of discriminative vs affective elements of touch. To this end, we simultaneously monitored heart rate and single unit activity from the primary somatosensory cortex (SI), which carries discriminative tactile

representations, and the amygdala, which carries affective tactile representations, under varying levels of autonomic arousal. To manipulate arousal, we delivered to regions of the monkey's face and head either gentle, non-aversive puffs of air or sweeping touches from a human handler meant to mimic social grooming. Heart rate was significantly lower when the monkeys received social touch (puff median = 119, touch = 116 BPM, Wilcoxon signed rank $p < 2e-7$, N = 26 sessions), indicative of a decreased autonomic response. Surprisingly, changes in heart rate resulted in concomitant changes in baseline firing rate in both SI and the amygdala but the directionality of these relationships was reversed between the two areas. Across the population of SI neurons, lower heart rate was associated with lower baseline activity (linear regression median slope = 0.21, $p < 4e-17$, N = 135 cells) whereas in the amygdala lower heart rate was associated with higher baseline activity (median slope = -0.05, $p < 0.0003$, N = 430). Next, we examined the relationship between heart rate and stimulus-evoked responses in SI and the amygdala. In both brain areas, we found that the fidelity of the stimulus-evoked responses was inversely related to baseline activity, where lower baselines were associated with more reliable stimulus responses (SI median slope = -0.85, $p < 3e-32$, N = 195; amygdala median slope = -0.95, $p < 8e-89$, N = 633). Thus, sensory representations were enhanced in SI and suppressed in the amygdala when the monkey was in an autonomic state characterized by a lower heart rate. In fact, at low arousal, neurons in the amygdala showed prolonged tonic elevation in firing rates that spanned multiple stimulus events rather than responding phasically to individual tactile stimuli ($p < 0.02$). We hypothesize that responses in the amygdala encode the overall affective state of the animal and not the individual stimuli that induce the state whereas responses in SI encode individual stimuli and these stimulus representations are enhanced in low arousal states.

Disclosures: **A.B. Martin:** None. **R. Andersen:** None. **L. Hillier:** None. **G. Somasekhar:** None. **M. Cardenas:** None. **N.N. Jacobson:** None. **S.J. Bensmaia:** None. **A.J. Fuglevand:** None. **K.M. Gothard:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.05

Topic: H.06. Social Cognition

Support: Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: The effects of testosterone on pro-social and pro-aggressive behaviours in male mice

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Abstract: Exhibiting successfully behavioural plasticity for appropriate responses to dynamic social environments is crucial to the survival of the individuals of social species. Among the social skills required for living in groups, social recognition (SR) is one of particular interest because it allows to identify previous investigated individuals and to emit appropriate pro-social

or -aggressive behaviours. Several studies highlighted the role of vasopressin (AVP) in aggressive and social behaviours, including SR. The vasopressinergic circuit includes highly sexually differentiated brain regions, such as the bed nucleus of the stria terminalis (BNST) and the lateral septum (LS), which show more AVP neurons and fibers in males than in females. Androgens play a key role in the organization and activation of this system. Furthermore, brain levels of sex hormones are rapidly impacted by social interactions, quickly regulating behaviours. The androgens/AVP interplay impact social and aggressive behaviours, even if the mechanisms involved are currently poorly understood. To elucidate testosterone (T)'s rapid effects on AVP neurons in the BNST of male mice, adult castrated male mice were intracerebrally infused with one of 4 doses of T (0.25, 0.5, 0.75, 1µg of T in 0.5µl of DMSO/side) in the BNST. They were then tested in a 'difficult' SR paradigm, in which castrated mice are impaired, and in a resident-intruder (RI) paradigm to assess aggressive behaviours. The 'difficult' version of the SR paradigm consisted in the exposure, 15 min after the infusion, to two 5-min sample phases with the same 2 stimulus mice and one 5-min test, whereas one of the stimuli was exchanged with a novel one. Each phase was separated by 5-min rests. For the RI paradigm, the focal animal was exposed to a castrated mouse for 5 min at 2 different time points (35 and 120 min) to evaluate rapid and genomic effects of T. Results revealed that T in the BNST rapidly facilitates SR, with male mice preferentially investigating a novel over a familiar castrated mouse with an inverted U-shaped dose-response. Moreover, even if T infusions did not elicit overt aggression towards the intruders, the highest doses altered the emission of dominants and submissive behaviours. The mice receiving 0.75 or 1µg of T increased their dominance score, with long-lasting effects in the 1µg group. Further investigations will determine the potential mechanisms, by evaluating the role of T metabolites and whether T modulates AVP release in the LS, potentially identifying a BNST-LS brain circuit of T/AVP interplay in the regulation of male social cognition.

Disclosures: **D. Aspesi:** None. **Z.R. Brill:** None. **G. Guillaume:** None. **E. Choleris:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.06

Topic: H.06. Social Cognition

Support: 2P50MH100023-06

Title: Longer looking time at high-ranking individuals depicted in videos of virtual social interactions

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Abstract: The status of interacting partners is a key determinant of social behavior, yet how the brain extracts this information from the behavior of others is unknown. The study of the neural representation of social status requires experimental manipulations that are seldomly possible under naturalistic conditions. To generate an adequate stimulus set we selected from videos of natural macaques' behavior segments that could be juxtaposed to mimic dominant-subordinate interaction between pairs of monkeys. In these videos (of 15-second duration), dominant animals threaten subordinates, who then react to these threats with appeasing displays. We generated one female and two male hierarchies with four members in each, allowing for six pairwise interactions. In these videos, the highest and lowest status individuals show only threatening and appeasing facial expressions, but the mid-ranking individuals show both dominant and submissive behaviors. Based on previous work in monkeys (Deaner *et al.*, 2005) we expected that the higher the social status of an individual, the more time the viewer monkey would spend looking at them. Indeed, a one-way ANOVA of looking time data from one male viewer monkey showed a significant difference in looking time with respect to hierarchy position ($F_{3,2624}$ = 152.9, $p < 0.0001$, n = 657 videos; Bonferroni post-hoc tests: $p < 0.001$ for all comparisons). We verified that the longer looking times elicited by dominant individuals are not explained by facial expressions, left-right side bias, or overall motion in the video. Ongoing experiments are expected to confirm these results in additional male and female monkeys. Furthermore, based on previous attempts to identify the neural corelates of social status (Munuera *et al.,* 2018) we expect neural activity in the amygdala to vary as we manipulate the perceived status of each individual.

Disclosures: S. Lee: None. **T.M. Champ:** None. **B.T. Seaton:** None. **L. Hillier:** None. **D. O'Neill:** None. **A.B. Martin:** None. **K.M. Gothard:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.07

Topic: H.06. Social Cognition

Support: BFU2017-89615-P
NIH R01 MH114269

Title: Altered in vivo cerebellar response to sensory inputs in the Cntnap2 mouse model of autism

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Abstract: Cerebellar alterations are among the most replicated neuropathological findings in postmortem brain samples of individuals with autism. Besides, the cerebellum has been shown to

be involved in several non-motor related behaviors, such as sensory processing, which is usually affected in autism. However, how these alterations contribute to the autism spectrum phenotype remains unknown. In this study, we performed an electrophysiological characterization of the cortical cerebellar circuit in relation to sensory processing in the Cntnap2 Knock-out mouse model of autism, a model previously reported to have cerebellar-learning dysfunction. We performed local field potentials (LFP) and extracellular unitary recordings of identified Purkinje cells (PC) in the cerebellar area Crus I/II of alert mice during spontaneous activity and in response to whisker stimulation. Our results show striking differences in Cntnap2-KO mouse compared to wild type both in LFP and in extracellular PC recording. The components of sensory evoked potentials were altered in KO mice, showing aberrant components across time compared to WT. These aberrant components seem to have correlations with PC simple spikes. Further, a thorough analysis of PC activity reveals a higher variability in the firing pattern in mutant mice. We conclude that Cntnap2 mice show dysfunctional cerebellar sensory processing due to asynchronous response of PC to sensory inputs.

Disclosures: **M. Fernandez:** None. **O. Penagarikano:** None. **T. Sierra:** None. **J. Márquez-Ruiz:** None. **C. Sánchez-León:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.08

Topic: H.06. Social Cognition

Support: IBS-R002-A1
MH099073, AG067008
2017M3C7A1048089
20-BR-04-03

Title: A role of anterior cingulate cortex in the emergence of worker-parasite relationship

Authors: ***S. AHN**[1], Y. KANG[2], J. LEE[2], S. JEONG[4], Y. LEE[4], S. LEE[4], J. KOO[4,5], J. J. KIM[6], M. JUNG[1,3];
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Abstract: We studied the brain mechanisms underlying action selection in a social dilemma setting where individuals' credits are unfairly distributed among group members. A stable 'worker-parasite' relationship developed when three individually operant-conditioned rats were placed together in a Skinner box equipped with response lever and food dispenser on opposite sides. Specifically, one rat, the 'worker,' engaged in lever-pressing while the other 'parasitic'

rats profited by crowding the feeder in anticipation. Anatomically, c-Fos expression in the anterior cingulate cortex (ACC) was significantly higher in worker rats than in parasite rats. Functionally, ACC inactivation suppressed the worker's lever press behavior under social, but not individual, settings. Genetically, $GABA_A$ receptor- and potassium channel-related mRNA expressions decreased in the worker's ACC. These findings indicate the requirement of ACC activation for the expression of exploitable effortful behavior in a social dilemma setting, which could be mediated by molecular pathways involving $GABA_A$ receptor/potassium channel proteins.

Disclosures: **S. Ahn:** None. **Y. Kang:** None. **J. Lee:** None. **S. Jeong:** None. **Y. Lee:** None. **S. Lee:** None. **J. Koo:** None. **J.J. Kim:** None. **M. Jung:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.09

Topic: H.06. Social Cognition

Support: NSERC

Title: Dorsal hippocampal D2-type dopamine facilitated social learning in male mice regulated by estrogenic mechanisms

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Abstract: Social learning is one of the most common forms of learning in almost all species and can be defined as learning that occurs via social interaction and/or social observation (Galef, 1988). The social transmission of food preference (STFP) paradigm is commonly used to test social learning in animals. During the STFP, intra-dorsal hippocampal (HPC) infusions of the D2-type dopamine (DA) receptor antagonist raclopride blocked social learning in female but not male mice, suggesting an interaction with sex hormones (Matta et al., 2017). Notably, later studies revealed that intra-HPC infusions of raclopride blocked social learning in gonadectomized male and female mice but not in gonadally intact male or female mice, suggesting a direct interaction between gonadal sex hormones and dorsal HPC D2-type DA receptors in social learning (Bass et al., 2019). However, the question of which specific sex hormone(s) are involved remains unanswered. The present study focused on the hormonal regulation of DA facilitated social learning in males because the previous findings were more robust in male mice. In the male brain testicular hormones may act via either androgenic or estrogenic mechanisms. In this study, we implanted castrated (CAS) “observer” (OBS) mice with subcutaneous slow releasing silastic capsules filled with either 1) the estrogen estradiol benzoate or a sesame oil control, or 2) the potent androgen dihydrotestosterone (DHT) or a cholesterol control. 10 days later (is this correct?) OBSs received bilateral intra-HPC infusions of raclopride (20 μg/μL) or a saline control 10-minutes prior to a 30-minute social interaction with a recently

fed, same sex, CAS "demonstrator" (DEM). Lastly, OBSs underwent an 8-hour choice test with free access to two novel flavored food diets, one of which their respective DEMs consumed before their social interaction. If social learning occurs, OBSs will prefer the DEM diet. Preliminary findings revealed that social learning was blocked following intra-HPC infusions of raclopride in CAS mice treated with the sesame oil control but not in CAS mice treated with estradiol. These findings appear to confirm the hypothesis that dorsal HPC D2-type DA receptors are interacting with estrogen receptors to regulate social learning in mice. Thus, D2-type DA-facilitated social learning appears to be regulated by estrogenic mechanisms in the male brain. However, these findings should be interpreted with caution until the DHT portion of the study is completed. This study was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

Disclosures: **N. Bass:** None. **M. Hickey:** None. **S. Van Den Berghe:** None. **E. Choleris:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.10

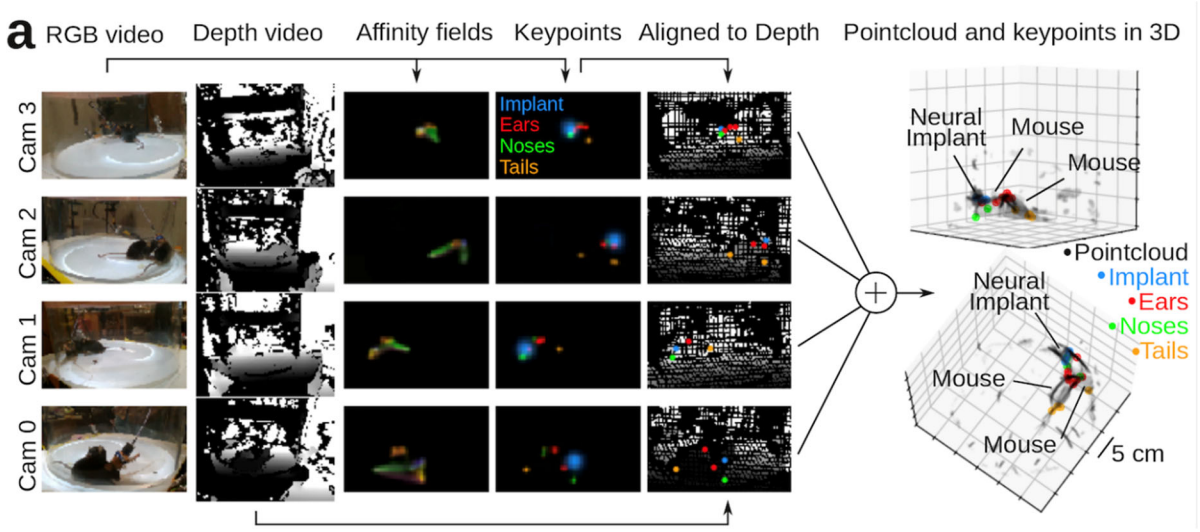
Topic: H.06. Social Cognition

Support: The Novo Nordisk Foundation (C.L.E.)
BRAIN Initiative (NS107616 to R.C.F.)
Howard Hughes Medical Institute Faculty Scholarship (R.C.F.)

Title: Automatic tracking of mouse social posture dynamics by 3D videography, deep learning and GPU-accelerated robust optimization

Authors: *C. L. EBBESEN[1], R. C. FROEMKE[2];
[1]New York Univ. Sch. of Med., New York, NY; [2]Otolaryngology, NYU Med., New York, NY

Abstract: Social interactions powerfully impact both the brain and the body, but high-resolution descriptions of these important physical interactions are lacking. Currently, most studies of social behavior rely on labor-intensive methods such as manual annotation of individual video frames. These methods are susceptible to experimenter bias and have limited throughput. To understand the neural circuits underlying social behavior, scalable and objective tracking methods are needed. We present a hardware/software system that combines 3D videography, deep learning, physical modeling and GPU-accelerated robust optimization. Our system is capable of fully automatic multi-animal tracking during naturalistic social interactions and allows for simultaneous electro-physiological recordings. We capture the posture dynamics of multiple unmarked mice with high spatial (~2 mm) and temporal precision (60 frames/s). This method is based on inexpensive consumer cameras and is implemented in python, making our method cheap and straightforward to adopt and customize for studies of neurobiology and animal behavior.



Disclosures: **C.L. Ebbesen:** None. **R.C. Froemke:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.11

Topic: H.06. Social Cognition

Support: NIMH T32 AG 49688-5

Title: Eye-gaze dynamics using nonsocial and social visual guides for decision-making

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[2]Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: There has been increased interest in the neural dynamics underlying social cognition in recent years. In conjunction, research in rhesus monkeys has mainly focused on aspects of social perception, reasoning, and social valuation of rewards. While experimental designs have looked at how agents make decisions for or with others, it remains unclear how social information coming from others impact the agent's choices with respect to nonsocial rewards. To address this question, we have developed a reward localization task (RLT) that incorporates visual guides in the form of social (eye-gaze) and nonsocial (arrows) cues. The RLT experimental design has monkeys deciding between two identical squares representing a potential fruit juice reward. Each visual cue provides contextual information about the location of the rewarded target. Preliminary results show that monkeys are able to learn to use both types of visual cues and perform above chance-level of 50%. Interestingly, the viewing duration was longer for nonsocial cues compared to social cues. Gaze data also revealed the monkey spent more time looking at the visual cue during correct trials compared to incorrect trials. This

behavioral design sets the foundation for future neurophysiological studies on how the brain incorporates social information with non-social decision processes.

Disclosures: J. Simon: None. **E.L. Rich:** None.

Digital Abstract Session

P317. Models and Systems of Social Behavior

Program #/Poster #: P317.12

Topic: H.06. Social Cognition

Support: Behavior studies were performed in Vitamin D supplemented HD induced animals followed by expression analysis of VDR, NGF, and BDNF in order to determine the neuroprotective role of VD supplementation to HD

Title: Therapeutic benefits of vitamin D3 supplementation to rescue motor and cognitive disability in mouse model of Huntington's disease

Authors: *V. SRIMATH KOIL KANDADAI;
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Abstract: Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder which leads to massive loss of neurons in the striatum. The onset of the disease is due to the presence of the mutation in the Huntingtin (HTT) gene present on the chromosome 4p16.3, where the number of CAG repeats present in HD affected patient is more than 40. Vitamin D3 (VD, cholecalciferol) is a known neurosteroid and affect myriad number of physiological processes. The present study was designed to explore neuroprotective role of VD supplementation in 3-nitropropionic acid (3-NP)induced mouse model of HD. 3-4 months old male C57BL/6 mice were divided into four groups based on their treatment i.e., Group I: Saline treated animals; Group II: 75mg/kg of 3-NP treated animals; Group III (HD): animals supplemented with 500IU/kg of VD for 15 days and GroupIV (HD + VD): animals supplemented with 500IU/kg of VD for 15 days’ post 3-NP treatment. Various behavioural tasks were undertaken to explore the effect of VD on movement, motor and memory like locomotion, stride length, rotarod and morris water maze. Thereby, striatal brain tissue was isolated from all the 4 groups on 30th day and mRNA expression of Vitamin D receptor(VDR), nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) were assessed. We found that Vitamin D3 (Cholecalciferol) intake significantly rescued striatal functions like motor activity, locomotion and spatial memory in HD animals with a significant enhancement in the expression of key neurotrophic factors like brain-derived neurotrophic factor (BDNF) and nerve-growth factor (NGF) together with increased Vitamin D receptor (VDR) mRNA expression. Altogether, our finding suggests that VD shows a therapeutic effect on motor dysfunction in HDv is a downstream neuroprotective pathway which involves a cross-talk between VDR and neurotrophic family of receptors. Key words: Huntington’s disease, 3-nitropropionic acid, vitamin D, behaviour, vitamin D receptor, nerve growth factor, brain derived neurotrophic factor.

Disclosures: V. Srimath Koil Kandadai: None.

Digital Abstract Session

P318. Neural Correlates of Social Behavior and Disorders in The Human Brain

Program #/Poster #: P318.01

Topic: H.06. Social Cognition

Title: 'nothing to see here': no structural brain differences as a function of neuroticism and extraversion from a systematic review and meta-analysis of gray matter volume studies

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Abstract: Personality reflects social, affective, and cognitive predispositions that emerge from genetic and environmental influences. Contemporary personality theories conceptualize five traits: Neuroticism, Extraversion, Agreeableness, Conscientiousness, and Openness to Experience. Starting around the turn of the millennium, neuroimaging studies have begun to investigate functional and structural features in the brain of these traits. Here, we present the first study to systematically investigate the association between Neuroticism and Extraversion, the two most commonly studied personality traits, and gray matter volumes, analyzing the literature of studies in a systematic review and meta-analysis. We observed some consistent results (e.g., Neuroticism and frontal regions; Extraversion and temporal regions) as well as some inconsistent results (e.g., Neuroticism and temporal, subcortical regions; Extraversion and frontal, subcortical regions) from the systematic review. These findings failed to be confirmed when we used a quantitative meta-analytic approach. Potential explanations to account for these discrepancies were discussed, including sample heterogeneity, five-factor trait measurements, structural image data acquisition, processing, and analytic strategies, statistical approach and statistical significance threshold, and complex nature and individual differences of personality construct and brain structures. Our conclusions will help to guide future research by adopting more rigorous designs and analytic approaches.

Disclosures: Y. Chen: None. **T. Canli:** None.

Digital Abstract Session

P318. Neural Correlates of Social Behavior and Disorders in The Human Brain

Program #/Poster #: P318.02

Topic: H.06. Social Cognition

Support: DGAPA-PAPIIT UNAM Grant IN203216
CONACyT Grant CB255462 (FAB)

Title: The dorsolateral prefrontal cortex presents structural variations associated with empathic capacity in psychotherapists

Authors: *M. E. DOMÍNGUEZ-ARRIOLA, V. E. OLALDE-MATHIEU, E. A. GARZA-VILLARREAL, F. A. BARRIOS;
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Abstract: Empathic capacity has been shown to be correlated with brain structural variations. This study investigates how this is the case in a group of psychotherapists who have a constant demand to modulate their empathic response. Eighteen professionally active psychotherapists and eighteen healthy controls underwent 3-Tesla MRI scanning and completed empathy-related psychometric assessments. Cortical thickness (CT) measures were estimated for each participant using the CIVET 2.1.0 pipeline. We evaluated how these measures differed between groups, and if they were associated with individual empathy scores at a series of regions of interest. Our analysis shows that psychotherapists display a significantly greater CT at the left dorsolateral prefrontal cortex (dlPFC; p < 0.05, FDR corrected). Moreover, psychotherapists' CT in this region is correlated with the tendency to feel empathically concerned for others (p < 0.01, FDR corrected). Finally, psychometric scores show that psychotherapists have a higher tendency to adopt the other's perspective to understand how they feel, and a lower tendency to use expressive suppression as an emotion regulation strategy. These findings are relevant because this dlPFC region participates importantly in emotion regulation and perspective taking processes. Thus, our findings support the idea that empathic capacity is reflected by brain structural variations, recruiting for the first time a sample of subjects for whom empathic responding is crucial in their profession.

Disclosures: M.E. Domínguez-Arriola: None. **V.E. Olalde-Mathieu:** None. **E.A. Garza-Villarreal:** None. **F.A. Barrios:** None.

Digital Abstract Session

P318. Neural Correlates of Social Behavior and Disorders in The Human Brain

Program #/Poster #: P318.03

Topic: H.06. Social Cognition

Title: Neural differentiation of self vs. other trait judgments by timing and magnitude of processing stages: implications for a theory of mind model of self-referencing

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Abstract: Simulation theory (ST) states that people understand others through simulation, which counters the probabilistic reasoning view of theory theory (TT). When thinking about traits of a close other, people use self-referential thought (Mitchell et al., 2006; Moran et al., 2011). It is

unclear which theory—ST or TT best describes the method by which self-referential thoughts occur. A combination of event-related potential (ERP), event-related spectral perturbation (ERSP), source localization, and hidden semi-Markov model multivariate pattern analysis (HSMM-MVPA) techniques are hypothesized to disentangle the neural underpinnings of self-versus-other information processing and distinguish competing theory of mind theories during a trait judgment task. EEG was recorded for 45 participants (30 females) ages 18-24 (M = 19.4) on resting and task measures, in which participants determined whether a series of character and appearance words match characteristics of the self and a close and distant other. Data analysis included repeated measures MANOVAs of reaction times, ERP amplitudes and latencies for the P300 generated from the parietal lobe (PCC/precuneus) and latter components of the frontal (mPFC) and parietal LSW. Time-frequency analysis included evoked and induced power through 100 Hz. ERP amplitudes were localized using minimum norm estimation to verify location and timing assumptions made for P300 and LSW. Lastly, an HSMM-MVPA approach provided an alternative look at differences in number and timing of processing stages. Results for the P300/LSW and source localization showed no differences between self, mother, and Fallon, which did not reflect prior BOLD activation (Moran et al., 2011). Overall, the ERP data did not have enough power or specificity to detect changes amid highly variable trials. Differences in self and mother were predicted by induced gamma power, suggesting the role of high frequency oscillations in information integration and categorization in the frontal and centroparietal regions. HSMM-MVPA models fit TT predictions and showed significant self-other differences in both duration of processing stages and magnitude of peaks. Findings suggest a timing and amplitude difference in self-other processing that parallels the TT probabilistic reasoning model of self-referencing. Future research should clarify the role of the mPFC in self-referential thought and its relation to ST and TT with simultaneous fMRI and EEG and populations with impaired self-recognition such as ASD and schizophrenia.

Disclosures: M. Reese: None. **L.E. Ethridge:** None.

Digital Abstract Session

P318. Neural Correlates of Social Behavior and Disorders in The Human Brain

Program #/Poster #: P318.04

Topic: H.06. Social Cognition

Support: NIH Grant 1U01NS098968-01

Title: Decoding communicative intentions using real-time brain activity

Authors: *A. E. HADJINICOLAOU[1], Y. AMIDI[1], G. BELOK[1], A. C. PAULK[1], Z. W. WILLIAMS[2], A. YOUSEFI[3], S. S. CASH[1];
[1]Neurol., Massachusetts Gen. Hosp., Boston, MA; [2]Neurosurg., Harvard Med. Sch., Boston, MA; [3]Computer Science/Neuroscience, Worcester Polytechnic Inst., Worcester, MA

Abstract: Humans constantly shift their goals and intentions. This is perhaps most pronounced during the act of conversation, in which the words of a companion change our thoughts and ultimately lead to the genesis of communicative intentions that unfold over a wide range of time scales. The study of intentions, as with many other high-level cognitive objects, is challenging as they are thought to arise from the activity of a distributed network of regions throughout the brain and are therefore difficult to measure directly. To examine such systems under natural conditions, we analyzed intracranial brain activity from N=6 epileptic study participants (implanted with electrodes for clinical purposes) who participated in recorded conversations with family, friends, and hospital staff. After transcribing spoken dialog to yield individual word timings for each participant and their companions, we modeled intent state as a latent variable whose dynamics were inferred from the onset of spoken words. We then built neural decoders for speech intent using spectral power estimates (theta, alpha, beta, gamma bands) of the recording channels as predictors. This was a two-stage process, in which we first determined the most informative predictors for intent state by fitting lasso-regularized maximum-likelihood coefficients for generalized linear models (GLMs) and standardizing these coefficients to account for their relative variance. In the final stage, GLM decoders were constructed for multiple predictor sets defined by a selection of (a) up to three different frequency bands, and (b) the best-p predictors for the selected frequency bands. Our results demonstrate that unexpected and widespread brain structures are predictive of speech intent. More specifically, spectral power predictors in theta (4-8 Hz) and gamma (30-50 Hz, 70-115 Hz, 125-200 Hz) bands were found to be most useful in decoding speech intent in the amygdala and hippocampus. To our knowledge, this study is the first to investigate the dynamics of communicative intent using ordinary human conversations and couple these dynamics with intracranial brain recordings.

Disclosures: A.E. Hadjinicolaou: None. **Y. Amidi:** None. **G. Belok:** None. **A.C. Paulk:** None. **Z.W. Williams:** None. **A. Yousefi:** None. **S.S. Cash:** None.

Digital Abstract Session

P318. Neural Correlates of Social Behavior and Disorders in The Human Brain

Program #/Poster #: P318.05

Topic: H.06. Social Cognition

Support: Grant-in-Aid for Scientific Research 18H04954

Title: Inter-brain electroencephalography synchronization during joint tapping: A comparison between stranger pairs and acquainted pairs

Authors: *Y. KURIHARA[1], T. TAKAHASHI[1,2], R. OSU[2];
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Abstract: Inter-brain synchronization (IBS) provides information on interpersonal relationships. For example, romantic couples showed higher IBS than pairs of individuals not familiar with one another (Kinreich et al., 2017). However, the identification of the brain regions whose IBS

correlates more with interpersonal relationships is still unknown. We simultaneously recorded electroencephalography (EEGs) from two individuals during the joint tapping task and compared IBS between strangers who meet for the first time and acquaintances who already knew each other. Seven stranger pairs (male pairs: four, female pairs: three, mean age:23.64, SD age: 4.31) and nine acquainted pairs (male pairs: four, female pairs: five, mean age:20.85, SD age: 1.27) volunteered for this study. IBS was evaluated using the Circular Correlation Coefficient (CCorr). For each pair of EEG electrodes between two participants, CCorr was measured. The average was calculated through the electrodes within each of the six regions of interest (ROIs; frontal, central, right-temporal, left-temporal, parietal, and occipital regions). The task comprised two resting conditions (Pre and Post) and four tapping conditions (Slow; 2 Hz, Fast; 4 Hz, Free; no reference of Hz, and Pseudo; tap to 2 Hz metronome). We conducted a two-way analysis of variance (ANOVA) considering the interpersonal relationship conditions (stranger pairs or acquainted pairs) and tapping conditions for each ROI. In the central region, significant differences in alpha IBS were observed between the interpersonal relationship conditions ($F_{1,14}$=7.39 ,p=.017,η_p^2=.346). However, tapping conditions and interaction of the two factors were not significant. ANOVA demonstrated the stranger pairs displayed higher IBS than the acquainted pairs in contrast with the earlier finding (Kinreich et al., 2017). The interpersonal interaction did not significantly affect the theta (4-7Hz) or beta (13-30 Hz). In the other five ROIs, there was no significant influence on interpersonal relationships. The central region with substantial IBS in the alpha included the motor area (C3, C4), where the alpha EEG was synchronized during joint tapping in Kawasaki et al. (2018). The observed significantly larger IBS in the stranger pairs than in the acquainted pairs may be explained by the possibility that the participants in the former pairing were more polite with each other. However, IBS depends on the type of behavioral task (Kingsbury and Hong, 2020), such as cooperation and competition. Therefore, future studies should examine the effect of interpersonal relationships on IBS during other behavioral tasks.

Disclosures: Y. Kurihara: None. **T. Takahashi:** None. **R. Osu:** None.

Digital Abstract Session

P318. Neural Correlates of Social Behavior and Disorders in The Human Brain

Program #/Poster #: P318.06

Topic: H.06. Social Cognition

Support: Viennese Science and Technology Fund; CS11-016 to CL
Austrian Science Fund; P32686 to CL, MR, and GS

Title: Modality-specific opioidergic modulation of empathy for pain

Authors: *M. RÜTGEN[1], E.-M. WIRTH[1], I. RIECANSKY[1], M. WOLETZ[2], C. WINDISCHBERGER[2], G. SILANI[1], P. PETROVIC[3], C. LAMM[1];
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Abstract: The shared representations account of empathy proposes that similar mechanisms underlie pain and its empathic experience. Previously, we found supportive evidence for this account, demonstrating that causal experimental manipulations of first-hand pain affect empathy for pain in a similar way. In a functional magnetic resonance imaging experiment (N = 102), placebo analgesia modulated both experiences, as indicated by decreases in subjective pain ratings and activity in brain regions that are part of a shared network of pain and empathy for pain. In a follow-up psychopharmacological behavioral experiment (N = 50), we then successfully blocked placebo effects for pain and its empathic experience using an opioid receptor antagonist (naltrexone). In the present study, we aimed at assessing whether these previously observed effects were specific for pain or a domain-general effect of placebo analgesia on affective processing. To this end, we analyzed imaging and self-report data of an empathy for affective touch paradigm, which participants of the aforementioned experiments performed immediately after the empathy for pain task. Placebo analgesia significantly reduced empathy for unpleasant touch and its first-hand experience, but did not affect pleasant touch. We found both overlapping and modality-specific neural placebo effects for pain and unpleasant touch in the bilateral anterior insula. Conversely, activity in the anterior midcingulate cortex was only modulated during experiences of pain. In contrast to previously observed effects on pain, naltrexone had no significant effects on any kind of affective touch. Our findings suggest a modality-specific involvement of opioidergic mechanisms in pain empathy and shed light on the neuro-chemical underpinnings of empathy.

Disclosures: M. Rütgen: None. **E. Wirth:** None. **I. Riecansky:** None. **M. Woletz:** None. **C. Windischberger:** None. **G. Silani:** None. **P. Petrovic:** None. **C. Lamm:** None.

Digital Abstract Session

P318. Neural Correlates of Social Behavior and Disorders in The Human Brain

Program #/Poster #: P318.07

Topic: H.06. Social Cognition

Support: FWF Austria Grant P29150

Title: In amygdala we trust: different contributions of the basolateral and central amygdala in social experiential learning

Authors: *R. SLADKY, F. RIVA, L. ROSENBERGER, C. LAMM;
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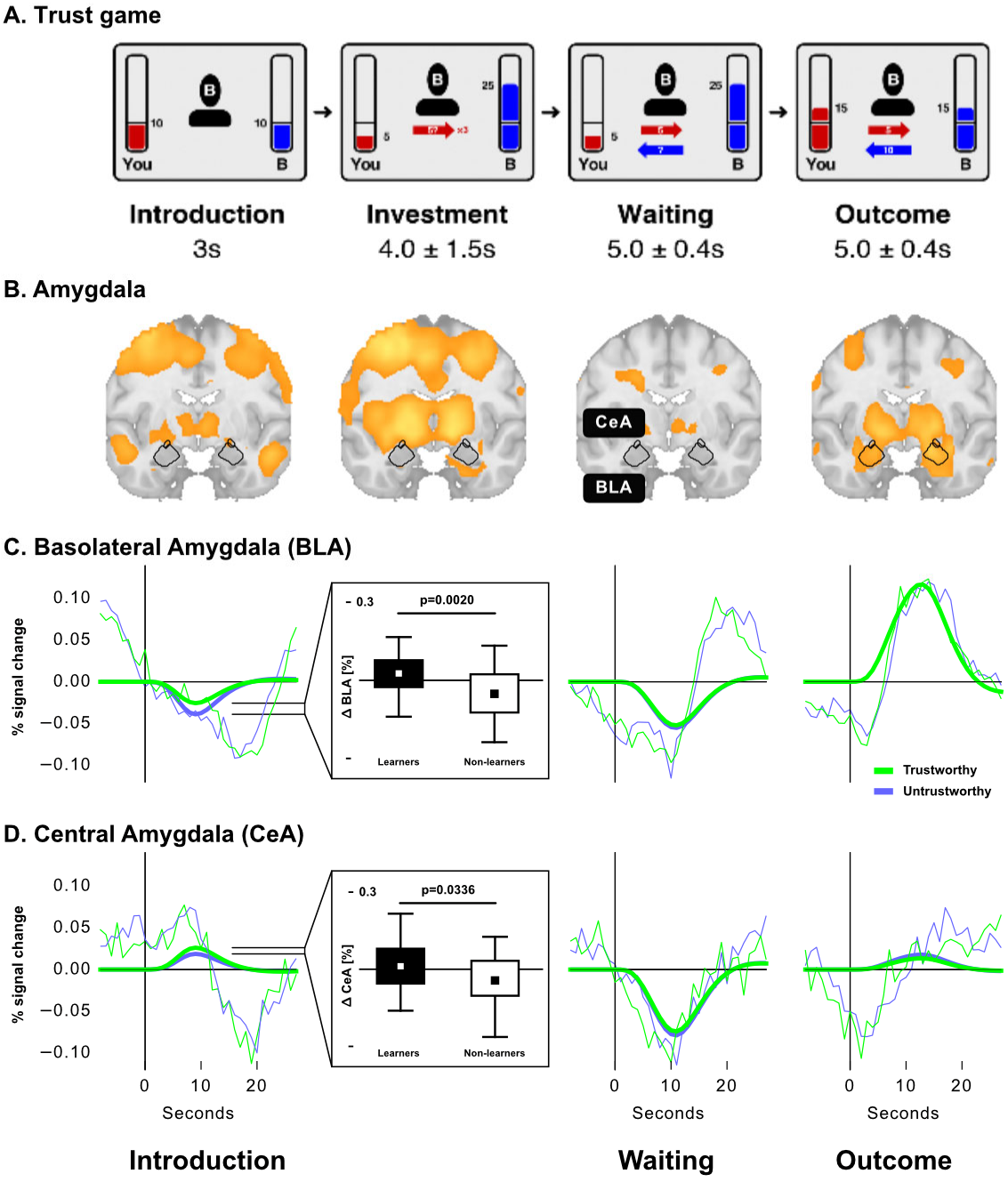
Abstract: Human societies are built on cooperation and mutual trust. Yet, not everybody is trustworthy. Through repeated interactions, we learn to adapt our social behavior accordingly. The amygdala, in general, is widely regarded as paramount for social cognition (Adolphs, 2010). Human patients with acquired BLA damage with acquired BLA damage failed to adapt their trust behavior towards trustworthy vs. untrustworthy interaction partners in a repeated trust game (Rosenberger et al., 2019). However, functional reorganizations and comorbidities might limit

the generalizability of these findings to neurotypical populations. Neuroimaging studies in neurotypical human samples indeed do not consistently report involvement of the amygdala in trusting others. This may be due to a lack of differentiation between subnuclei and their different functions in the development of trust. Here we used the same repeated trust game previously used in patients (Figure A) in neurotypical volunteers (age=23.83±3.15 years, f/m=31/31) while undergoing functional MRI (3 T Siemens Skyra, MB-EPI factor=4, TR/TE = 704/34 ms, $2.2\times2.2\times3.5$ mm^3, $96\times92\times32$ voxels). We observed that both the BLA and the central amygdala (CeA) played a role, but they were relevant for different aspects (Figure B). While the BLA was most active when obtaining feedback on whether invested trust had been reciprocated (or not), the CeA was most active when participants were anticipating their trust decisions (Figure C,D). Only in those who learned to differentiate between the trustees (measured by a difference in their investments and their subjective ratings), BLA and CeA were significantly more active for untrustworthy trustees before choosing whether to trust or mistrust this trustee (Figure C,D boxplots). In sum, we show that the amygdala is highly relevant for learning about the trustworthiness of an interaction partner, also in neurotypical subjects. However, its involvement is heterogeneous with respect to anatomical (BLA vs. CeA), functional (introduction vs. outcome), and individual factors (learners vs. non-learners).



Disclosures: **R. Sladky:** None. **F. Riva:** None. **L. Rosenberger:** None. **C. Lamm:** None.

Digital Abstract Session

P318. Neural Correlates of Social Behavior and Disorders in The Human Brain

Program #/Poster #: P318.08

Topic: H.06. Social Cognition

Title: Electroencephalography (EEG) hyperscanning measurements in children with neurodevelopmental disorders, their mothers, and music therapists

Authors: *K. KANG[1,2], S. ORLANDI[2], N. LORENZEN[3], T. CHAU[2,1], M. H. THAUT[1]; [1]Univ. of Toronto, Toronto, ON, Canada; [2]Holland Bloorview Kids Rehabil. Hosp., Toronto, ON, Canada; [3]Univ. of Guelph, Guelph, ON, Canada

Abstract: Music arouses emotions and is also one of the most powerful tools to bring people together. Previous findings showed that physiological indicators can align spontaneously and contemporaneously between people during social interaction. Electroencephalography (EEG) research has shown this also for music-based interaction, how interbrain synchronization emerges while performers in music ensembles interact musically with each other. In such context music drives a mutually calibrated state of emotional and physiological 'synchronization'. However, this interbrain synchronization in clinical settings has less been studied. Thus, we investigated interbrain synchronization in the child with disabilities-mother (CM) dyads and the child with disabilities-music therapist (CT) dyads during a music session. A total of seven dyads participated in a 15-minute-long music session (Table 1). During the music session, the music therapist played the guitar and sang the child's favorite songs. The parent observed their child's responses through the screen in a partitioned area. Interestingly, the results showed a significantly higher interbrain synchronization in CM dyads (M = .24, SD = .02) compared to CT dyads (M = .17, SD = .001), $p < .001$, possibly due to music familiarity, and maternal cognitive-empathy and emotional predisposition. In addition, higher interbrain synchronization occurred in empathy-related frequency bands (delta: 0-3 Hz) in CM and in cognition-related frequency bands (alpha: 8-15 Hz) in CT. Both dyads also experienced higher synchronization in the frontal and temporal lobes which are associated with socio-emotional responses. These findings opened the possibility of using interbrain synchronization as an objective measurement of socio-emotional responses in children with neurodevelopmental disorders. For example, if there is less synchrony in CM and/or CT dyads than expected, a therapist or parent may alter their approach to engage with the child.

Table 1*Participants' Demographic Information*

Participant	Age	Gender	Diagnosis
P01	18	F	Cerebral Palsy
P02	11	M	Cerebral Palsy
P03	11	F	Keefstra Syndrome / Autism Spectrum Disorder
P04	12	M	Autism Spectrum Disorder
P05	12	F	Autism Spectrum Disorder
P06	16	F	Autism Spectrum Disorder and Fragile X syndrome
P07	12	M	Autism Spectrum Disorder

Disclosures: K. Kang: None. **S. Orlandi:** None. **N. Lorenzen:** None. **T. Chau:** None. **M.H. Thaut:** None.

Digital Abstract Session

P318. Neural Correlates of Social Behavior and Disorders in The Human Brain

Program #/Poster #: P318.09

Topic: H.06. Social Cognition

Support: ESRC ES/S001964/1

Title: The Role of Autobiographical Memory in Empathy. An EEG and fMRI investigation.

Authors: *F. MECONI[1], C. FERREIRA[1], J. LINDE-DOMINGO[2], S. MICHELMANN[3], B. STARESINA[1], I. A. APPERLY[1], S. HANSLMAYR[4];
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Abstract: Empathy is the ability to feel and understand others' inner states at the basis of any social interaction. Theoretical accounts suggest that past experiences play a role in empathy but direct evidence of a reactivation of autobiographical memories (AM) in empathy is yet to be shown. We addressed this question in two experiments. In experiment 1, electrophysiological activity (EEG) was recorded from 28 healthy students who were required to judge their empathy for individuals depicted in contexts for which participants either did or did not have an AM. This empathy task was followed by a task that explicitly required memory retrieval of the AM and non-AM contexts. The retrieval task acted as a localizer aimed to extract the neural fingerprints of the mental representation of AM and non-AM contexts, which were then used to probe data from the empathy task. An EEG pattern classifier was trained and tested across tasks and showed evidence for AM reactivation when participants were preparing their judgement in the empathy task. Participants self-reported higher empathy for people depicted in situations they had experienced themselves as compared to situations they had not experienced. This behavioural finding was replicated in experiment 2 in which hemodynamic activity was recorded from an independent sample of 28 participants performing the same empathy task as in experiment 1. Consistently with previous neuroimaging studies showing brain activations underlying empathic processes, we observed greater activation in precuneus, posterior parietal cortex, superior and inferior parietal lobule and superior frontal gyrus when targets of empathy were depicted in AM as compared to non-AM contexts. Taken together, our findings show evidence for the reactivation of AMs in empathy that magnifies self-perception of empathic feelings.

Disclosures: F. Meconi: None. **C. Ferreira:** None. **J. Linde-Domingo:** None. **S. Michelmann:** None. **B. Staresina:** None. **I.A. Apperly:** None. **S. Hanslmayr:** None.

Digital Abstract Session

P319. Social Recognition

Program #/Poster #: P319.01

Topic: H.06. Social Cognition

Support: NRF-2017M3C7A1026959
NRF-2018H1A2A1061381

Title: Social isolation impairs the prefrontal-nucleus accumbens circuit responsible for social recognition in mice

Authors: G. PARK, C. RYU, S. KIM, S. KIM, *Y.-S. LEE;
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Abstract: Social isolation impairs the prefrontal-nucleus accumbens circuitresponsible for social recognition in mice
It is well known that social experience in early life causes prolonged deficits in cognitivefunctions. Previously, social isolation in the postnatal critical period has been shown to affect variousmedial prefrontal cortical (mPFC) functions including social preference in mice. However, it is stillelusive how other aspects of social functions and which downstream targets for the mPFC areaffected by the insubstantial social experience in young age. In this study, we observed that sociallyisolated mice in their postnatal developmental period show an impairment in social recognitionwhich was not rescued by following re-socialization period. Interestingly, we found that thefunctional connectivity between mPFC and the shell part of nucleus accumbens (NAc shell) is alteredafter the social isolation. Monitoring neural activity either by c-fos staining or by in vivo fiberphotometry revealed that mPFC neurons projecting to NAcSh selectively activated whenencountered by familiar conspecifics, suggesting that this circuit may encode social familiarity.Chemogenetic inhibition of mPFC to NAc shell projecting neuron showed a significant impairmentin the social recognition without affecting social preference while activation of such neuron insocially isolated mice exhibited a rescued social behavior. This result suggests that early socialexperience of mice can have a lifelong-lasting impact on their social behavior, which phenomenoncritically involved by mPFC-NAc shell circuit.

Disclosures: G. Park: None. **C. Ryu:** None. **S. Kim:** None. **S. Kim:** None. **Y. Lee:** None.

Digital Abstract Session

P319. Social Recognition

Program #/Poster #: P319.02

Topic: H.06. Social Cognition

Support: NIH Grant NS094643
Child Neurology Foundation PERF Elterman Grant
UT Austin startup funds
UT System STARs award
Child Neurology Society Philip R. Dodge Young Investigator Award

Title: Prefrontal thalamocortical and corticothalamic networks in mouse social behavior

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Abstract: The medial prefrontal cortex (mPFC) reciprocally connects with the mediodorsal nucleus of the thalamus (MD). This bidirectional circuit has been implicated in a range of cognitive and social / emotional behaviors. Here, we tested the hypothesis that the mPFC⇄MD circuitry is required for novel social exploration behavior in mice. To do this, we optogenetically activated mPFC→MD and MD→mPFC projection neuron terminals in wildtype mice in the following behavioral assays: novel mouse exploration, novel object exploration, open field, olfactory habituation / dishabituation. We observed that optogenetic activation of the mPFC→MD or MD→mPFC axon terminals decreased social exploration behavior. Notably, the other behaviors tested were largely unaffected. These results imply that the bidirectional MD⇄mPFC circuit is required for social exploration behavior.

Disclosures: C. Haberl: None. **A.C. Brumback:** None.

Digital Abstract Session

P319. Social Recognition

Program #/Poster #: P319.03

Topic: H.06. Social Cognition

Support: Beatrice and Samuel A Seaver Foundation

Title: Role of Hypothalamic Paraventricular Oxytocin Neurons in Social Recognition Memory

Authors: *K. THIRTAMARA RAJAMANI[1], K. NIBLO[1], V. GRINEVICH[2], H. HARONY-NICOLAS[1];
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Abstract: TitleRole of Hypothalamic Paraventricular Oxytocin Neurons in Social Recognition MemoryAuthorsKeerthi Thirtamara Rajamani, Kristi Niblo, Valery Grinevich, Hala Harony-NicolasAbstractOxytocin is a nine amino acid neuropeptide that is synthesized and released by neurons in the paraventricular (PVN), supraoptic and accessory nuclei of the hypothalamus. It is implicated in social behaviors including maternal care, social bonding, and social recognition memory. Social recognition memory (SRM) in mammals is a corner stone for several behaviors which together contribute to their evolutionary fitness, starting from being able to distinguish prey from conspecifics to reproductive behaviors and kinship. Despite the clear role of oxytocin in SRM, it is still unclear which of the three nuclei within the hypothalamus is necessary for the formation of this form of memory. We hypothesized that oxytocin neurons within the PVN of the hypothalamus are necessary for both short- and long-term SRM. Using designer receptors activated by design drugs (DREADDS), we specifically silenced oxytocin neurons (OT-hM4DGi) in the PVN of wild type Sprague Dawley rats and assessed their performance on both

short and long term SRM.We found that following saline injection, rats exhibited normal short and long-term SRM. However, upon clozapine N oxide (CNO) injection and, via silencing of OXT neurons in the PVN, the same animals failed to exhibit either forms of memory. In order to account for nonspecific effects of CNO, we performed the same behavior in a different cohort of rats that were injected with a control virus (OT-mcherry), which lacks the DREADD. We found that CNO does not have a nonspecific effect, suggesting that the behavioral effects observed in the DREADD injected animals are a result of silencing of oxytocin neurons in the PVN.Taken, together these findings attribute a novel role for PVN oxytocin neurons in social recognition memory. Future studies are aimed at identifying the downstream targets of these OXT projections neurons that originate in the PVN and their ability to modulate OXT dependent social recognition memory.

Disclosures: K. Thirtamara Rajamani: None. **H. Harony-Nicolas:** None. **V. Grinevich:** None. **K. Niblo:** None.

Digital Abstract Session

P319. Social Recognition

Program #/Poster #: P319.04

Topic: H.06. Social Cognition

Support: NIH Grant MH-094268
Stanley
S-R/RUSK

Title: Anterior insula-associated social novelty recognition: orchestrated regulation by a local retinoic acid cascade and disynaptic oxytocin-serotonin signaling

Authors: *K. AN[1], S.-H. KIM[1], H. NAMKUNG[1], M. MIHALJEVIC[1], K. YANG[1], B. MAHER[2], M. NIWA[1], A. SAWA[1,3];
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Abstract: Social cognition is critical for human behavior in complex social environments, and deficits in social cognition consistently underlie functional disabilities in individuals with a wide range of psychiatric disorders. Neuroimaging studies have suggested that the anterior insula (AI) is a ‘common core’ brain region that is impaired across neurological and psychiatric disorders, which include deficits in social cognition. Nevertheless, neurobiological mechanisms of the AI for social cognition remain elusive. Here we demonstrate that the AI is a crucial center for social novelty recognition and decipher its regulatory mechanisms at both cell-autonomous and cell non-autonomous levels. At the molecular and cellular level, AI-associated social novelty recognition is maintained by proper activity of AI layer 5 pyramidal neurons, for which retinoic acid (RA)-mediated gene transcription, regulated by an AI-enriched RA-degrading enzyme

Cyp26B1, plays a pivotal role. At the circuitry level, social novelty recognition is regulated by 5-HT2C receptor-mediated serotonergic inputs from the dorsal raphe nucleus (DRN) to the AI. Furthermore, we demonstrate that oxytocin (OT) influences the AI-mediated social cognition not by a direct projection of OT neurons, nor by direct diffusion of OT in the AI, but instead, by influencing the serotonergic projections in the DRN. Together, we demonstrate an orchestrated regulation of AI-mediated social novelty recognition by a local RA cascade and disynaptic OT-serotonin signaling.

Disclosures: **K. An:** None. **S. Kim:** None. **H. Namkung:** None. **M. Mihaljevic:** None. **K. Yang:** None. **B. Maher:** None. **M. Niwa:** None. **A. Sawa:** None.

Digital Abstract Session

P319. Social Recognition

Program #/Poster #: P319.05

Topic: H.06. Social Cognition

Support: BMBF 01GQ1006
BMBF 01GQ1603
DFG TRR 169
WWTF VRG13-007

Title: The left temporoparietal junction causally supports goal emulation in human observational learning

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Abstract: Humans learn from their own trial-and-error experience and observing others by inferring their goals (i.e., goal emulation) in order to guide future decisions. Previous studies have established a comprehensive neurocomputational account of the integrated value computation between directly earning and social observational learning (e.g., Zhang & Gläscher, 2020, Science Advances). Crucially, these studies have identified that bilateral temporoparietal junction (TPJ) is functionally associated with goal emulation. To date, however, the causal role of bilateral TPJ in such observational learning processes remains unclear. Here, employing a within-subject double-blind continuous theta-burst stimulation (cTBS; Huang et al., 2005) over bilateral TPJ as well as vertex (control), we tested how the (bilateral) TPJ supports goal emulation in observational leaning, with a probabilistic reversal learning task (N = 31, female: 17). Participants on each trial were first required to choose between two stimuli, after they have seen the choices from four other co-players, they had the opportunity to adjust their choices. A probabilistic reward was then delivered given their second choice. Notably, the four co-players were instructed to the participants as “intelligent computers,” and choices from those computers were in fact generated from our computational algorithm model (Zhang & Gläscher, 2020), such

that they matched with actual human behavior. To increase ecological validity, we used human faces to indicate each computer. Behavioral results indicate that disrupting activity in the left TPJ resulted in reduced choice switch probability paired with longer response time when confronted with opposing social information, as opposed to the right TPJ and vertex conditions. In addition, choice accuracy was declined when participants actually switched their choices in the left TPJ condition, showing opposite pattern relative to right TPJ and vertex conditions. Computational modeling with the hierarchical Bayesian approach suggests that the corresponding parameter quantifying social information significantly decreased in the left TPJ condition, as compared to the right TPJ and vertex conditions. And the associated between this model parameter and behavioral performance is the weakest in the left TPJ condition. Together, our results provide evidence for the causal role of left TPJ in supporting goal emulation in human observational learning.

Disclosures: L. Zhang: None. **C. Lamm:** None. **J. Gläscher:** None. **C.C. Hilgetag:** None. **F.I. Kandil:** None.

Digital Abstract Session

P319. Social Recognition

Program #/Poster #: P319.06

Topic: H.06. Social Cognition

Support: NSF BCS- 1655300
UCLA Faculty Senate Grant

Title: Brain networks for self-recognition from whole-body movements

Authors: *A. KADAMBI, G. ERLIKHMAN, M. JOHNSON, M. MONTI, H. LU;
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Abstract: Humans can identify own-body movements presented in whole body point-light actions despite little visual experience of seeing themselves from third person. Though this ability taps into the core of dynamic self-awareness, the neural mechanisms remain unknown. In the present study, we utilized functional neuroimaging to examine which brain networks may support self-recognition from whole-body movements. Twelve right-handed participants (males = 6, females = 6, M_{age} = 20.00) performed six visually instructed actions (e.g., "perform *argue"*) and six verbally instructed actions ("*imitate* the movements in the video"), which were recorded by a motion capture system. One of each participant's friends (gender-matched) was also recruited to record motion capture data for performing the same actions. After a delay period of 12 - 20 days, the participants ran a fMRI session in which they viewed point-light actions either performed by the participant self, gender-matched friend or stranger. Participants were asked to make a self-recognition judgment among three choices (i.e., self, friend, or stranger). Each point-light action was presented for five seconds. The experiment consisted of four runs of 36 trials each. Behavioral accuracy in self-recognition from point-light displays (0.48) was significantly

above-chance (0.33). Brain activity was first compared between self-action and stranger-actions. Perception of own-body movements activated bilateral inferior parietal lobule (IPL; supramarginal gyrus), left inferior frontal gyrus (IFG; pars opercularis), posterior superior temporal sulcus (pSTS; input to the mirror neuron system), as well as the prefrontal cortex (dlPFC; dorsolateral). Self-specific activity controlling for familiarity (i.e., self-action versus friend-action) similarly showed involvement of bilateral IPL and left IFG activity. Greater engagement of lower-level visual (extrastriate body area), and primary motor (M1) and somatosensory (S1) regions was found during processing of stranger and friend-actions versus self-actions. These results extend theories of visuomotor recruitment during domain-general action processing, and highlight a possible self-specific action circuitry, consisting of fronto-parietal mirroring (pSTS, IFG, IPL) and control network (dlPFC) regions recruited during dynamic movements.

Disclosures: **A. Kadambi:** None. **G. Erlikhman:** None. **M. Johnson:** None. **M. Monti:** None. **H. Lu:** None.

Digital Abstract Session

P320. Human LTM: Encoding and Retrieval

Program #/Poster #: P320.01

Topic: H.07. Long-Term Memory

Support: DARPA N66001-14-C-4016

Title: An Ensemble Classifier for Decoding Hippocampal Spike Patterns During a Delayed Match-to-Sample Task in Human

Authors: *X. SHE[1], B. MOORE[1], B. M. ROEDER[3], G. NUNE[1], B. LEE[1], S. SHAW[5], A. NGUYEN[5], C. HECK[1], C. LIU[1], S. A. DEADWYLER[4], R. E. HAMPSON[4], T. W. BERGER[2], D. SONG[2];
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Abstract: To understand how visual memories are encoded in the hippocampal spiking activities in human, an ensemble memory decoding model is developed to classify spike patterns into memory labels on a single trial basis. Model inputs are spatio-temporal patterns of spikes recorded in the hippocampal CA3 and CA1 regions of epilepsy patients performing a delayed match-to-sample (DMS) task. Each DMS trial is initiated by touching the screen within the focus ring on the computer screen. The “Sample” phase starts after the focus ring clicks, and a sample image shows up at a random location on the touch screen. Once subjects touch the sample image, a random delay occurs, and then the “Match” phase starts. In the “Match” phase, the same sample image is displayed with four other non-match distracter images. Subjects are required to touch the correct sample image to achieve a successful trial. Sample images shown in the task are categorized with binary memory labels indicating their categories and features. Such memory

labels are used as outputs of the decoding model. The ensemble memory decoding model consists of two layers. In the first layer, to solve the underdetermined problem resulting from small sample size and the very high dimensional input and output signals, B-spline basis functions are applied to reduce dimensionality; L1-regularized logistic regression (LASSO) classifiers are used to achieve sparse model estimation. Multiple classifiers with different B-spline resolutions are employed to include a wide range of temporal resolutions of neural signals. Each classifier serves as a base-learner to classify spatio-temporal patterns with a given temporal resolution. A bagging-based sampling strategy is used to further reduce the estimation variances of these classifiers. In the second layer, another LASSO classifier takes outputs of first-layer classifiers as inputs to make the final output prediction. This ensemble classifier serves as a meta-learner that combines multiple temporal resolutions to classify the spatio-temporal patterns of spikes. Results show that this method can effectively avoid overfitting and yield significant prediction of memory labels. The ensemble classifier consistently outperforms the best single-layer classifier by utilizing multi-resolution spatio-temporal features of spike patterns in classification. In addition to decoding memory contents, the proposed model can also be used to design optimal stimulation patterns for eliciting specific memories in the hippocampus and thus has important implications for the development of hippocampal memory prostheses.

Disclosures: **X. She:** None. **B. Moore:** None. **B.M. Roeder:** None. **G. Nune:** None. **B. Lee:** None. **S. Shaw:** None. **A. Nguyen:** None. **C. Heck:** None. **C. Liu:** None. **S.A. Deadwyler:** None. **R.E. Hampson:** None. **T.W. Berger:** None. **D. Song:** None.

Digital Abstract Session

P320. Human LTM: Encoding and Retrieval

Program #/Poster #: P320.02

Topic: H.07. Long-Term Memory

Support: ISF Individual Reseach Grant 1485/18 to SGD

Title: Size matters: larger images are better remembered during free viewing

Authors: *S. MASARWA, O. KREICHMAN, S. GILAIE-DOTAN;
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Abstract: We are constantly exposed to multiple visual scenes, and while freely viewing them without an intentional effort to memorize or encode them, only some are eventually remembered. It has been suggested that image memorability is influenced by multiple factors as depth of processing, familiarity, and visual category. However, this is typically investigated with intentional or unintentional encoding tasks. Furthermore, since visual memory relies on size-invariant high-level visual perception that is less sensitive to image size, image size is not typically considered a contributing factor. Here we reasoned that during naturalistic visual behavior of free viewing, of images without an encoding task, bigger images would be better remembered due to multiple factors (as vaster expanse of activated visual cortex, deeper

processing and others). In an extensive set of experiments participants (n=117) freely viewed small to large images (3-24 vis. deg.) without any encoding task or knowledge about a memory test that may follow. Subsequently, they were presented with mid-sized images (50% already seen) and were asked to report if they recall seeing them or not. Larger images were better remembered (~23% more than smaller images), image memorability was proportional to image size, faces were better remembered, and outdoors the least. These were independent of image set, presentation order, or screen resolution. Furthermore, when the amount of information in the images and its world size were kept constant but viewed from different distances (changing the retinal image size), memory performance was influenced by the retinal image size and not by the other factors. While multiple factors affect image memorability, here we show that during free viewing with no encoding task, a basic physical image dimension influences its memorability.

Disclosures: **S. Masarwa:** None. **O. Kreichman:** None. **S. Gilaie-Dotan:** None.

Digital Abstract Session

P320. Human LTM: Encoding and Retrieval

Program #/Poster #: P320.03

Topic: H.07. Long-Term Memory

Title: Investigating the neural correlates of retroactive interference at encoding: an EEG study

Authors: *M. MUNSEN, J. D. KOEN;
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Abstract: It is well established that encountering a new, but similar, experience can induce retroactive interference that results in forgetting a previous experience. Evidence from multivariate analyses of fMRI data suggests that reactivating a previous experience during potentially interfering experience might protect against retroactive interference. However, little is known about if the time course of memory reactivation differentially predicts resistance to retroactive interference. The present study addressed this question by leveraging the temporal resolution of EEG with an AB/AC interference paradigm. We recorded EEG from 32 participants during a task where they encoded a series of words while performing one of four judgment tasks. Words in the Interference (AB/AC) condition were presented twice (associated with two different encoding judgments) whereas words in the Control (DE) condition were presented only once. Following encoding, participants completed a source memory test where they were required to recall the judgment(s) that were performed with each word seen during the encoding phase. As expected, source memory accuracy was lower for both tasks on Interference trials relative to the Control trials. The main analysis of the EEG data focused on two putative correlates of episodic memory retrieval—the FN400 and LPC - because, if the AB task is being reactivated during AC learning, we should see differences in the amplitude in one or both of these components during AC trials as a function of subsequent AB source memory. However, a one-way repeated measures ANOVA on AC trials with a within-subjects factor of AB subsequent memory showed no significant AB memory effects for the FN400 nor LPC.

Moreover, a similar analysis using a scalp-wide cluster mass test did not identify any AB subsequent memory effects during AC trials. Furthermore, a scalp-wide within-subjects cluster mass test performed showed only canonical front-central subsequent memory effects that were similar for AB, AC, and DE trial types. The present results suggest that the electrophysiological correlates of memory encoding do not differ with retroactive interference. Future analyses will employ multivariate classification of the EEG data to further address the question of reactivation.

Disclosures: M. Munsen: None. **J.D. Koen:** None.

Digital Abstract Session

P320. Human LTM: Encoding and Retrieval

Program #/Poster #: P320.04

Topic: H.07. Long-Term Memory

Support: NIH R01MH107512

Title: Replicability of fMRI Subsequent Memory Effects in Children and Adults

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Abstract: With the advent of neuroimaging techniques such as functional MRI (fMRI), scientists have been able to characterize the neural correlates of memory functions and investigate the neural basis underlying changes in memory function across development. However, in recent years, the replicability and consistency of task-based fMRI effects have been broadly questioned, and specifically, the replicability of memory-related fMRI effects such as those obtained by the commonly used subsequent memory paradigm remains unknown. Consistency and replicability are particularly important in developmental studies in which interpretations are often based on the assessment of age-related individual differences in observed fMRI effects.
In this study, we investigated the reliability of behavioral and brain measures observed using a subsequent memory paradigm in a sample of children, adolescents, and adults (n = 85; ages 8 to 25, 16.51 ± 4.73). We found excellent replicability for contrasts related to general task performance (all encoding trials vs. implicit baseline; ICC = .94) and reduced but good replicability for a specific contrast targeting subsequent memory (Hit trials vs. Miss trials; ICC = .73). Better replicability was found in cortical regions, including frontal, parietal, and occipital cortex, but reduced in subcortical regions, including the hippocampus.
We argue that the group-level subsequent memory effects are replicable for children and adults, although the level of confidence varies by the choice of contrast and brain regions.

Disclosures: L. Tang: None. **Q. Yu:** None. **P.J. Pruitt:** None. **Q. Yin:** None. **R. Homayouni:** None. **J. Damoiseaux:** None. **N. Ofen:** None.

Digital Abstract Session

P320. Human LTM: Encoding and Retrieval

Program #/Poster #: P320.05

Topic: H.07. Long-Term Memory

Support: Sloan Research Fellowship
Google Faculty Research Award

Title: Cortical signal at major boundaries between internally-generated mental contexts during narrative recall

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Abstract: Growing evidence suggests that humans identify boundaries between events in a continuous stream of stimuli based on the mismatch between predicted and actual sensory inputs, and that boundary detection is associated with transient cortical and subcortical responses. However, little is known about neural responses at transitions between mental contexts during experiences that are internally rather than externally driven. Here, boundaries cannot be based on mismatches between predictions and sensory inputs, but are instead self-generated. To test whether internally-driven boundaries produce neural responses comparable to those observed during stimulus-driven experience, we conducted an fMRI experiment. Subjects watched a series of short movies and then verbally recalled the movie plots regardless of the order of presentation. During recall, transitions between movies were considered major event boundaries, determined purely by subjects' internal mentation; no external stimulus was presented to prompt the onset/offset of the recall of each movie. We observed activity changes at boundaries between movies during recall, as well as during movie watching, across widespread brain areas. We focused our analyses on the posterior medial cortex (PMC), a brain area thought to be involved in representing 'situation models' of events, and also reported to show boundary-related responses in prior studies. At between-movie boundaries during recall, PMC showed a transient increase in activation followed by decreased activation. This signal was present even when there was little or no pause between the recall of two different movies, suggesting that it was not mainly caused by a break in the speech. PMC multi-voxel patterns at recall boundaries were positively correlated with each other and less distinctive across different movies compared to non-boundary patterns, suggesting that the boundary response was insensitive to the specific content of each movie. Boundary-related PMC patterns were also task-general, as the patterns at between-movie boundaries during recall were highly similar to those measured during movie watching. Early sensory control regions showed a transient change in overall activation similar to PMC, but their patterns at recall boundaries showed neither decreased movie-level distinctiveness nor a positive correlation with the movie watching phase. Together, our results demonstrate that internally-driven boundaries between self-generated mental contexts evoke task- and content-general neural responses in higher associative areas, potentially reflecting a cognitive state related to the flushing and updating of situation models.

Disclosures: **H. Lee:** None. **J. Chen:** None.

Digital Abstract Session

P320. Human LTM: Encoding and Retrieval

Program #/Poster #: P320.06

Topic: H.07. Long-Term Memory

Support: NRF-2019R1A2C2005213
NRF-2020R1A2C2007770
NRF-2020M3E5D9079908
IBS-R015-D1

Title: Neural Representation of Long-term Value Memory in Human Ventral Striatum

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Abstract: Learning object values is essential in dealing with environmental problems and increasing our benefits through better decisions. While the ventral striatum in the basal ganglia is thought to be critical to the learning process of object values, recent studies of addiction suggest that activation of the ventral striatum is related to memory of addicted objects, indicating that the ventral striatum may be involved in the long-term maintenance of object value memory. To investigate whether the ventral striatum is involved in long-term value memory processing, we performed an event-related functional magnetic resonance imaging (fMRI) experiment, comprising separate pre-learning, learning, and post-learning sessions. Both during the pre-learning and the post-learning sessions, participants were scanned while they passively viewed fractal object images. During the learning session, which took place between the pre-learning and post-learning sessions for five days outside the scanner, participants were trained to associate each object image with monetary gain, no gain, or loss. Focusing on the responses in the ventral striatum, we found that the neural responses to the objects associated with monetary gain increased significantly after learning. Moreover, the responses were greater than those for objects paired with monetary loss after learning. Furthermore, using multi-voxel pattern analysis, we revealed that the similarity of neural response patterns for individual objects associated with monetary gain increased significantly after learning compared to the similarity before learning, and that this increase in neural pattern similarity was positively correlated with the memory performance after learning. Taken together, our data suggest that the ventral striatum contains long-term memory that underlies evaluations of learned values of objects.
This work was supported by the Basic Science Research Program (NRF-2019R1A2C2005213, NRF-2020R1A2C2007770), the Neurological Disorder Research Program (NRF-2020M3E5D9079908), and IBS-R015-D1 through the National Research Foundation (NRF) of Korea.

Disclosures: **J. Kang:** None. **H. Kim:** None. **S. Hwang:** None. **H.F. Kim:** None. **S. Lee:** None.

Digital Abstract Session

P320. Human LTM: Encoding and Retrieval

Program #/Poster #: P320.07

Topic: H.07. Long-Term Memory

Title: The anterior temporal lobe retains shared semantic representations of distinct verbal information during successful memory retrieval

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Abstract: When an observer retrieves a word from memory, the anterior temporal lobe (ATL) reinstates patterns of neural activity present when the word was first encountered. While these patterns have been associated with item-specific information represented in the ATL, they only account for a small amount of variance in the ATL activity during memory retrieval. It remains unclear whether these ATL retrieval patterns also reflect other aspects of retained memory contents. We hypothesize that ATL retrieval patterns also contain the activation of a gist representation shared across items in addition to item-specific information. To test this hypothesis, we asked a group of participants (n = 19) to perform a paired associates verbal memory task while they were undergoing intracranial electroencephalogram (iEEG) monitoring. In this task, participants studied word pairs from a pre-selected word pool and were later cued with a single word from each pair. We extracted spectral power from the iEEG signal using Morlet wavelets between 3-150 Hz and developed a trial-by-trial neural similarity matrix for every participant over the time course of retrieval. Next, using the semantic features as quantified by their co-occurrence statistics in natural language, we quantified the similarity among the to-be-retrieved words across trials. We used this word similarity pattern across trials to quantify the gist representation of these words. We found that ATL retrieval pattern similarity was strongly correlated with the similarity among words. As the words were more related to other words on average, namely more centrally situated within the semantic space, they triggered more similar ATL retrieval patterns. We ruled out several alternative interpretations of these observations, including attention, motor response, and lower-level word features at the single-item level. Collectively, our findings suggest that ATL retrieval patterns contain not only item-specific information, but also a gist representation that is shared across items in memory.

Disclosures: **S. Bhasin:** None. **W. Xie:** None. **A. Vaz:** None. **S. Inati:** None. **K.A. Zaghloul:** None. **J.H. Wittig:** None.

Digital Abstract Session

P320. Human LTM: Encoding and Retrieval

Program #/Poster #: P320.08

Topic: H.07. Long-Term Memory

Support: NIH Grant MH100121
NIH Grant MH114869

Title: Semantic knowledge of famous people and places is represented in hippocampus and distinct cortical networks

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Abstract: Studies have found that anterior temporal lobe is critical for detailed knowledge of object categories, suggesting that it has an important role in semantic memory. However, in addition to information about entities, such as people and objects, semantic memory also encompasses information about places. We tested predictions stemming from the PMAT model, which proposes there are distinct systems that support different kinds of semantic knowledge: an anterior temporal (AT) network that represents information about entities and a posterior medial (PM) network that represents information about places. We used representational similarity analysis to test for activation of semantic features when human participants viewed pictures of famous people and places, while controlling for visual similarity. We used machine learning techniques to quantify the semantic similarity of items based on encyclopedic knowledge in the Wikipedia page for each item and found that these similarity models accurately predict human similarity judgments. We found that regions within the AT network, including anterior temporal lobe and inferior frontal gyrus, represented detailed semantic knowledge of people. In contrast, semantic knowledge of places was represented within PM network areas including precuneus, posterior cingulate cortex, angular gyrus, and parahippocampal cortex. Finally, we found that hippocampus, which has been proposed to serve as an interface between the AT and PM networks, represented fine-grained semantic similarity for both individual people and places. Our results provide evidence that semantic knowledge of people and places is represented separately in anterior temporal and posterior medial areas, while hippocampus represents semantic knowledge of both categories.

Disclosures: **N.W. Morton:** None. **E.L. Zippi:** None. **S.M. Noh:** None. **A.R. Preston:** None.

Digital Abstract Session

P320. Human LTM: Encoding and Retrieval

Program #/Poster #: P320.09

Topic: H.07. Long-Term Memory

Support: UNAM DGAPA PAPIIT IG300608 IG300121

Title: Effects of chronic low and high blood pressure on source memory and working memory

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Abstract: The effects of chronic low (hypotension) and high blood pressure (hypertension) on memory are unclear due to divergent results, originating in part, due to participant misclassifications. The aim of this study was to compare source memory and working memory performance in individuals diagnosed as hypotensive or hypertensive receiving medical treatment with the source and working memory performance in normotensive participants. From a sample of 1656 participants, 219 were identified as hypertensive and 37 were identified as hypotensive. Each of these two groups was compared with normotensive individuals matched by age, education and sex. Individuals who were diagnosed with diabetes mellitus, hypercholesterolemia or hypertriglyceridemia were excluded from all groups. Source memory performance and working memory performance were assessed through computerized tasks. Source memory accuracy was lower in hypotensive and hypertensive individuals than in normotensive individuals, and spatial working memory discrimination was inferior in hypertensive participants compared to that of normotensive individuals. Moreover, the more time that has passed since hypertension or hypotension was formally diagnosed, the worse recognition and source memory accuracy were. Blood pressure impairment should be considered a major concern not only because it is linked to severe cardiovascular and cerebrovascular diseases but also because of its negative effects on the two types of memory that are the most essential for preserving a self-sufficient lifestyle.

Disclosures: S. Cansino: None. **F. Torres-Trejo:** None. **C. Estrada-Manilla:** None. **A. Mercado-Canales:** None. **D. Medina-Velázquez:** None. **R. Esquivel-García:** None. **S. Ruiz-Velasco:** None.

Digital Abstract Session

P321. Functional Studies of Human Memory Networks

Program #/Poster #: P321.01

Topic: H.04. Executive Functions

Title: The effects of a mindfulness intervention on the neural activity of musicians with musical performance anxiety during a working memory task

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Abstract: Rationale and Objective: Studies have shown that music performance anxiety (MPA) impacts all types of musicians, affecting their personal and/or professional functioning. Anxiety in general has also been shown to affect cognitive functioning, such as working memory, which is critical to musicians' performances. Mindfulness, an effective treatment for those with general anxiety, has been used as a potential treatment for MPA, while the efficacy of mindfulness on working memory has shown mixed results. The objective of this study was to determine the effectiveness of a short mindfulness intervention on musicians' neural activity during a working memory task. **Methods:** Twenty participants (10 participants who underwent a mindfulness intervention and 10 control participants) were recruited for this exploratory study. All participants were young adult musicians who experienced MPA. Ten participants identified as female and 10 identified as male, and the age range for this sample was 18 to 35 years. For this study, participants were allocated to either a mindfulness condition where they practiced structured mindfulness for six to nine sessions over a course of two weeks or a control group where they did not practice mindfulness. At time one, participants underwent a one-hour MRI scan that included a letter N-Back task, a common task used to measure participants' working memory. Participants also completed the same scan sequence two weeks later (time two). **Results:** When examining neural activity in the mindfulness group pre- and post-intervention, a repeated measures t-test showed that there was significantly less activity post-intervention in the bilateral dorsolateral prefrontal cortex and in the left supplementary motor area, regions required for successful working memory. More activity pre-intervention might suggest more effort required to accomplish the task. When examining the difference in neural activity between the mindfulness and control groups at time two, a two-sample t-test showed that there was significantly less activity in the visual cortex bilaterally, including in the cuneus and the lingual gyrus in the mindfulness group compared to controls. **Conclusions:** These findings demonstrate that mindfulness interventions have efficacious results on working memory in those who experience MPA. Musicians who incorporate mindfulness into their daily lives may benefit from potentially lessened effort required during working memory tasks, allowing for more neural resources to focus on their music.

Disclosures: K. Boileau: None. **U. Hansen:** None. **N. Stanson:** None. **G. Comeau:** None. **L. Fang:** None. **A. Smith:** None.

Digital Abstract Session

P321. Functional Studies of Human Memory Networks

Program #/Poster #: P321.02

Topic: H.08. Learning and Memory

Title: Tradeoff between hippocampal learning and numerical processing in young children

Authors: *M. ZAFAR, S. SELVAM, K. K. NAIDOO, R. NARAHARISETTY, C. K. MATTINGLY, T. E. PILGER, N. C. HINDY;
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Abstract: Children can perceive the number of objects in a configuration in different ways: either through numerical processing or pattern recognition. An example of numerical processing is a child counting or subitizing a small number of disorganized blocks. Numerical cognition is related to neural processes in the intraparietal sulcus (IPS). On the other hand, a child might instantly know the number of blocks stacked in a familiar configuration. Remembering previously seen patterns is related to neural processes in the hippocampus. Using fMRI and an at-home training program on an iPad, we examined how these neural mechanisms are affected in young children through repeated exposure to particular patterns. A multi-touch iPad game, Fingu, was used for at-home training; players see configurations of 1-10 fruits moving on the screen and have to indicate the number of fruits by pressing down the correct number of fingers. Before and after at-home training, participants completed tasks similar to those in Fingu in an fMRI scanner. Half of the fMRI tasks contained configurations that were repeatedly viewed during iPad training with Fingu, while the other half contained configurations that were not seen in Fingu. Prior to training, we observed greater functional connectivity between the IPS and visual cortex than between the hippocampus and visual cortex. However, after a week of training on the iPad, functional connectivity was enhanced between the hippocampus and visual cortex, and diminished between the IPS and visual cortex. This trend remained consistent across Fingu and novel configurations. Thus, while perception of spatial configurations appeared to rely on numerical processing in the IPS before training, perception of the same configurations after a week of training may have primarily relied on pattern recognition by the hippocampus. These findings suggest an inverse relationship between the hippocampus and IPS in perception as a function of repeated exposure to visuospatial patterns. As children learn patterns around them, visual perception may become increasingly mediated by mnemonic processes in the hippocampus.

Disclosures: M. Zafar: None. **S. Selvam:** None. **K.K. Naidoo:** None. **R. Naraharisetty:** None. **C.K. Mattingly:** None. **T.E. Pilger:** None. **N.C. Hindy:** None.

Digital Abstract Session

P321. Functional Studies of Human Memory Networks

Program #/Poster #: P321.03

Topic: H.08. Learning and Memory

Support: UNMC Department of Neurological Sciences
Fremont Area Alzheimer's Collaboration
IDeA-CTR Pilot
IDeA-CTR Superstar

Title: Effects of multiday repetitive transcranial magnetic stimulation of functional brain networks supporting memory in healthy young adults.

Authors: *C. J. PHIPPS, A. RANGEL, A. M. HELLER, V. S. PHATAK, D. L. MURMAN, D. E. WARREN;
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Abstract: Declarative memory has been associated with a network of brain regions including the hippocampus. Investigations suggest that non-invasive brain stimulation (NBS) applied to components of this network may affect memory performance. For example, repetitive transcranial magnetic stimulation (rTMS) applied to lateral parietal regions has been shown to enhance declarative memory performance in young adults by up to 20% (Wang et al., 2014). To test generalizability of this approach, we are administering a clinical trial (ClinicalTrials.gov #NCT03574207) applying NBS to healthy young adults, healthy older adults, and patients with amnestic mild cognitive impairment (aMCI). Here, we report preliminary findings from the trial arm studying young adults. Our approach replicates methods from Wang et al. (2014). Young adults (N=15, age=18-35 years) completed two rounds of participation combining pre/post-NBS brain and cognitive measurement with stimulation (treatment or sham for five consecutive days) in the form of rTMS. Interventions were identical except for stimulation intensity. Pre-/post-NBS, brain measures included structural and resting-state functional MRI data; cognitive measures included cognitive abilities (e.g., hippocampal-dependent memory). Interventions applied a β-frequency pulse alternating 2-sec. 20 Hz stimulation with 28-sec. rest for 20 min; stimulation location was constant between conditions (left lateral parietal cortex coactive with a hippocampus-centered network). Intensity was tailored to participants' resting motor threshold (RMT) and varied by condition: treatment, 100% RMT; sham, 10% RMT. Conditions were completed in a counterbalanced order, with at least two weeks between conditions. Using rs-fMRI data from each participant, we mapped a hippocampal functional networks and identified individualized left lateral parietal targets for rTMS. Changes were observed in brain and cognitive measures related to the stimulation condition. Brain measures revealed changes in patterns of RSFC between several areas of the hippocampus-centered network targeted with rTMS in the treatment condition. Our ongoing clinical trial measures changes in brain and cognitive variables associated with NBS of a functional brain network supporting memory. The current trial arm, focused on healthy young adults, found evidence consistent with plasticity in the intrinsic brain network supporting memory. Findings from the remaining arms (older adults, healthy or aMCI) will inform the generalizability and translational potential of our observations to populations with clinical impairments of memory.

Disclosures: C.J. Phipps: None. **D.E. Warren:** None. **D.L. Murman:** None. **V.S. Phatak:** None. **A. Rangel:** None. **A.M. Heller:** None.

Digital Abstract Session

P321. Functional Studies of Human Memory Networks

Program #/Poster #: P321.04

Topic: H.08. Learning and Memory

Support: NSERC RGPIN-2016-06721

Title: The effect of hippocampal damage on integrating information across time

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Abstract: Introduction: The hippocampus (HPC) supports some forms of integration of information across time. Two tasks prominently used to test temporal integration are i) associative inference (AI) tasks, which require inferring an association between stimuli that are indirectly related but were never presented together, and ii) statistical learning (SL) tasks, which involve learning item relationships by extracting regularities across many experiences. There is general agreement that the HPC is required for AI, and recent studies also argue it is required for SL- contrary to longstanding theories. **Methods:** We tested four patients with varying degrees of bilateral HPC damage and their matched healthy controls. During AI, participants studied overlapping paired associates (AB, BC) then were tested on premise pairs (AB, BC) and indirect associations (inferred AC). Past research predicts deficits to both direct and indirect pairs in patients with any degree of HPC damage. During SL, participants passively viewed continuous picture sequences that contained an underlying structure of triplets to later be recognized. Based on a recent model we predicted that an intact CA1 field of the HPC would be sufficient to support SL, even with damage to the dentate gyrus (DG) and CA3. **Results:** Unexpectedly for AI, patients with more localized HPC damage could infer the indirect associations as well as controls when performance for the premise pairs was equated. Thus, HPC damage alone does not impair inference as long as the direct associations are acquired. For SL, binomial tests were used to determine above chance performance. None of the patients exhibited above chance statistical learning, including patient BL with focal DG damage. Notably, however, neither did many of the controls. **Conclusions:** Temporal integration of indirect relationships through AI can be intact even with damaged HPC so long as the premise pairs are available. This may indicate that extra-HPC structures support inference at retrieval, or that residual HPC tissue that is insufficient for normal acquisition of the premise pairs is sufficient for normal inference. For SL, we believe it is premature to draw any conclusion about either impairment or preservation of function with intact CA1. Our data of presumed impairment in SL are consistent with previous studies that used the same task, however many controls in previous research also show chance performance, complicating the interpretation of deficits in individuals with HPC damage. A more reliable task should be developed before conclusions about SL ability can be made in a small group of patients.

Disclosures: **Z. Belchev:** None. **H. Marlatte:** None. **M. Fraser:** None. **A. Gilboa:** None.

Digital Abstract Session

P321. Functional Studies of Human Memory Networks

Program #/Poster #: P321.05

Topic: H.08. Learning and Memory

Support: Lycaki-Young Funds (State of Michigan)
DMC Foundation
Ethel and James Flinn Foundation
Mark Cohen Neuroscience Endowment
National Institutes of Mental Health (MH111177)

Title: Disordered network topology during associative learning in schizophrenia

Authors: *E. D. MERAM, S. BAAJOUR, A. CHOWDURY, J. STANLEY, V. A. DIWADKAR;
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Abstract: Schizophrenia (SCZ) is characterized by impairments in associative learning (Diwadkar et al., 2008) but differences from controls in brain network topology *during* phases of learning have not been investigated. We used the graph theoretic measure of betweenness centrality (BC) to summarize network topology in 4 different conditions of an object-location associative learning task: Memory Formation (MF), Post-Encoding Consolidation (PEC), Retrieval (MR), and Post-Retrieval Consolidation (PRC) (Baajour et al., 2020). BC estimates the number of "shortest functional paths" traversing through a node and thus indexes a node's hubness or "integrative value" (Sporns et al., 2010). fMRI data were collected from 59 subjects (32 SCZ, 18<Age<50, 3T Siemens Verio). During MF, 9 objects were presented in associated locations (3x3 spatial grid) for naming. During PEC, participants covertly rehearsed memoranda as they fixated on a marker. During MR, locations were cued, & participants were required to name the associated object. PRC was identical to PEC. The sequence cycled eight times. fMRI data were processed in SPM12. For each condition, time series were extracted from 90 cerebral parcels (Tzourio-Mazoyer et al., 2001). BC was estimated for each node in each participant's network. Inter-group differences in BC were estimated for each node in each condition.
During MF, SCZ had *decreased* BC in the thalamus, putamen, precuneus, & frontal nodes, but increased BC across visual, temporal, parietal, & frontal lobe nodes. During PEC, differences in the superior frontal, precuneus, thalamus, and putamen were identical to those in MF with additional decreases in posterior cingulate and Heschl's Gyrus, & increases in frontal-hippocampal nodes. During MR, decreased BC was observed in multiple nodes, & increases in parietal, frontal, and visual nodes. During PRC, decreased BC was in frontal & striatal nodes, with increased BC observed in frontal, temporal & parietal nodes. Ranking nodes by BC within groups revealed that generally, significantly reduced BC in SCZ was observed for highly ranked nodes. Increased BC was observed for lowly ranked nodes.
We observed a disordered network topology in every condition, emphasizing the global nature of network deficits in SCZ during associative learning. Second, in all conditions, decreases

occurred in nodes more central to the network whereas increases were present in nodes less central to the network, suggesting a predictable pattern of network dysfunction. Third, disordered topology was observed even in conditions devoid of overt sensori-motor processing, suggesting a crucial role of these refractory periods in associative learning.

Disclosures: **E.D. Meram:** None. **S. Baajour:** None. **A. Chowdury:** None. **J. Stanley:** None. **V.A. Diwadkar:** None.

Digital Abstract Session

P322. Neuromodulation, Learning and Memory

Program #/Poster #: P322.01

Topic: H.08. Learning and Memory

Support: European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 785907 (Human Brain Project SGA2)
European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 945539 (Human Brain Project SGA3)
The Research Council of Norway under Grant Agreement No. 269774 (INCF Norwegian Node)
The Research Council of Norway Grant 227769
The Kavli Foundation
The Centre of Excellence scheme of the Research Council of Norway-Centre for Neural Computation Grant 223262
The Research Council of Norway, National Infrastructure scheme Grants 197467 and 295721 (NORBRAIN)

Title: Brain-wide quantitative analysis reveals complementary patterns of parvalbumin and calbindin neurons in executive and modulatory regions of the mouse brain

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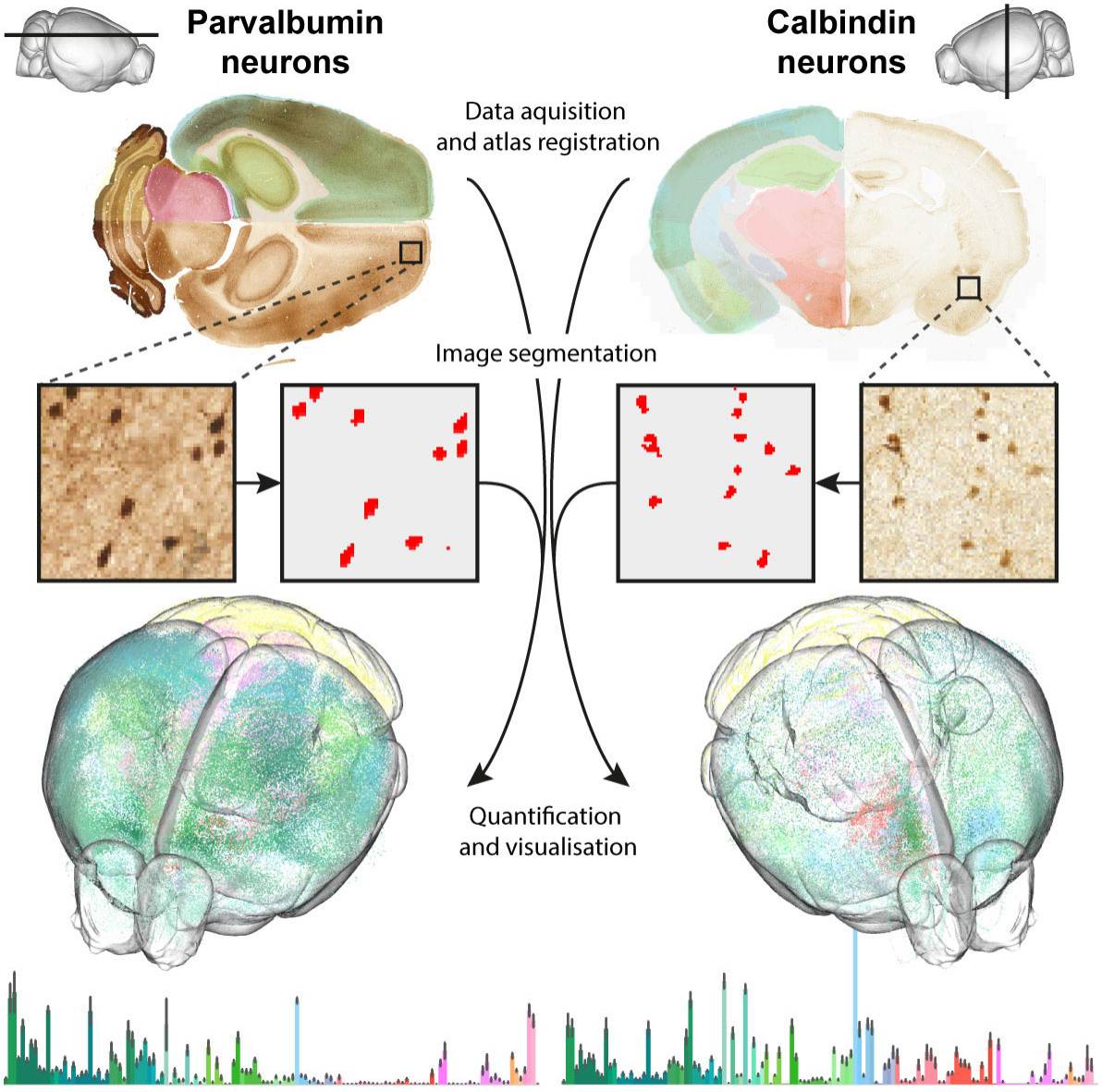
Abstract: The calcium-binding proteins parvalbumin and calbindin are expressed in largely non-overlapping types of neurons across the murine brain. These cell types are known to play important roles in neural networks involved in sensorimotor processing, spatial navigation, and fear memory. Knowledge about the number and spatial distribution of these cell types within and across brain regions will be important to understand their contributions in functional networks, but such data are so far only available for a few regions. To amend this we performed a brain-wide quantification of immunohistochemically stained parvalbumin and calbindin neurons across

the adult mouse brain. We used the QUINT workflow, which combines anatomical information from the Allen Mouse brain Common Coordinate Framework with machine-learning based segmentation of cells. Our results indicate that these two neuron types distribute in complementary patterns. Parvalbumin neurons are more prevalent in sensorimotor cortices, hippocampal and parahippocampal areas, and in the brainstem, while calbindin neurons are relatively more dominant in olfactory and amygdalar areas, as well as in the thalamus and hypothalamus. These results point to interesting differences in the relative importance of these cell types in different brain regions. We share all raw data and analytic results via EBRAINS.eu to facilitate further exploration and re-use of the data.



Disclosures: **I.E. Bjerke:** None. **S.C. Yates:** None. **A. Laja:** None. **M.P. Witter:** None. **M.A. Puchades:** None. **J.G. Bjaalie:** None. **T.B. Leergaard:** None.

Digital Abstract Session

P322. Neuromodulation, Learning and Memory

Program #/Poster #: P322.02

Topic: H.08. Learning and Memory

Support: NIMH R01MH108729
NSF IOS-1656488

Title: Perirhinal and postrhinal cortices contribute to the configural process of contextual learning

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Abstract: Behaviors do not occur in a vacuum: many paradigms in humans and animals have shown that behaviors are often critically dependent on the physical context. The medial temporal lobe (MTL) is an important structure underlying context-dependent behavior. Within the MTL, a preponderance of the literature has targeted the role of the hippocampus. In addition to the hippocampus proper, the MTL also comprises parahippocampal areas including the perirhinal (PER) and postrhinal cortices (POR). These areas, however, have received less attention regarding their contribution to context-dependent behavior. It has been proposed that the hippocampus supports the configural approach of contextual processing, in which elements of the environment are first combined into a unitary representation before being associated with other stimuli. It is unknown whether the PER and POR are necessary for this process. In this study, bilateral excitotoxic lesions of the PER or POR were created in adult male rats, and their performances in two context-dependent procedures were compared with sham-lesioned rats. One procedure is a fear conditioning variant that specifically demonstrates the context pre-exposure facilitation effect (CPFE), which results from the configural process of context learning and critically depends on the hippocampus. Preliminary data indicates that either PER or POR lesions impaired CPFE when compared to shams. The second procedure is contextual spontaneous object recognition (SOR). Normal rats show increased exploration when encountering a familiar object but in a different context, because the rats not only encode the object but also bind it with the context where it previously appeared. PER and POR lesioned rats also displayed deficits in this procedure. These results suggest that both the PER and POR are necessary for forming a configural representation of the context for both fear learning and spontaneous exploratory behavior. This finding will compliment our existing knowledge of the hippocampus and provide a fuller picture of how the medial temporal lobe functions to support contextual learning.

Disclosures: X. Peng: None. **D.L. Poeta:** None. **R.D. Burwell:** None.

Digital Abstract Session

P322. Neuromodulation, Learning and Memory

Program #/Poster #: P322.03

Topic: G.01. Fear and Aversive Learning and Memory

Support: Wellcome Trust Senior Investigator Award (104631/Z/14/Z)
Gates Cambridge Scholarship
Angharad Dodds John Bursary in Mental Health and Neuropsychiatry

Title: Serotonin depletion impairs both Pavlovian and instrumental reversal learning in healthy humans

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Abstract: Serotonin is implicated in aversive processing and updating responses to changing environmental circumstances. Optimising behaviour to maximise reward and minimise punishment may require shifting strategies upon encountering new situations. Likewise, emotional reactions to threats are critical for survival yet must be modified as danger shifts from one source to another. Whilst numerous psychiatric disorders are characterised by behavioural and emotional inflexibility, few studies have examined the contribution of serotonin in humans. We modelled both processes in two independent experiments (N = 97; 50 females, mean age 24), using instrumental and aversive Pavlovian reversal learning paradigms, respectively. Upon depleting the serotonin precursor tryptophan - in a double-blind randomised placebo-controlled design - healthy volunteers showed impairments in updating both behaviour and emotion to reflect changing contingencies. Reversal deficits in each domain, furthermore, were correlated with the extent of tryptophan depletion. These results translate findings in experimental animals to humans and have implications for the neurochemical basis of cognitive inflexibility.

Disclosures: **J.W. Kanen:** None. **A.M. Apergis-Schoute:** None. **R. Yellowlees:** None. **F.E. Arntz:** None. **F.E. van der Flier:** None. **A. Price:** None. **R.N. Cardinal:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Enterprise, Routledge, Cambridge University Press. F. Consulting Fees (e.g., advisory boards); Campden Instruments. **D.M. Christmas:** None. **L. Clark:** None. **B.J. Sahakian:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Cognition. F. Consulting Fees (e.g., advisory boards); Cambridge Cognition, Greenfield BioVentures, Cassava Sciences. **M.J. Crockett:** None. **T.W. Robbins:** B. Contracted

Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Shionogi & Co, GlaxoSmithKline. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Cognition. F. Consulting Fees (e.g., advisory boards); Cambridge Cognition, Greenfields Bioventures, Unilever. Other; editorial honoraria from Springer Verlag, editorial honoraria from Elsevier.

Digital Abstract Session

P323. Prefrontal, Medial, and Orbital Cortices in Cognition, Learning, and Memory

Program #/Poster #: P323.01

Topic: H.08. Learning and Memory

Title: Behavioral and neural substrates of learning attentional rules

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Abstract: We must constantly adapt the rules we use to guide our attention. To understand how the brain learns attentional rules, we trained two monkeys to perform a novel task, which required them to learn which color is the most rewarded at a given time (the current 'attentional rule'). However, just as in real life, monkeys were never explicitly told the rule. Instead, they had to learn it through trial and error by choosing one of three presented colors, receiving feedback (amount of reward), and then updating their internal attentional rule. Then, after the monkeys reached a behavioral criterion, the rule changed, and they had to re-learn the new rule. This change was not cued but could be inferred based on reward feedback. Behavioral modeling found that monkeys used rewards to learn attentional rules. After the rule changed, animals adopted one of two strategies. If the change in the rule was small, reflected in a small reward prediction error, the animals continuously updated their rule. However, for large changes in the rule (large reward prediction errors), monkeys 'reset' their belief about the rule and re-learned the rule from scratch. To understand the neural correlates of learning new attentional rules, we simultaneously recorded single units in the lateral prefrontal cortex (lPFC, 402 neurons), frontal eye fields (FEF, 300 neurons) and lateral intraparietal cortex (LIP, 185 neurons). Preliminary results suggest that lPFC neurons encode the rule, developing stable rule representations early in learning and maintaining them throughout the block of trials. This representation was lost immediately after the rule switch, reflecting the reset seen in behavior. In contrast, LIP neurons encoded the rule only once the rule was well-known and behavioral accuracy was high. This suggests that attentional rules are learned first in lPFC and then passed to LIP. Together, our results provide insight into the behavioral and neural basis of learning new abstract rules.

Disclosures: C.I. Jahn: None. **N. Markov:** None. **B. Morea:** None. **T. Buschman:** None.

Digital Abstract Session

P323. Prefrontal, Medial, and Orbital Cortices in Cognition, Learning, and Memory

Program #/Poster #: P323.02

Topic: H.08. Learning and Memory

Support: NSERC Grant RGPIN-2016-490978
CIHR Grant MOP125958

Title: Differential influence of ventromedial prefrontal cortex lesions on neural representations of schema and category knowledge

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Abstract: This project investigated the oscillatory dynamics of prior knowledge (PK)-mediated processing, whether or not we can differentiate kinds of PK, and how these processes are altered by ventromedial prefrontal cortex (vmPFC) damage. PK influences how we perceive and interpret information. For this to transpire, PK must first be reinstated (i.e. activating PK templates) and instantiated (i.e. using the activated template to interact with incoming information). We focused on two kinds of PK: schemas and categories. Schemas are flexible networks of PK that represent information gathered over multiple similar experiences (e.g. going to the doctor); schema knowledge is presumed to be mediated by the vmPFC. Categories are groups of concepts that are related based on certain characteristics (e.g. bugs) and are mediated by the anterior and lateral temporal lobes (ATL/LTL). While two separate systems are proposed to underlie each respective PK type, there is evidence of overlap. While electroencephalography was recorded, patients with vmPFC damage (n = 11) and matched controls (n = 13) brought to mind a schema or category (reinstatement) and were asked to decide whether words appearing on the screen were related to the schema or category (instantiation). We predicted reinstatement would be supported by low-frequency oscillatory activity between the vmPFC and angular gyrus for schemas, and between the ATL/LTL and inferotemporal cortex for categories. For instantiation, we expected focal, high-frequency oscillatory activity in the vmPFC for schemas, and ATL/LTL for categories. Of particular interest was whether or not vmPFC patients performed poorly for both knowledge types, which would indicate system overlap. Reinstating PK was associated with pre-stimulus theta and alpha desynchronization; for schemas, this involved communication between the vmPFC and posterior parietal lobe, including the angular gyrus, whereas category reinstatement was associated with LTL and inferotemporal cortex desynchrony. In both conditions, patients showed reduced desynchrony. Instantiating PK was indexed by post-stimulus low and high-frequency desynchrony between the same respective regions for schemas and categories. While some differences emerged between schemas and categories, desynchrony between the regions above often predicted performance on trials of both knowledge types. Notably, however, certain patients showed deficits specific to schematic processing: those with damage to the subcallosal vmPFC. We conclude that damage to vmPFC

influences processing of both schemas and categories, but the underlying network-level mechanisms of this disruption differ.

Disclosures: **A.E. Giuliano:** None. **K. Bonasia:** None. **V.E. Ghosh:** None. **M. Moscovitch:** None. **A. Gilboa:** None.

Digital Abstract Session

P323. Prefrontal, Medial, and Orbital Cortices in Cognition, Learning, and Memory

Program #/Poster #: P323.03

Topic: H.08. Learning and Memory

Title: The role of mPFC projecting sub-populations in recent and remote expression of memories

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Abstract: As memories age, they undergo reorganization at the systems level. In particular, memories are known to initially depend on the hippocampus for expression, but become more dependent on the medial prefrontal cortex (mPFC) at remote time-points. Although retrieval-induced activity of mPFC neurons increases with time, it is unclear whether mPFC projecting subpopulations contribute to retrieval in distinct ways at different time points. mPFC has many different projection targets in the brain, including the basolateral amygdala (BLA), nucleus reunion (NRe), and nucleus accumbens (NAc). Using a contextual fear conditioning paradigm in mice, we aimed to delineate how these distinct projection-specific sub-populations of mPFC neurons contribute to memory expression at recent vs. remote time-points. To examine this, we used retrograde viral vectors to label projection-specific sub-populations in the mPFC. First, we examined the extent to which these distinct mPFC sub-populations are activated during contextual fear learning (i.e. encoding), and subsequently reactivated during recent vs. remote recall. Second, we used an activity-dependent tagging method to express excitatory and inhibitory opsins in these projection-specific mPFC sub-populations during learning. We then asked whether reactivating these sub-populations is sufficient for memory expression at recent and remote time-points in a novel context. Our results provide evidence that different mPFC projection-specific sub-populations differentially contribute to recent vs remote recall. In particular, BLA projecting mPFC neurons are reactivated at both recent and remote time-points, while NRe and NAc projecting populations exhibited increased reactivation at the remote compared to recent time-point. Consistent with this, photo-stimulation of BLA projecting cells induced freezing at both recent and remote time points, whereas photo-stimulation of NRe and NAc projecting neurons only induced freezing at the remote time point. The involvement of BLA projecting sub-population at both recent and remote time-points suggests that mPFC-BLA circuit contributes to the fear memory recall independent of memory age. However, mPFC-NRe and mPFC-NAc contribute only at the remote time-point. The differential dependency of these

three circuits to memory age may relate to the role of their down-stream targets in regulating fear memories. BLA is well-known for the encoding and expression of emotional memories regardless of the memory age. On the other hand, NRe and NAc are, respectively, related to the generalization and extinction, properties of memories which may change as a function of memory age.

Disclosures: A. Golbabaei: None. **S.A. Josselyn:** None. **P.W. Frankland:** None.

Digital Abstract Session

P323. Prefrontal, Medial, and Orbital Cortices in Cognition, Learning, and Memory

Program #/Poster #: P323.04

Topic: H.08. Learning and Memory

Support: Natural Sciences and Engineering Research Council of Canada (NSERC) - Postgraduate Doctoral Scholarship
NSERC Graduate-Master's Program Scholarship
Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (RGPIN-04933-2018)
Canada Foundation for Innovation JELF
Ontario Research Fund (36876)
University of Toronto startup funds

Title: Differential engagement of prefrontal and medial temporal lobe cortex during memory formation under semantic versus perceptual attention conditions

Authors: *S. VIJAYARAJAH, M. L. SCHLICHTING;
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Abstract: Although it is known that learning new information in relation to prior knowledge can promote successful remembering, the mechanisms that support this memory benefit remain unclear. Recent work suggests the integration of new information into one's existing knowledge engages ventromedial prefrontal cortex (PFC) networks. However, most work examining the mechanisms that facilitate integration have focused on the presence versus absence of a relationship to prior knowledge. Within experiences that do contain features related to prior knowledge, it is unclear how attending to such features might impact medial PFC network engagement. Here, we used functional magnetic resonance imaging (fMRI) to ask whether attending to semantic features related to prior knowledge (i.e., well-known stories) recruits medial PFC memory networks more so than attention to perceptual features unrelated to prior knowledge (i.e., artist style). We manipulated participants' (N=42 adults) attention to the semantic (story) or perceptual (artist style) features of storybook illustrations during incidental encoding by asking them to search for consecutive feature repeats along the cued dimension. Participants then completed an old/new recognition memory test for the studied illustrations. To assess whether semantic versus perceptual attention recruited different regions to support

remembering, we compared univariate activation at encoding for subsequently remembered versus forgotten illustrations. We found that a number of regions throughout the brain showed greater activation for subsequently remembered than forgotten trials across both attention conditions, including both lateral and medial PFC; medial temporal lobe cortex (parahippocampal cortex, PHC); and ventral visual stream (inferior temporal gyrus and fusiform). When interrogating activation as a function of the attentional encoding state, PHC as well as both lateral and medial PFC showed greater activation for semantic versus perceptual attention overall, with a significantly greater attention-related difference in medial than in lateral PFC. Our results demonstrate common neural substrates for successful memory formation when attending to either semantic or perceptual features of experiences, with increased activation in a subset of these regions (i.e., PHC; medial and lateral PFC) when attending to meaning—a dimension that would enable connections to be formed with prior knowledge. These findings suggest that the *availability* of information consistent with prior knowledge may be sufficient to engage PFC and medial temporal lobe, with *attention towards* such features amplifying this engagement.

Disclosures: S. Vijayarajah: None. **M.L. Schlichting:** None.

Digital Abstract Session

P323. Prefrontal, Medial, and Orbital Cortices in Cognition, Learning, and Memory

Program #/Poster #: P323.05

Topic: H.08. Learning and Memory

Support: NIH Grant MH117873
NIH Grant MH117103
NIH Grant DA044297
NIH Grant DA036737

Title: Cocaine-sensitive orbitofrontal circuits encode action variables for flexible decision making

Authors: *D. LI, E. PITTS, N. DIGHE, B. BARBEE, B. KOCHOIAN, S. GOURLEY; Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Goal-directed decision making is critically important for navigating dynamic environments, and requires the flexible encoding, representation, and retrieval of learned action-outcome associations (contingencies) in order to guide future choices. Meanwhile, failures of flexible contingency-guided action represent a core feature of many neuropsychiatric disorders including drug addiction. First, we find that individual differences in cocaine-induced decision-making deficits are associated with post-synaptic protein and dendritic spine alterations in the orbitofrontal cortex (OFC). We thus sought to identify specific OFC circuits that underlie flexible goal-directed action. Using projection-selective chemogenetic manipulations, we reveal that basolateral amygdala (BLA)>OFC projections are necessary for the encoding, but not

retrieval, of contingency memories, and that these connections bidirectionally modulate contingency encoding across multiple environmental domains. Meanwhile, we also find that OFC>dorsomedial striatum (DMS) projections are necessary during choice epochs themselves to facilitate "online" contingency memory retrieval. Thus, we demonstrate that learned contingency information is transmitted in a directionally defined manner across a discrete BLA>OFC>DMS circuit, which functions in concert across temporal scales to enable flexible choice behavior. We then utilized activity-dependent genetic tools to demonstrate that a memory trace for learned contingency information is stably represented by encoding-activated neuronal ensembles within the OFC, whose reactivation is necessary for subsequent memory retrieval. We then sought to identify potential cellular plasticity mechanisms which are necessary for long-term storage of contingency information by OFC neurons. Accordingly, we employed di-synaptic circuit mapping and connection-selective gene silencing to show that contingency learning is associated with both dendritic spine plasticity and neurotrophin signaling specifically within a BLA>OFC>DMS circuit. Our findings therefore suggest that the OFC is indispensable for flexible decision making by serving as the critical locus within an extended amygdalo-fronto-striatal network, providing the temporal link between memory encoding and retrieval, thus bridging the initial learning of new contingency information with its future application.

Disclosures: D. Li: None. **S. Gourley:** None. **E. Pitts:** None. **N. Dighe:** None. **B. Kochoian:** None. **B. Barbee:** None.

Digital Abstract Session

P323. Prefrontal, Medial, and Orbital Cortices in Cognition, Learning, and Memory

Program #/Poster #: P323.06

Topic: H.08. Learning and Memory

Support: Reseach Fellowship from the German Research Foundation (DFG, MA8509/ 1-1)

Title: The orbitofrontal cortex is necessary for learning to ignore irrelevant cues

Authors: *K. M. COSTA, G. SCHOENBAUM;
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Abstract: We learn not only what is potentially useful, but also what is meaningless and should be ignored. Failures in this ability have been associated with psychosis and attentional disorders, yet how it is accomplished is a seldom explored question in neuroscience. Learning to ignore irrelevant cues is evident in latent inhibition - where presenting a cue several times without consequences leads to retardation of subsequent conditioning to that cue. Importantly, this pre-exposure learning is a form of learning that requires the recognition of hidden task states (contingencies not directly distinguishable by external events). We hypothesized that this should depend on the orbitofrontal cortex (OFC), since this region has been implicated in such processing. We tested this by using chemogenetics to selectively inactivate the OFC in rats during the pre-exposure phase of a within-subjects latent inhibition task. Adult male rats were

transfected with either hM4d (a Gi-coupled DREADD; N=16) or mCherry (control; N=15) in the OFC; after recovery, they experienced four pre-exposure sessions in which an auditory cue was presented 12 times without any consequences, after injection of 0.2 mg/kg of the high-potency DREADD agonist JHU37160 (JH60) to inactivate OFC principal neurons. Rats then underwent six conditioning sessions in which the pre-exposed auditory cue (PE) and a novel, non-pre-exposed cue (NPE) were presented, each immediately followed by the delivery of three sucrose pellets. Control rats showed latent inhibition, developing stronger conditioned responding to the NPE than to the PE. In contrast, hM4d rats did not show latent inhibition, but developed strong conditioning to both cues at a similar rate. We also tested if OFC inactivation affected what was learned about the PE cue. Rats received additional conditioning to equalize responding to the PE and NPE cues, then they underwent extinction and satiety-based devaluation tests. The effects of extinction and devaluation did not differ between cues or groups, suggesting that the impact of OFC inactivation during pre-exposure was limited to the latent inhibition effect, at least as assessed by these tests. Our findings implicate the OFC in learning that a given cue is irrelevant, a cardinal form of latent learning. Inactivation during pre-exposure ensured that the observed effects are restricted to the integration of information prior to its use in any sort of behavior, which is congruent with the concept that the OFC supports this critical aspect of learning.

Disclosures: K.M. Costa: None. **G. Schoenbaum:** None.

Digital Abstract Session

P324. Functional Studies of Nucleus Reuniens

Program #/Poster #: P324.01

Topic: H.08. Learning and Memory

Support: NIH Grant R01MH107886
Alzheimer's Association grant SAGA-17-419092
Sigma Xi Grant-In-Aid of Research
UW Milwaukee Support for Undergraduate Research Fellows

Title: Inactivation of the nucleus reuniens impairs spatial memory in mice

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Abstract: Coordinated activity between the hippocampus and medial prefrontal cortex (mPFC) is required for memory encoding and retrieval. Our laboratory demonstrated that simultaneous subthreshold chemogenetic inactivation of the dorsal hippocampus (DH) and medial prefrontal cortex (mPFC) impairs the consolidation of object placement (OP) memory in female mice (Tuscher et al., 2018), suggesting that these two brain regions work in concert to promote memory consolidation. However, mechanisms through which these brain regions interact to promote memory consolidation remains poorly understood. A small cluster of cells in the midline thalamus known as the nucleus reuniens (RE) facilitates communication between the

hippocampus and mPFC through bidirectional excitatory projections. Furthermore, recent work from other labs indicates that the RE is necessary for spatial working memory and fear extinction learning. The goal of this study was to determine whether activity in the RE is necessary for OP memory. Kappa-opioid receptor DREADD (KORD) virus activated by salvinorin B was used to inactivate excitatory neurons in the RE. Mice infused with GFP virus or saline were used as controls. During training, mice were allowed to explore 2 identical objects placed near the corners of a large white box, and received a 10 mg/kg injection of salvinorin B either 10 minutes prior to training or immediately after training to target effects to the encoding and consolidation phases of memory, respectively. Testing was conducted 4h after training, a timepoint at which control mice remember the location of training objects. During testing, one object was moved to a different quadrant of the testing box. Activation of the KORD prior to or immediately after training blocked OP memory relative to chance and controls, supporting a key role for the RE in spatial memory consolidation. Current work is investigating the roles of specific projections among DH, mPFC, and RE. We include preliminary findings from retrograde labeling work using AAV-Cre-eGFP in a retrograde-specific serotype.

Disclosures: **M.R. Schwabe:** None. **C.M. Lincoln:** None. **L. Taxier:** None. **K.M. Frick:** None.

Digital Abstract Session

P324. Functional Studies of Nucleus Reuniens

Program #/Poster #: P324.02

Topic: H.08. Learning and Memory

Title: Role of the Nucleus Reuniens in Long Term Memory Consolidation

Authors: ***K. CALVIN**[1], R. A. WIRT[2], E. FLORES[1], B. UDIN[1], J. M. HYMAN[3];
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Abstract: Anterior cingulate cortex (ACC) and hippocampal interactions are well characterized in episodic memory. The dorsal hippocampus is critical for memory-guided behavior, but experimental evidence suggests that spatial and contextual memories depend not only on the hippocampus itself, but the ACC and hippocampal circuit. Investigations disrupting the hippocampal and medial prefrontal cortex (mPFC) circuit have shown a failure to transfer spatial and contextual information that is processed by the hippocampus to mPFC circuitry responsible for goal-directed behavior and decision making. In spatial and contextual memory tests, oscillatory synchrony between these regions has been show to increase. However, very little is known about how this synchrony arises. The nucleus reuniens (RE) of the thalamus has reciprocal connections to hippocampus as well as ACC and has been shown to be vital to a variety of episodic and contextual memory tasks. This suggests that the hippocampus and mPFC oscillatory synchrony noted above may be modulated by activity in the RE. Prior findings have demonstrated that this circuit between mPFC, RE, and the hippocampus is necessary for

processing and regulating fear memory generalization. Additional research has shown that neurons in the RE provide trajectory-specific coding during a continuous alternation T-maze task, similar to coding observed in the dorsal hippocampus. There have also been recent findings of head direction cells in the RE. Recent research has also shown an essential role of the RE in coordinating slow oscillatory activity between hippocampus and neocortex, suggesting a role for slow-wave sleep-related episodic memory consolidation. RE has also demonstrated a role in suppressing the retrieval of memories, and inhibiting the activity of the reuniens has a significant effect on the retrieval of remote contextual memories. These mechanisms of control seem like a rich area for further research, especially considering somewhat analagous mechanisms in anterior cingulate cortex (ACC). Similar to hippocampus and prefrontal cortex, the RE may participate in a variety of memory-related behaviors, and may act to coordinate hippocampal-mPFC interactions. To investigate this role of the RE further, we recorded from ACC, RE and hippocampus while animals performed a simple place preference task. In preliminary findings, animals that received a chemogenic block of the reuniens immediately following behavior and prior to memory consolidation showed a marked deficit in their ability to recall remote memories of place preference, while they demonstrated a clear bias from preference memory in all control conditions.

Disclosures: K. Calvin: None. **R.A. Wirt:** None. **E. Flores:** None. **J.M. Hyman:** None. **B. Udin:** None.

Digital Abstract Session

P325. Thalamic and Brainstem Circuits

Program #/Poster #: P325.01

Topic: H.08. Learning and Memory

Support: NIH UH3 NS095495

Title: Ambulatory cognitive testing of verbal memory with a chronically implanted neural sensing and stimulation device in the hippocampus and anterior nucleus of the thalamus

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Abstract: Brain processes underlying encoding and recall of verbal memory can be probed by intracranial electroencephalography (iEEG) during the presentation of words in a free recall task. The purpose of this study was to investigate the spectral and temporal properties of brain activity underlying human verbal memory encoding with recordings from bilateral hippocampus and

anterior nucleus of the thalamus (ANT). Recordings were obtained during the encoding of words from a patient with drug resistant epilepsy implanted with an investigational Medtronic Summit RC+S^{TM} sensing and stimulation device. The patient was able to complete verbal memory tasks in their home environment with iEEG and behavioral data streamed to a handheld device and cloud repository. The participant was presented with lists of words on a handheld tablet screen for a delayed test of free recall. Lists were composed of 12 randomly chosen nouns. After a distractor task, the participant was asked to recall as many of the words as possible. The participant completed 8 independent sessions over several weeks with 15 lists per session for 1440 total word epochs recorded. Epochs with interictal epileptiform spikes were removed from analysis. For each electrode, we determined the average power spectral density at each time point across all word presentation epochs and found the overall induced power during word presentation. We then calculated the average subsequent memory effect (SME) per trial in each frequency band. Frequency bands analyzed were low theta (2-4 Hz), high theta (4-8 Hz), alpha (8-12 Hz), beta (13-25 Hz), low gamma (25-55 Hz), and high gamma (65-115 Hz). The SME was found to have a dependence on the frequency band analyzed (ANOVA, $F=3.87$, $p<0.01$), and post-hoc group analysis (Tukey-Kramer, $p<0.05$) showed that SME was higher in the low gamma band than in alpha or beta bands. SME was dependent on brain area (repeated measures ANOVA, $F=20.72$, $p<0.05$). SME also had an event related dependence (repeated measures ANOVA, $F = 515.39$, $p<0.001$) with SME being higher during word presentation than both before or after presentation. The spectral, spatial, and temporal patterns identified in the human iEEG recordings from this preliminary study are consistent with previous studies of large-scale, distributed dynamics of verbal memory encoding, and are the first to investigate the effects in the ANT. Emerging devices for electrical stimulation of the brain enabling smart sensing and continuous streaming of the iEEG data are crucial for long-term studying of underlying brain dynamics during cognitive tasks.

Disclosures: V.S. Marks: None. **V. Kremen:** None. **V. Sladky:** None. **J. Dolezal:** None. **B. Brinkmann:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic Inc is supplying a device for a research study. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); equity and licensed IP in Cadence Neuroscience. **T. Pal Attia:** None. **M.T. Kucewicz:** None. **G.A. Worrell:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH UH3NS095495. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); equity and licensed IP in Cadence Neuroscience.

Digital Abstract Session

P325. Thalamic and Brainstem Circuits

Program #/Poster #: P325.02

Topic: H.08. Learning and Memory

Support: R01 AG003376, McKnight Brain Research Foundation

Title: Age-associated alterations in locus coeruleus neuronal, glial cell, and vascular elements in cognitively assessed aged macaque monkeys

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Abstract: The Locus Coeruleus (LC) is a brainstem nucleus best known for being the primary central nervous system site of noradrenaline production and is involved in the modulation and optimization of behavioral performance in mammals. The LC is a molecularly and anatomically heterogeneous region that is densely innervated by blood vessels and glial cells. The LC appears to be especially vulnerable to age-related neurodegeneration and is one of the first regions to show evidence of pathological changes associated with Alzheimer's disease (Mather & Harley 2016). While previous work has investigated age-related changes in neurochemical markers in the LC, the impacts of normative aging on LC neuron, glial cell, and vascular characteristics in non-human primates remains an open question. Our research utilizes coronal brainstem sections from a colony of 30 cognitively assessed rhesus macaque monkeys ranging in age from 7 to 32 years (human equivalent ~21 - 96 years). All monkeys underwent tests of spatial short-term memory (delayed response), object recognition memory (delayed nonmatching-to-sample), and object discrimination. We used immunofluorescence techniques to identify neuronal nuclei (NeuN), catecholaminergic neurons (TH), vasculature (STL), and astrocytes (GFAP). The entirety of the locus coeruleus region for each immunolabeled section was imaged bilaterally at 40X on a high-resolution confocal microscope to obtain 3-dimensional volumes of the LC for the quantification of neuron, glial cell, and vascular densities using unbiased stereological techniques. These data were assessed with respect to the age and cognitive status of the monkeys. Preliminary results indicate that overall TH+ density and total neuron density remain stable in the older monkeys compared to the adults. Furthermore, vascular and reactive astrocyte density was not different between the adult and aged animals. No anatomical variable has shown a relationship with any of the three behaviors used to assess cognition in these animals. If these results hold it would indicate that cell loss, changes in vascular innervation, and increases in astrocyte reactivity do not occur in the LC region in normative aging. These results support the hypothesis that LC degradation is a key component of pathological aging but is not compromised in healthy normative aging.

Disclosures: K. McDermott: None. **I. Sinakevitch:** None. **D.T. Gray:** None. **S.O. Khattab:** None. **W.S. Pyon:** None. **C.A. Barnes:** None.

Digital Abstract Session

P325. Thalamic and Brainstem Circuits

Program #/Poster #: P325.03

Topic: H.08. Learning and Memory

Support: RO1 AG003376, McKnight Brain Research Foundation

Title: A 3D interactive representation of Locus Coeruleus nucleus morphology in aged macaque monkeys

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Abstract: The Locus Coeruleus (LC) is a brainstem nucleus best known for being the primary central nervous system site of noradrenaline production. Dysregulation of LC systems contribute to cognitive dysfunctions in both healthy aged brains and brains that succumb to Alzheimer's disease. Importantly, the LC is heterogeneous along both the rostral-caudal and dorsal-ventral axes with respect to neuron morphology, projection targets, and vulnerability to the impacts of normative brain aging and neurodegenerative disease. In particular, the rostral LC projects to cortical and hippocampal brain regions critical to cognition. The aim of the present study is to examine the LC in a nonhuman primate model of aging, using a system that standardizes the 3D morphology of the LC nucleus to enable precise comparisons of neuronal morphology and densities in immunolabelled brain sections from individual monkeys. To this end, coronal brainstem sections were examined every 240 microns along the rostral-caudal axis of the LC from a colony of 30 cognitively assessed rhesus macaque monkeys ranging in age from 7 to 32 years (human equivalent ~21 - 96 years) were used. Three young and three aged monkeys from this sample were used in the present study. To create the 3-dimensional volume, digitized Nissl-stained images of 30 micron thick frozen brain sections at the level of the pons were first collected. High-resolution 5x microscopy images of each LC that had been immunolabeled with a fluorescent morphological marker of catecholaminergic neurons (tyrosine hydroxylase) were then acquired, followed by a 40x high-resolution confocal stack of the LC on the same section. AMIRA software was employed to reconstruct the LC. The volume of the nucleus was delineated using cell bodies positive for TH and large cell bodies in Nissl stains characteristic of this nucleus as boundaries. Preliminary data from these animals indicate that the macaque LC extends approximately 2000 um along the rostro-caudal axis. We observed a core LC nucleus with high TH+ cell densities that became more scattered in more rostral brain sections. Overall volumes varied between 1 and 2.2 mm^3, and the older monkeys tended to have smaller LC volumes compared to the younger individuals, but this will need to be replicated in the full sample of 30 animals. This analysis pipeline will allow specific sites of vulnerability along the rostral-caudal axis of the LC to be identified for further molecular analyses aimed at understanding the mechanisms responsible for LC vulnerability and its impacts of cognition in normative aging and disease.

Disclosures: I. Sinakevitch: None. **C. Deer:** None. **K. McDermott:** None. **S.O. Khattab:** None. **D.T. Gray:** None. **C.A. Barnes:** None.

Digital Abstract Session

P325. Thalamic and Brainstem Circuits

Program #/Poster #: P325.04

Topic: H.08. Learning and Memory

Support: NIH Grant R01EY028219
Picower Innovation Fund

Title: Temporal dynamics of locus coeruleus norepinephrine during a learned behavior

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Abstract: Neurons in the locus coeruleus (LC) are the main source of the neuromodulator, norepinephrine (NE), in the forebrain. Despite the brain-wide effects of LC-NE release, the conditions under which LC-NE neurons are activated and the modes of NE action during learned behavior are poorly understood. Previous reports on LC-NE have sought to explain its role in gain control or network reset, yet how these two functions get integrated at the level of LC dynamics remain unknown. Here, we recorded and manipulated genetically identified LC-NE neurons in mice trained on an instrumental learning behavior incorporating stimulus-reward uncertainty. In this auditory detection task, head-fixed mice have to press a lever to a go tone to obtain a water reward, and refrain from pressing the lever to a no-go tone to avoid an air puff punishment. We modulated task difficulty by using tones of four intensities within the two frequencies. Targeted recordings of LC-NE neurons with opto-tagging, or with calcium imaging via two-photon microendoscopy, show that the majority of LC-NE neurons responds before execution - the lever press - and after punishment, and a smaller fraction responds to reward. The amplitude of the execution and reward response of LC-NE neurons varies with tone intensity: the execution signal increases with tone intensity, while the reward signal decreases with tone intensity (lower uncertainty of reward). LC-NE transient activity peaks following a punishment regardless of tone intensity to signal greater uncertainty or surprise. The effect of photo-inactivation of LC-NE activity during selected trials is twofold. First, inactivating LC-NE produces a decrease in lever presses during go trials with low tone intensity, suggesting a role for LC-NE activity during behavioral response in high uncertainty conditions. Second, inactivating LC-NE impairs active reinforcement learning, or the ability of the mice to optimize its task strategy following a punishment trial. Together, our results suggest that distinct temporal dynamics enable the simultaneous regulation of execution and active learning by LC-NE during learned behavior.

Disclosures: V. Breton-Provencher: None. **G.T. Drummond:** None. **M. Sur:** None.

Digital Abstract Session

P325. Thalamic and Brainstem Circuits

Program #/Poster #: P325.05

Topic: H.08. Learning and Memory

Support: NIH grant R01EY028219, PIIF

Title: Modular projections and engagement of locus coeruleus norepinephrine in a learned behavior

Authors: *G. DRUMMOND, V. BRETON-PROVENCHER, M. SUR;
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Abstract: The locus coeruleus (LC) is a small brainstem nucleus and the primary source of the neuromodulator norepinephrine (NE) in the brain. Receiving diverse, widespread inputs, while also projecting throughout the brain, LC-NE is thought to signal global brain states such as arousal and attention. However, LC-NE is implicated in more precise roles such as mediating learning, promoting task execution, and signaling unexpected uncertainty. In our own lab, we have found that in mice performing an instrumentally conditioned auditory go/no-go task with rewards, punishments, and varying tone intensities to modulate trial difficulty, LC-NE neurons are active pre-lever press and post-reinforcement, with responses scaling with the degree of uncertainty. LC-NE activity affects task execution on the current trial, as well as performance on the following trial. How these execution and reinforcement signals are used to facilitate different aspects of behavior—task execution and active learning—remains unknown. Here, we explore the idea that modular projections and spatiotemporal targeting of LC-NE release enable modulation of multiple aspects of a learned behavior. Using retrograde tracing, we examine whether LC-NE neurons exhibit a modular organization, such that subsets of LC-NE neurons selectively project to specific cortical outputs, the motor or dorsomedial prefrontal cortex. Further, we assess how the activity of LC-NE neurons projecting to the motor or dorsomedial prefrontal cortex can promote different aspects of behavior. To do so, we use two-photon calcium imaging of LC-NE axons and imaging of NE release using the genetically encoded fluorescent sensor, GRAB-NE, while the mice perform the auditory go/no-go task. Our preliminary results show that though there is evidence for some degree of modularity at the anatomical level, both prefrontal cortex and motor cortex may receive similar LC-NE activity. Hence, functional modularity of LC-NE action may be limited to differential integration of NE in subcortical targets, or require selective, dynamic engagement of task-specific NE signals in specific cortical areas.

Disclosures: G. Drummond: None. **V. Breton-Provencher:** None. **M. Sur:** None.

Digital Abstract Session

P326. Striatal and Corticostriatal Circuits

Program #/Poster #: P326.01

Topic: H.08. Learning and Memory

Support: University of Oklahoma Vice President for Research

Title: Neural activity quickly and accurately differentiates iron deficient from iron sufficient women early in visual category learning

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Abstract: Iron deficiency is prevalent in both developed and developing environments and is typically ignored medically until it reaches the point of anemia. However, iron deficiency can lead to negative consequences before it reaches anemia, including negative impacts on measures of work productivity; measures of perception, attention, and memory; and measures of academic success. We have documented that the negative relationship between iron deficiency and cognition is mediated by negative changes in electroencephalography (EEG). These results suggest that iron deficiency changes brain function in ways that are manifest in behavior, which implies that brain function in the subclinical condition of iron deficiency without anemia is affected. The present study sought to determine the possibility of identifying someone as either iron deficient or sufficient based solely on brain activity, and, if possible, how quickly (based on processing time on a cognitive task) this could be done. Both iron sufficient and deficient (non-anemic) women learned two visual categorization tasks while concurrent EEG was acquired. Both tasks involved classifying gray-scale gabor patches on the basis of spatial frequency and orientation; one task used an easily-verbalized rule, the other required complex integration of the information. Moving windows (20 ms width) of EEG data from 100 electrodes were used to predict the participant's iron status using logistic regression; model form was determined using stepwise variable selection. Results show that iron status can be predicted with more than 90% accuracy in less than 150 ms of processing time, within the first 200 trials of learning, with fewer than 12 electrodes. This suggests that iron deficiency, before it becomes a medical problem (anemia), produces measurable changes in brain function, highlighting the need to consider interventions earlier than standard practice. We discuss the implications with respect to the role that the visual system plays in understanding the mechanisms by which iron deficiency exerts negative influences on perception and cognition.

Disclosures: M.J. Wenger: None. **L.A. De Stefano:** None. **S.E. Rhoten:** None. **L. Boozary:** None. **A. Barnett:** None.

Digital Abstract Session

P326. Striatal and Corticostriatal Circuits

Program #/Poster #: P326.02

Topic: H.08. Learning and Memory

Support: NIH DA044297
NIH 5T32NS096050-24
Brain and Behavior Foundation (NARSAD)
Emory University Research Council
Children's Healthcare of Atlanta
Silvio O Conte Center

Title: Striatal melanocortin-4 receptor controls behavioral flexibility in mice

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Abstract: Goal-directed action refers to behaviors that are dynamic, sensitive to unexpected events, and require the dorsomedial striatum (DMS). Meanwhile, habitual behaviors are reflexive and unchanging and rely on the dorsolateral striatum (DLS). Molecular factors underlying an organism's ability to flexibly shift between goal-directed and habitual behavior are incompletely understood. We recently discovered that striatal melanocortin-4 receptor (*Mc4r)* expression correlates with this behavioral flexibility in adult male and female mice. To identify functional consequences, we used viral-mediated gene silencing to reduce *Mc4r*. *Mc4r* knockdown in the DMS enhanced the ability of mice to select actions based on reward likelihood and value, while reduction in the DLS facilitated habit formation. Thus, inhibiting MC4R appears to enhance the functions of distinct striatal subregions. Changes in *Mc4r* expression are thought to modulate activity of medium spiny neurons, leading to the hypothesis that chemogenetic manipulation of *Mc4r*+ DMS neurons will impact expression of behavioral flexibility. Stimulation of *Mc4r*+ DMS neurons via Gq-coupled Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) facilitated animals' ability to select actions based on reward likelihood. Meanwhile, inhibition via Gi-DREADDs rendered animals insensitive to changes in reward likelihood, promoting habits. Finally, we discovered using trans-synaptic tracing that several brain regions necessary for goal-directed action synapse onto *Mc4r*+ neurons in the DMS, and we confirmed using combinatorial viral vector strategies that inputs from one such region, the orbitofrontal cortex, are necessary for MC4R-related behavioral flexibility.

Disclosures: E.C. Heaton: None. **S.T. Yount:** None. **S.L. Gourley:** None.

Digital Abstract Session

P326. Striatal and Corticostriatal Circuits

Program #/Poster #: P326.03

Topic: H.08. Learning and Memory

Support: DA042111
DA048931
GM07628

Title: Dopamine release patterns in the nucleus accumbens core are influenced by novelty detected in environment

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Abstract: Dopamine in the nucleus accumbens (NAc) is critically involved in signaling prediction error; however, findings indicate that attention-related constructs such as novelty are also involved in associative learning and suggest that NAc dopamine may play an important role in mediating this relationship. Prior investigations of dopamine's involvement in associative learning have often been constrained by the use of traditional techniques that preclude the ability to precisely examine dopamine's role in associative processes. Here we use fiber photometry in conjunction with a fluorescent dopamine sensor with high temporal resolution to examine novelty-induced dopamine responses in the NAc core. Using a "latent inhibition" design, we first showed that repeated exposure to a stimulus decreased the peak dopamine response to that stimulus progressively. The preexposed conditioned stimuli (CS) elicited a weaker dopamine response than the non-preexposed CS, which was also associated with a decreased learning rate for the preexposed CS when paired with a footshock. We further demonstrated that both the presentation of unexpected and the omission of expected stimuli produced a similar protracted increase in dopamine baseline. Consistent with the observation of external inhibition, we also showed that presentation of an unexpected stimulus resulted in a decreased conditioned response to the predictive stimulus. Finally, we found that the presentation of an unexpected stimulus reversed the decreased dopamine response caused by repeated stimulus presentation, an effect known as "dishabituation". While our findings are largely consistent with prevailing theories of dopamine's role in signaling prediction error, the data presented here highlight the importance of attention and novelty in dopamine's involvement in associative learning.

Disclosures: M.G. Kutlu: None. **P. Melugin:** None. **J.E. Zachry:** None. **G. Schoenbaum:** None. **M.J. Sharpe:** None. **E.S. Calipari:** None.

Digital Abstract Session

P327. Consolidation and Reconsolidation: Neural Circuit Mechanisms

Program #/Poster #: P327.01

Topic: H.07. Long-Term Memory

Support: VA Merit 5I01CX001375

NIMH Grant R01 MH024600

Title: Human brain activity and functional connectivity as memories age from one hour to one month

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Abstract: Theories of memory consolidation describe changes in the role of brain regions and changes in connectivity between brain regions as memories age. Human lesion studies suggest memories become hippocampus-independent over years, whereas animal studies suggest this process occurs across relatively short intervals, from weeks to months. Recent human neuroimaging studies provide evidence that changes in hippocampal and cortical activity and connectivity can be detected over these short intervals, but many of these studies examined only two time periods. We assessed brain activity and brain connectivity over four time periods to allow for increased temporal resolution of these neural changes. Memory and whole-brain FMRI activity was measured for unique sets of photos of indoor and outdoor scenes studied 1 hour, 1 day, 1 week, and 1 month prior to scanning. During scanning, participants (N=21 young adults) viewed scenes, made old/new recognition judgments, and gave confidence ratings. Memory accuracy, confidence ratings, and response times changed with memory age. We examined brain activity associated with scenes to identify regions where activity changed with the age of memory (following a power function). A widespread cortical network was observed where activity either increased or decreased with memory age [prefrontal cortices (PFC), anterior cingulate, sensorimotor cortices, posterior parietal cortices (PPC), and middle occipital gyrus; $p < 0.001$]. Hippocampal activity was not related to memory age. These findings were almost identical when we minimized the effect of behavioral changes across time periods. Whole-brain connectivity associated with the bilateral ventromedial PFC (ROI-based, generalized psychophysiological interaction analysis associated with memory age [following a power function]) revealed a significant increase in connectivity with the left PPC ($p < 0.001$). Connectivity analysis for the bilateral hippocampus revealed decreasing connectivity with the hippocampus, parahippocampal gyrus, lateral temporal cortex, and PPC ($p < 0.02$). In sum, we detected changes in cortical activity and changes in hippocampal and cortical connectivity with memory age across short intervals. These findings provide support for the predictions of systems consolidation and suggest that these changes begin soon after memories are formed.

Disclosures: C.W. Tallman: None. **C.N. Smith:** None.

Digital Abstract Session

P327. Consolidation and Reconsolidation: Neural Circuit Mechanisms

Program #/Poster #: P327.02

Topic: H.07. Long-Term Memory

Title: Retrieval Spikes: A dendritic mechanism for retrieval-dependent memory consolidation

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Abstract: Retrieval of a new memory can improve its long-term retention. We investigated neuronal mechanisms underlying this phenomenon, by training mice with the classical fear conditioning paradigm under two-photon imaging. We used a protocol in which cued retrieval during training improved fear learning, and imaging focused on the primary motor cortex (M1), a region that is essential for conditioned freezing. We found that cued retrieval elicited strong calcium spikes in apical dendrites of layer 5 pyramidal neurons (L5 PNs), the principal cortical output cells. Dendritic spines that were very active shortly before, and during, these retrieval spikes underwent persistent depotentiation: becoming smaller, and less active at later presentations of the tone. Transient optogenetic inhibition of CaMKII, a calcium-dependent master regulator of plasticity, abrogated the depotentiation of active spines, only if applied shortly after retrieval spikes. Retrieval spikes activity correlated negatively with dendritic and somatic activities at later recalls. These inverse relationships were not observed in L5 PNs whose plasticity was disrupted by optogenetic inhibition of CaMKII. We further show that the generation of retrieval spikes coincides with reduced activity of dendritic-targeting inhibitory neurons (SST^+, $NDNF^+$) during retrieval, suggesting that the local inhibitory circuit is poised for retrieval-dependent spike formation. Collectively, these findings indicate that retrieval evokes dendritic spikes that weaken the synaptic inputs that were active during spiking. This negative feedback leads to a reduced representation of the conditioned stimulus in M1, which supports stillness during freezing. We describe a dendritic consolidatory mechanism in which retrieval of a new memory increases its strength.

Disclosures: E. Geron: None. **W. Gan:** None.

Digital Abstract Session

P327. Consolidation and Reconsolidation: Neural Circuit Mechanisms

Program #/Poster #: P327.03

Topic: H.07. Long-Term Memory

Support: BrainsCAN at Western University through the Canada First Research Excellence Fund (CFREF)

Title: What is the spatial scale of sleep oscillations in cortex?

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Abstract: In the two-stage model of memory consolidation, memories are first formed in the hippocampus and then transferred to neocortex for long-term storage (McClelland et al.,

Psychological Review, 1995; Rasch and Born, Curr Opin Neurobiol, 2007). This transfer is mediated by synaptic plasticity, for which co-occurrence of pre- and post-synaptic activity plays a critical role (Bi and Poo, J Neurosci, 1998). While it has become increasingly clear that sleep oscillations actively and causally contribute to this process, it remains unclear to what extent these oscillations coordinate activity across areas in neocortex. Based on early recordings in animals under anesthesia, sleep rhythms were generally considered to occur across a wide range of cortex, creating a state of large-scale synchrony (Andersen et al., J Physiol, 1967; Contreras et al., J Neurosci, 1997; Achermann and Borbély, Neuroscience, 1998).Recent work, however, has challenged this idea by reporting isolated sleep rhythms such as slow-oscillations and spindles. What is the spatial scale of sleep rhythms in cortex? To answer this question, we adapted deep learning algorithms initially developed for detecting earthquakes and gravitational waves in high-noise settings to analysis of neural recordings in sleep (Perol et al., Science Advances, 2018; George and Huerta, Physics Letters, 2018). We studied sleep spindles in non-human primate electrocorticogram (ECoG), human electroencephalogram (EEG), and clinical intracranial electroencephalogram (iEEG) recordings. Our approach, which detects a range of clearly formed large- and small-amplitude spindles during sleep, reveals that sleep spindles co-occur over a broad range of cortex. In particular, multi-area spindles, where more than 10 electrode sites exhibit this rhythm simultaneously, are much more frequent than previously estimated by amplitude-thresholding approaches, which tend to select only the highest-amplitude spindles and can miss events that temporarily fall below threshold. Because spindles are intrinsically related to sleep-dependent consolidation of long-term memories, these results have important implications for the distribution of engrams in cortex of primate and humans.We next hypothesized that, if spindles are actively involved in consolidating distributed networks in cortex, they may exhibit an increased prevalence across areas following a memory task. To address this additional hypothesis, we analyzed human EEG recordings following a low- and high-load visual working memory task. Consistent with this hypothesis, we find our method detects an increase in multi-area spindles in the high-load memory condition.

Disclosures: **M. Hasanzadeh Mofrad:** None. **A. Suller Marti:** None. **L. Muller:** None.

Digital Abstract Session

P327. Consolidation and Reconsolidation: Neural Circuit Mechanisms

Program #/Poster #: P327.04

Topic: H.07. Long-Term Memory

Support: NIH MH063901

Title: Tracking the emergence of novel memory representations across hippocampal-cortical networks

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Abstract: While the hippocampus is necessary for associative memory formation, extensive evidence indicates that memory representations become distributed across hippocampal-cortical networks over time. Memory reorganization across hippocampal-cortical networks is thought to be accompanied by a change in the nature of memory representations, including the extraction of structured knowledge across experiences. Most studies typically probe memory representations at a few timepoints, with some studies reporting immediate changes after learning, while others find changes across days to weeks. Here, we characterized the development of de novo memory representations by performing longitudinal fMRI on the same individuals across three months. Three human participants were repeatedly exposed to abstract fractal images (referred to as trained stimuli) in the context of a serial reaction time (SRT) task. A subset of fractals was presented in temporal sequences during the SRT task. Sequence learning was reflected in reduced response time for intact sequences that emerged over time. Neural representations were characterized in fMRI scans while individual stimuli were presented in isolation. A set of brain regions became increasingly responsive to trained fractals over time, including the hippocampus, medial prefrontal cortex (mPFC), medial parietal cortex, angular gyrus, and anterior temporal cortex. This set of brain regions significantly overlapped with large-scale cortical networks implicated in memory retrieval ('core' and 'dorsomedial' subsystems of the 'default network'). Moreover, shared sequence-level representations (greater pattern similarity for stimuli within versus between sequences) emerged over time in the hippocampus, mPFC, and medial parietal cortex, providing evidence for integration across items within sequences. Evidence for sequence representations persisted in the hippocampus over months, in contrast to the notion that memories are 'transferred' from the hippocampus to neocortical sites as posited by some theoretical accounts of memory consolidation. These findings provide novel evidence for the formation of de novo associative memory representations over the course of months across hippocampal-cortical networks.

Disclosures: A. Tambini: None. **J. Miller:** None. **L. Ehlert:** None. **A. Kiyonaga:** None. **M. D'Esposito:** None.

Digital Abstract Session

P327. Consolidation and Reconsolidation: Neural Circuit Mechanisms

Program #/Poster #: P327.05

Topic: H.07. Long-Term Memory

Support: KAKENHI 19H03328
Shionogi & CO., LTD
KAKENHI20K15910

Title: Association of a neutral contextual memory with a fearful experience induces synapse formation selectively in Arc-expressing neurons of the mouse retrosplenial cortex

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Abstract: Exposure to a novel context induces the immediate-early gene expression in a subset of neurons that constitute neuronal networks for memory trace. These neural ensembles may be modulated by memory updating such as fear association through synaptic reorganization. However, the steps of the circuit modulation have yet to be visualized at the level of individual synapses. Contextual fear conditioning is a behavioral paradigm in which an aversive stimulus is associated with a neutral context. To visualize and manipulate neural circuits activated during behaviors by recombinant adeno-associated virus vectors, we developed a doxycycline-inducible reporter expression system (Tet-ON) under the control of enhanced synaptic activity response elements (E-SARE) from the *Arc* promoter. By using this method, we first expressed channelrodopsin-2 (ChR2) into neuronal ensembles in the retrosplenial cortex (RSC) of the mouse brain activated during exposure to a neutral context, and then tested whether light-induced activation of these ensembles could elicit fear responses 1 day after the fear conditioning. We found that ensembles recruited by a preceding exposure to the same context with the fear conditioning could induce fear responses (*P = 0.013, paired t-test, n = 8) whereas neurons recruited by a distinct context exposure could not (P = 0.49, paired t-test, n = 8). Next, by using 2-photon microscopy, we visualized and monitored dendritic spines of the context-activated RSC neurons that were labeled with enhanced green fluorescent protein using the E-SARE Tet On system. We detected dendritic spines newly formed after the fear conditioning in the neuronal ensembles recruited by the preceding exposure to the same context rather than by the exposure to a distinct context (**P < 0.01, one-way ANOVA post hoc turkey's test, n = 6). Finally, we explored brain regions projecting to the RSC by a retrograde viral injection (AAV2 retro) into the RSC. We found several brain regions projecting to the RSC including claustrum, subiculum, frontal association cortex, anterior cingulate cortex, parietal association cortex, and basolateral amygdala. These results suggest that, accompanied with synapse formation, the RSC neuronal ensembles responding to the neutral context are incorporated into fear memory traces after subsequent fear conditioning training in the same context.

Disclosures: K. Minatohara: None. **S. Ohnami:** A. Employment/Salary (full or part-time):; SHIONOGI &CO., LTD.. **S. Okabe:** None. **H. Bito:** None. **H. Okuno:** None.

Digital Abstract Session

P327. Consolidation and Reconsolidation: Neural Circuit Mechanisms

Program #/Poster #: P327.06

Topic: H.07. Long-Term Memory

Support: NIH-NIA 1R01AG048908-01A1
the James and Audrey Foster MGH Research Scholar Award
Ellison Family Grant
Alzheimer's Association research grant
DFG research fellowship

Harvard Brain Initiative Young Scientist Travel Award
Bertarelli Program in translational Neuroscience and Neuroengineering

Title: Feed-forward inhibition in Dentate Gyrus-CA3 drives time-dependent re-organization of memory ensembles in prefrontal cortex

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Abstract: Hippocampal-cortical communication is thought to determine the extent to which memories generalize over time, but the underlying circuit mechanisms are poorly understood. By identifying Ablim3, a cytoskeletal F-actin binding protein that is exclusively localized to dentate granule cell (DGC) mossy fiber terminals, as a molecular brake of DGC connectivity with parvalbumin inhibitory neurons (PV-INs), we demonstrated a critical role for DGC recruitment of feed-forward inhibition (FFI) onto CA3 in time-dependent memory generalization. Viral downregulation of *Ablim*3 in DGCs enhanced excitatory drive onto stratum lucidum PV INs, elaborated PV IN synaptic contacts with CA3 neurons and increased FFI in DG-CA3. Mice with increased FFI in DG-CA3 exhibited reduced remote memory generalization. These observations motivated investigation of how FFI in DG-CA3 affects neuronal ensemble dynamics in hippocampal-prefrontal cortical networks over time. Towards this goal, we performed single-photon calcium imaging in hippocampal CA1 and the anterior cingulate cortex (ACC) of awake, behaving mice - in which we increased FFI in DG-CA3 - to longitudinally track neuronal ensembles during encoding and recall of contextual fear memory in the fear conditioned and neutral contexts at recent and remote timepoints. We first developed a Matlab-based automated workflow to standardize the extraction and sorting of temporal and spatial information from both CA1 and ACC datasets to allow for comparison between these two brain regions. Consistent with the notion that memory traces re-organize during consolidation in hippocampal-cortical networks, we directly observed time-dependent refinement of context-associated ensembles in ACC over time. Importantly, increasing FFI in DG-CA3 potentiated this refinement and we observed a decrease in the overlap between ensembles of the fear-conditioned and neutral contexts in CA1 and ACC at remote timepoints. Analysis of active neurons revealed that FFI reduced numbers of correlated pairs of active neurons in ACC, but not CA1, following context exposure. Together, these findings provide direct evidence for time-dependent evolution of memory ensembles and identify FFI in DG-CA3 as a neural substrate for memory consolidation in hippocampal-cortical networks. Targeting Ablim3 may represent a strategy to promote hippocampal inhibition, dampen hippocampal hyperactivity and promote memory consolidation in aging and Alzheimer's disease.

Disclosures: H. Twarkowski: None. **V. Steininger:** None. **M. Kim:** None. **A. Sahay:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); US Patent 10, 287, 580 Issued May 14, 2019: Methods for inhibition of Ablim3 to improve memory in aging, Alzheimer's disease and PTSD.

Digital Abstract Session

P327. Consolidation and Reconsolidation: Neural Circuit Mechanisms

Program #/Poster #: P327.07

Topic: H.07. Long-Term Memory

Support: JST CREST JPMJCR1655,FPD P19310,JSPS KAKENHI 16K18359, 15F15408, 26115502, 25116530, JP16H06280, 19F19310, and 20H03552
World Premier International Research Center Initiative from MEXT
Takeda Science Foundation
Shimadzu Science Foundation
Kanae Foundation
Research Foundation for Opto-Science and Technology
Ichiro Kanehara Foundation

Title: Sparse activity of hippocampal adult-born neurons during REM sleep for memory consolidation

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Abstract: The occurrence of dreaming during rapid eye movement (REM) sleep prompts interest in the role of REM sleep in hippocampal-dependent episodic memory. Within the mammalian hippocampus, the dentate gyrus (DG) has the unique characteristic of exhibiting neurogenesis persisting into adulthood. Despite their small numbers and sparse activity, adult-born neurons (ABNs) in the DG play critical roles in memory; however, their memory function during sleep is unknown. Here, we investigate whether young ABN activity contributes to memory consolidation during sleep using Ca^{2+} imaging in freely moving mice. We found that contextual fear learning recruits a population of young ABNs that are reactivated during subsequent REM sleep against a backdrop of overall reduced ABN activity. Optogenetic silencing of this sparse ABN activity during REM sleep alters the structural remodeling of spines on ABN dendrites and impairs memory consolidation.

These findings provide a causal link between ABN activity during REM sleep and memory consolidation.

Disclosures: **S. Srinivasan:** None. **D. Kumar:** None. **I. Koyanagi:** None. **A. Carrier-Ruiz:** None. **P. Vergara:** None. **Y. Sugaya:** None. **M. Kasuya:** None. **T. Yu:** None. **K. E. Vogt:** None. **M. Muratani:** None. **T. Ohnishi:** None. **S. Singh:** None. **C. M. Teixeira:** None. **Y. Chérasse:** None. **T. Naoi:** None. **S. Wang:** None. **P. Nondhalee:** None. **B. A.H. Osman:** None. **N. Kaneko:** None. **K. Sawamoto:** None. **S. G. Kernie:** None. **T. Sakurai:** None. **T. J. McHugh:** None. **M. Kano:** None. **M. Yanagisawa:** None. **M. Sakaguchi:** None.

Digital Abstract Session

P328. Effects of Diet On The Hippocampus

Program #/Poster #: P328.01

Topic: H.08. Learning and Memory

Support: Snyder Institute for Chronic Disease
International Microbiome Centre

Title: Dietary fibre attenuates novel object impairment resulting from high fat/sucrose diet and increases hippocampal DeltaFosB

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Abstract: A high fat/sucrose diet (HFS), one of the most important risk factors for the development of metabolic diseases such as type 2 diabetes, has also been shown to affect cognitive performance. A HFS diet can trigger a cascade of inflammatory responses which may be a factor in triggering neuroinflammation and associated cognitive decline. The hippocampus, which is paramount for memory consolidation and retention, shows increased neuroinflammatory markers and decreased neurogenesis following a HFS diet. The stability of hippocampal memories requires changes in transcription factors that are involved in synaptic plasticity/function. DeltaFosB is a stable transcription factor that is involved in forming associations between stimuli and the environment and a decrease in DeltaFosB, such as in depression, is associated with cognitive decline. In rodents, a high fibre diet has been shown to attenuate HFS-induced cognitive decline in hippocampal-dependent tasks, but the mechanisms by which this occurs remains elusive. The current study examined the effects of HFS and high fibre diets on novel object recognition (NOR), a hippocampal-dependent task, and explored associated relative DeltaFosB expression in the hippocampus using western blotting. 100 male Sprague-Dawley rats underwent obesity induction with a HFS diet for 8-weeks. The top 50% of weight gainers were allocated to the following groups (n=10/group) for an 8-week fibre intervention: chow-fed control, HFS, HFS+oligofructose (HF/O), HF/O+beta-glucan (HF/OB), HF/O+resistant starch (HF/OR), or HF/OB + resistant starch (HF/OBR). During week 6 rats

underwent NOR procedures consisting of habituation, familiarization, and testing, each occurring for 5-min over three days. Insulin tolerance testing occurred during week 7 to determine the effects of the diets on metabolic function. The HFS diet group was unable to distinguish between the novel or familiar objects suggesting impaired memory consolidation or retention, these rats also showed impaired insulin sensitivity confirming the presence of metabolic dysfunction. Dietary fibre groups significantly distinguished the novel from familiar objects and had improved insulin sensitivity. Relative DeltaFosB expression was increased in the HF/OB and HF/OR fibre groups which positively correlated with time spent exploring the novel object, alluding to increased hippocampal activity. These results suggest that not only can a high fibre diet attenuate metabolic outcomes associated with a HFS diet, a diet high in fibre has the potential to attenuate HFS-induced cognitive dysfunction possibly via increased neuronal activity within the hippocampus.

Disclosures: E.W. Tuplin: None. **E. Alukic:** None. **D.E. Lowry:** None. **F. Chleilat:** None. **W. Wang:** None. **N.A. Cho:** None. **R.A. Reimer:** None.

Digital Abstract Session

P328. Effects of Diet On The Hippocampus

Program #/Poster #: P328.02

Topic: H.08. Learning and Memory

Support: R21ES027119
P30ES005022
T32ES007148

Title: Intermittent fasting alters cognition, anxiety-like behavior, and hippocampal norepinephrine content in aged mice

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Abstract: Intermittent fasting (IMF) is associated with many health benefits in animal models and humans. Yet, little is known if an IMF diet affects mood and cognitive processing. We have previously identified that IMF in diet-induced obese males increases norepinephrine content in the hypothalamus and increase arcuate neuropeptide Y (NPY) gene expression more than in *ad libitum* males. This suggests that IMF may improve cognition through activation of the hindbrain norepinephrine neuronal network and reverse the age-dependent decline in NPY expression. Less is known about the association between anxiety and IMF. In humans, IMF during Ramadan may alleviate anxiety. Here, we address the impact of IMF on hippocampal-dependent memory using the Y-maze and spatial object recognition (SOR), hippocampal-independent memory using novel object recognition (NOR), and anxiety-like behavior using the open field task (OFT) in middle-aged male (12 mo) and aged female (18 mo) mice. Y-maze data indicate that IMF males perform worse in the identification of the unknown arm than same-sex controls, suggesting an IMF-

induced deficit in interpreting spatial navigation routes. Conversely, no difference was detected in females on this task. In the SOR task, which evaluates hippocampal-dependent object orientation, IMF males were not affected; however, IMF females exhibited a decrease in performance. This suggests that, in females, IMF impairs spatial information about the arrangement of objects. In the NOR, which detect differences in hippocampal-independent memory, IMF males performed better than their same-sex counterparts; however, we did not detect an effect in females. IMF treated males displayed and increase in hippocampal norepinephrine content, suggesting IMF may alter hindbrain norepinephrine signaling. Our overall cognitive results suggest that, in males, IMF results in deficits only in hippocampal-dependent tasks, yet an enhancement in performance when hippocampal-independent tasks are conducted. In females, only the hippocampal-dependent task of SOR was affected by IMF, suggesting IMF disrupts spatial object configuration. In the OFT, IMF male and female mice spent more time in the center zone, indicative of an anxiolytic phenotype. Collectively our research suggests that IMF is enough to produce both an impairment and enhancement in memory dependent upon age, sex, and hippocampal involvement, as well as an anxiolytic phenotype in both male and female aged mice.

Disclosures: K. Wiersielis: None. **A. Yasrebi:** None. **K. Conde:** None. **T. Roepke:** None.

Digital Abstract Session

P329. Hippocampal Circuits: Anatomical Studies

Program #/Poster #: P329.01

Topic: H.08. Learning and Memory

Support: NSERC Discovery Grant RGPIN-2018-06765
CIHR Project Grant PJT – 173244
CFI John R. Evans Leaders Fund 38098

Title: Mapping glutamatergic projections from the median raphe to the hippocampus in mice

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Abstract: The raphe nuclei contain a vast diversity of neuronal populations, including glutamatergic neurons characterized by the expression of the vesicular glutamate transporter type 3 (VGLUT3). VGLUT3-positive neurons project to many areas of the brain, including the hippocampus (HP). The aim of our study was to reveal the topography of glutamatergic projections from the raphe to the HP. We performed anterograde and retrograde tracing studies in male and female VGLUT3-Cre mice (n=12), using Cre-dependent viral vectors expressing fluorescent proteins. Local injection of an anterograde viral tracer in the median raphe, but not in the dorsal raphe, revealed VGLUT3 positive fibers in all subfields of the HP (CA1, CA2, CA3 and dentate gyrus). A high density of glutamatergic fibers was observed at the junction between

stratum radiatum and stratum lacunosum-moleculare. Next, we used retrograde viral vector injections to map the position of HP-projecting glutamatergic neurons within the different raphe sub-nuclei. VGLUT3 neurons projecting to the HP were predominantly found in the median raphe region (paramedian and median raphe) and the B9 nuclei. A large majority of VGLUT3-positive neurons were situated in the rostral parts of these nuclei and their number decreased caudally. Finally, we sought to determine if distinct glutamatergic pathways projected to the dorsal and ventral parts of the HP, known to have different roles. We found that most VGLUT3-positive neurons projected to either dorsal of ventral HP. In addition, a medio-lateral gradient was observed with ventral HP-targeting neurons located close to the midline whereas dorsal HP targeting neurons were found more laterally. Our results uncovered a clear segregation of inputs from raphe VGLUT3 neurons to the dorsal and ventral parts of the HP, suggesting roles for raphe glutamatergic projections in the modulation of functions such as spatial learning and memory or anxiety.

Disclosures: J. Fortin-Houde: None. **F. Henderson:** None. **B. Amilhon:** None. **G. Ducharme:** None.

Digital Abstract Session

P330. Interactions Between Hippocampus and Entorhinal Cortex: Rodent Studies

Program #/Poster #: P330.01

Topic: H.08. Learning and Memory

Support: NIH grants R01 NS086947
R01 MH119179

Title: Spatial working memory can be supported by differential firing of medial entorhinal cortex cells in the delay zone

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Abstract: The ability to retain information in working memory (WM) over periods of seconds is central to numerous cognitive tasks. Included in the large brain network that is necessary for encoding and maintaining spatial WM are the medial entorhinal cortex (MEC) and the hippocampus. Even though both brain regions contribute to WM, we have previously shown that an intact MEC can at least partially compensate for the loss of hippocampal function and vice versa. Such compensation raises the question which aspects of neuronal coding remain intact after partial damage to the entorhinal-hippocampal circuit. For example, we have shown that hippocampal spatial firing patterns during spatial WM are partially preserved without MEC. To determine to what extent MEC depends on hippocampal input to represent task-related information we recorded neuronal activity patterns in MEC with and without hippocampal input while rats performed a spatial alternation task on a figure-8 maze. As previously described, WM over delays of 10 s and 60 s was partially impaired by the loss of hippocampal neurons, while

behavioral performance without a delay remained intact. To describe the entorhinal contribution to spatial WM, we first examined the firing patterns in the memory task in control rats (n = 3 rats, n = 147 cells). Similar to the open field, the majority of MEC neurons showed spatial selectivity on the maze with their firing preference distributed across different maze zones, such as delay zone, stem, and return arms. Of the cells that were active during the delay and on the stem, ~20 % were differentially active between trials when animals were turning either right or left at the choice point, although the proportion was lower with 60 s delays. Large hippocampal lesions (n = 4 rats) did not abolish either spatial selectivity or left/right choice selectivity, but altered the firing patterns of MEC cells (n = 79). Spatial selectivity was reduced in layer II cells and occurred preferentially in the delay zone or the stem. When cells were active in these zones, they surprisingly showed increased differences between left and right choice trials, but only during trials without delay. In trials with either 10 s delay or 60 s delay, differential activity was retained in < 10 % of MEC cells. Taken together, MEC efficiently codes for memory-related information, and such information is reduced but not abolished by removing hippocampal feedback. The physiological patterns thus resemble the effects of the lesion on memory performance, which supports the possibility that task-relevant information in MEC does not depend on an intact hippocampus and can directly support spatial WM.

Disclosures: **L. Yuan:** None. **M. Sabariego:** None. **T. Tang:** None. **J. Leutgeb:** None. **S. Leutgeb:** None.

Digital Abstract Session

P330. Interactions Between Hippocampus and Entorhinal Cortex: Rodent Studies

Program #/Poster #: P330.02

Topic: H.08. Learning and Memory

Support: NIH Grant 1R01NS109362-01
Mathers Foundation Award
Klingenstein-Simons Fellowship in Neuroscience
The Esther A. & Joseph Klingenstein Fund
Sloan Research Fellowship
Leon Levy Fellowship
Whitehall Three Year Research Grant

Title: Modulation of cortical sensory processing by direct hippocampal feedback

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Abstract: Our brains constantly extract salient information from the environment and compare it to our experiences. The modulation of ongoing sensory processing in the context of prior experiences helps us to perceive an environment and experiences within it as rewarding or harmful; novel or familiar. This interplay between stored representations and sensory processing

relies on the functional interaction between the hippocampus (HC), critical for learning and memory, and its neighboring entorhinal cortex (EC), a hub of multi-sensory information processing. The canonical circuit posits that HC receives sensory information from superficial layers of EC for long-term memory representation but does not send direct hippocampal feedback to these cortical layers. Instead, HC is believed to project to the deep layers of EC, which then relay the information back to the superficial layers of EC forming an indirect feedback loop. Our study aims to elucidate the true direct reciprocal circuit between the HC and the EC to explore how 'memory' inputs from the hippocampus modulate 'real-time' cortical sensory processing allowing the animal to adapt to its changing environment. Using virus-based anatomical mapping and optogenetic functional dissection of hippocampal-EC inputs, we demonstrate the presence of monosynaptic hippocampal connections to both deep and superficial layers of EC. We find that the hippocampal inputs to EC deep layers are more extensive in terms of innervation surface area, have higher probability of monosynaptic connections and significantly greater connection strength than the HC inputs to EC superficial layers. To understand the circuit mechanism underlying the EC layer specific differences in synaptic strength of the HC input, we are characterizing the dynamics of excitation-inhibition balance and short-term plasticity of the hippocampal inputs onto cortical neurons. Using paired stimulation of hippocampal and sensory inputs converging on to EC neurons, we will further identify the mechanism by which hippocampus modulates cortical sensory processing. Our findings suggest anatomically and functionally segregated feedback from hippocampus to EC resulting in a reciprocal circuit architecture for parallel information processing.

Disclosures: **T. Butola:** None. **L. Peng:** None. **J. Basu:** None.

Digital Abstract Session

P330. Interactions Between Hippocampus and Entorhinal Cortex: Rodent Studies

Program #/Poster #: P330.03

Topic: H.08. Learning and Memory

Support: NIH NINDS 1R01NS109362-01
NIH BRAIN Initiative 1R01NS109994
Klingenstein-Simons Fellowship Award in Neuroscience
Mathers Foundation Award
Whitehall Three Year Research Grant
Sloan Research Fellowship
Molecular, Cellular, and Translational Neuroscience Training Grant
5T32NS86750

Title: Lateral entorhinal cortex: a distal input that drives strong inhibition and dendritic excitation onto CA1 pyramidal neurons

Authors: *O. BILASH, J. BASU;
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Abstract: The functional interaction between the entorhinal cortex (EC) and hippocampus (HC) is critical for episodic memory formation. Direct projections from the medial and lateral entorhinal cortex (MEC and LEC) provide the hippocampus with spatial and non-spatial (contextual) sensory information, respectively. Previous studies have largely focused on MEC-HC interactions, and only recently have behavioral studies started to demonstrate that LEC is involved in olfactory learning, novelty and object-related learning, and contextual learning. Still, the circuit-level functional interactions between LEC and the hippocampus remains underexplored. LEC inputs synapse onto the distal dendrites of CA1 pyramidal neurons (PNs), where they are poised to integrate with other extra- and intra-hippocampal inputs, likely influencing compartment-specific hippocampal learning rules. Our study examines how distal dendritic input from LEC modulates hippocampal information flow through specific GABAergic microcircuits, short-term plasticity rules, and non-linear dendritic computations. Using slice electrophysiology and optogenetics, we demonstrate that glutamatergic LEC projections drive excitation onto CA1 PNs, while simultaneously recruiting strong feed-forward inhibition (FFI). LEC inputs elicit subthreshold post-synaptic potentials, but are not strong enough to elicit action potentials in CA1 PNs. However, using dendritic recordings, we find that optogenetic stimulation of LEC axons in CA1 can drive dendritic spikes in CA1 PN distal dendrites. To better understand the mechanism underlying the LEC-driven dendritic spikes, we began to probe the GABAergic microcircuit elements recruited by LEC inputs. We performed targeted recordings from GABAergic neuron-specific transgenic mouse lines and found that LEC inputs directly recruit specific inhibitory neurons (INs) in area CA1, notably vasoactive intestinal peptide (VIP)-expressing INs. VIP INs are known to be disinhibitory in the hippocampus and neocortex, so we propose that LEC inputs gate dendritic spikes in CA1 PNs by engaging local disinhibitory VIP INs. Given that *in vitro* and *in vivo* studies have previously demonstrated the role of dendritic spikes in hippocampal plasticity, our findings suggest that LEC plays an important role in promoting supralinear dendritic computations, thus enabling it to influence long-term representation in the hippocampus.

Disclosures: O. Bilash: None. **J. Basu:** None.

Digital Abstract Session

P330. Interactions Between Hippocampus and Entorhinal Cortex: Rodent Studies

Program #/Poster #: P330.04

Topic: H.08. Learning and Memory

Support: NIH BRAIN INITIATIVE 1R01NS109994
NIH 1R01NS109362-01
Mathers Foundation Award
Klingenstein-Simons Fellowship Award in Neuroscience
Sloan Research Fellowship
Whitehall Three Year Research Grant

Title: Lateral entorhinal cortex modulates contextual and spatial memory processing in CA1 hippocampal area through long range circuitry

Authors: *M. HERNANDEZ FRAUSTO, L. PENG, J. BASU;
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Abstract: The entorhinal cortex (EC) and Hippocampus (HC) are crucial structures for episodic memory, and spatial and contextual dependent learning. Lateral entorhinal cortex (LEC) conveys contextual features of the environment, related to objects, novelty, fear, and rewards, while the medial entorhinal cortex (MEC) conveys spatial information, encoding an animal position in space[1,2]. LEC sends both long range excitatory and inhibitory[3] projections to hippocampal CA1 area through direct pathways. While indirect excitatory pathway has been implicated in memory storage, direct pathway from LEC to CA1 area functions remains unclear. Here, we propose that LEC long range projections in CA1, modulates contextual non-spatial and spatial representations while the animal is executing different cognitive tests. To address the role of long excitatory projections (LREP) we use a chemogenetic silencing in CA1 during contextual Novel Object Recognition test (NOR), Novel Object Location test (NOL) and the natural tendency of the mouse to escape from open environments and hide in a safe area through association of spatial external cues in a Barnes maze. Our results suggest an important role of excitatory projections from LEC in CA1 in discrimination of objects as well as spatial memory recall.We are currently exploring the contribution of each of the GABAergic projection neuron subtypes, as we found that long range inhibitory projections (LRIP) comprise of parvalbumin (PV), somatostatin (SOM) and vasoactive intestinal peptide (VIP) expressing GABAergic neuron subtypes.1. Ohara, S. *et al.* Disentangling the role of the MEC and LEC in the processing of spatial and non-spatial information: Contribution of lesion studies. *Neuron* **31**, 652-665 (2017).2. Basu, J. & Siegelbaum, S. A. The Corticohippocampal Circuit, Synaptic Plasticity, and Memory. *Cold Spring Harb. Perspect. Biol.* **7**, 1-27 (2015).3. Basu, J. *et al.* Gating of hippocampal activity, plasticity, and memory by entorhinal cortex long-range inhibition. *Science (80-.).* **351**, (2016).

Disclosures: M. Hernandez Frausto: None. **J. Basu:** None. **L. Peng:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.01

Topic: H.09. Spatial Navigation

Title: Natural switches in attention and active-sensing rapidly modulate hippocampal spatial codes

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Abstract: Navigation is a rich and dynamic behavior. It requires the animal to know its own location within the environment, while also paying attention to incoming sudden events such as

obstacles, other conspecific or predators that may appear along the way. Most studies on the neurobiology of navigation focus on the representation of the animal's current position in small, empty and static setups that do not imitate well the complexity and rich dynamism of real-world navigation. Here we set out to test how brief attentional switches to 'things out-there' affect the representation of space in the hippocampus. To this end, we designed a task in which two bats flew together in a 120-meter flight tunnel, and had to be attentive both to the environment and to the position of the other bat, in order to avoid collisions during events of 'cross-over' between the bats. We recorded the neural activity in hippocampal area CA1 and tracked the positions of the bats in the tunnel using wireless-electrophysiology and custom tracking devices. We recorded the bats' echolocation calls and found that bats increased their call rate and amplitude ~20 meters before a cross-over event, indicating that the bats were attentive during these events. Our neural recordings showed that during cross-overs, CA1 neurons encode the distance to the other bat. Thus, as the bats approached each other, the neurons switched from an allocentric coding of the tunnel (place tuning) to egocentric × allocentric coding (inter-bat distance × place tuning) – and then switched back to allocentric coding after the bats passed each other. These neuronal switches were very rapid, as fast as 100 ms. This switching to a neuronal representation of the other bat was correlated on a trial-by-trial basis with the attention signal, as indexed by the bat's echolocation calls – suggesting that spatial attention and active-sensing are controlling these major switches in neural coding. Interestingly, we found that in place-cells, the different place-fields of the same neuron could exhibit very different tuning to inter-bat distance – creating a non-separable coding of allocentric position × egocentric distance. Theoretical analysis demonstrated that such a non-separable code leads to better decoding. Together, our results suggest that attentional switches during navigation – which in bats can be measured directly based on their echolocation signals – elicit rapid dynamics of hippocampal spatial coding. More broadly, this study demonstrates that during natural behavior, when animals often switch between different behaviors, neural circuits can rapidly and flexibly switch their core computations.

Disclosures: **A. Sarel:** None. **S. Palgi:** None. **D. Blum:** None. **J. Aljadeff:** None. **L. Las:** None. **N. Ulanovsky:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.02

Topic: H.09. Spatial Navigation

Title: Hippocampal cells encode time or distance, depending on the rat's prior expectation

Authors: *S. ABRAMSON[1], B. KRAUS[2], J. A. WHITE[3], M. E. HASSELMO[2], G. MORRIS[1], D. DERDIKMAN[1];
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Abstract: The hippocampus is known since the 1970ies for playing an important role in spatial processing and in episodic memory. The discovery of place cells within the hippocampus has pointed to the importance of these cells for navigation. The more recent discovery of hippocampal time cells has broadened the perspective of encoding in the hippocampus. One hypothesis is based on the notion that hippocampal cells deduce location by integrating travelled distance ("path integration"). According to this hypothesis, time cells, which fire at particular times when animals are running on a treadmill without changing location, actually encode accumulated distance on the treadmill. To examine this hypothesis, Kraus et al.[1] performed treadmill experiments in which animals either ran for a fixed-time or a fixed-distance with varying velocities. The two distinct coding modes of that hippocampal principle cells, whereby some cells encoded travelled distance and others elapsed time, thus excluding the notion that all hippocampal cells were performing path integration. Using the data from these experiments, we asked whether the two populations depended on the type of task the rats were engaged in. We show that indeed the type of experiment determined the cells' encoding: In experiments in which the rat ran for a fixed time interval with varying distance, the cells tended to encode a fixed time. In contrast, in the experiments in which the rat ran for a fixed distance with varying time intervals, the cells tended to encode a fixed distance. These results demonstrate that the specific response of the cells depends on the rat's expectation of the rules determining its behavior
[1] Kraus, B. J., Robinson II, R. J., White, J. A., Eichenbaum, H., & Hasselmo, M. E. (2013). Hippocampal "time cells": time versus path integration. *Neuron*, *78*(6), 1090-1101

Disclosures: **S. Abramson:** None. **B. Kraus:** None. **J.A. White:** None. **M.E. Hasselmo:** None. **G. Morris:** None. **D. Derdikman:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.03

Topic: H.09. Spatial Navigation

Title: Multi-scale representation of very large environments in the hippocampus of flying bats

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Abstract: Most animals navigate daily over distances spanning from hundreds of meters to many kilometers. However, over the last fifty years, hippocampal research focused on spatial representations in small laboratory environments. Nothing is known about hippocampal neural codes for large spatial scales - the scales of natural navigation of rodents, bats and other mammals. Here we addressed this question for the first time, by developing a unique setup - including a very large environment with an ethologically-relevant spatial scale. We studied Egyptian fruit bats - flying mammals that are excellent navigators over large scales, and which have rodent-like hippocampal spatial representations in small laboratory environments. We

developed an on-board wireless neural-logger, which allows recording single-units over unlimited distances. We built a 200-meter long tunnel where bats can fly freely; bat's position was tracked using an RF localization device that measures distances to a ground-based antenna array - yielding high accuracy of ~9-cm, much better than GPS. Bats flew back-and-forth along the tunnel, more than 100 laps per-session (over 20-km total distance). Recordings from dorsal hippocampal area CA1 of 10 bats in the long tunnel showed spatial firing that was very different from findings in small-scale laboratory environments: Across neurons, the size of place-fields ranged from 0.6 m to 32 m. Single-neurons exhibited multiple fields, and a multi-scale representation of space - whereby place-fields of the same neuron differed up to 20-fold in size. These large variations in scale were unrelated to local landmarks, and could not be explained by the animal's flight speed, which was extremely stable along the tunnel - suggesting that dorsal CA1 neurons have a genuine multi-scale representation of space. This coding scheme was observed from the first exposure to the environment, and was similar between lab-born and wild-born bats, suggesting the multi-scale code is a very robust phenomenon, which does not require neither recent experience nor early experience with large environments. Theoretical decoding analysis showed that this multi-scale code enables representing very large environments with extremely low decoding-errors. We modeled these results using a set of overlapping continuous attractors of different scales that randomly share neurons between them - which created a continuum of field-sizes, and recapitulated the multi-field multi-scale code observed in the data. Together, the experiments and theory suggest that in large environments, the hippocampus exhibits an efficient coding scheme that is radically different from small environments.

Disclosures: **T. Eliav:** None. **S.R. Maimon:** None. **J. Aljadeff:** None. **M. Tsodyks:** None. **G. Ginosar:** None. **L. Las:** None. **N. Ulanovsky:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.04

Topic: H.09. Spatial Navigation

Support: India Alliance Grant IA/S/13/2/501024
Data collection was supported by NIH R01 NS039456 (J. Knierim, PI)

Title: Local field potential theta, spike-theta phase modulation and precession along the CA1 transverse axis

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Abstract: Brain rhythms help coordinate neural activity to support cognitive functions. Theta oscillations (5-10Hz) in the rodent hippocampus have been hypothesized to facilitate learning and spatial memory. Theta mediated coordination of neural activity can allow effective communication between different spatially tuned populations for processing spatial information

and context. Pyramidal cells in the hippocampus can use rate and temporal codes to convey spatial information. Hippocampal neurons exhibit temporal codes through theta phase precession: the preferred phase of firing advances as the animal progresses through the neuron's place field. Along the CA1 transverse axis, distal CA1 (dCA1) has been shown to be less spatially selective, and exhibit weaker theta modulation and phase precession than proximal CA1 (pCA1) (Henriksen et al., 2010, Oliva et al., 2016). In contrast, we recently reported that spatial selectivity is comparable along the CA1 transverse axis when the animals run on a circular track with distinct local texture cues and global visual cues in a circular arena (Deshmukh, 2020, bioRxiv). In the current study, we sought to compare theta oscillatory dynamics in pCA1 and dCA1 in the same environment by studying theta power in the local field potentials (LFP), theta modulation of neural spiking, and phase precession along the CA1 transverse axis in 10 male Long Evans rats. We find that a similar proportion of neurons are theta modulated (pCA1: 62/83 cells, dCA1: 79/115 cells, Chi-square, $p = 0.357$) and show phase precession (pCA1: 81/83 cells, dCA1: 112/115 cells, Chi-square, $p = 0.929$) between pCA1 and dCA1. The strength of modulation (median: pCA1 = 0.73, dCA1 = 0.67, Wilcoxon rank-sum, $p = 0.087$) and phase precession (median: pCA1 = 0.28, dCA1= 0.27, Wilcoxon rank-sum, $p = 0.205$) is also comparable between the two CA1 sub-regions. This trend does not change when the local and global cues are mismatched by rotating local cues counterclockwise and global cues clockwise by equal amounts. We also demonstrate that absolute theta power in LFP is higher in dCA1 than in pCA1 (Wilcoxon Sign rank, $p=0.02$). These findings challenge the established notion of dCA1 being inherently less spatially selective than pCA1. The combination of higher theta amplitude in LFPs in dCA1 than pCA1 and the presence of theta phase precession in almost all neurons indicates that theta mediated spatial information processing in the two areas is comparable in our experimental paradigm.

Disclosures: **A. Bishnoi:** None. **S.S. Deshmukh:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.05

Topic: H.09. Spatial Navigation

Support: P.J. is supported by Biotechnology and Biological Sciences Research Council Doctoral Training Programme studentship
BB/N019008/1
BB/P019560/1

Title: Dorsal and ventral CA1 representations of space and reward during spatial learning

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Abstract: Dorsal CA1 (dCA1) place cells accumulate at rewarded locations. Because the ventral CA1 (vCA1) projects to the amygdala and the nucleus accumbens — brain regions canonically involved in reward learning — we hypothesized that the reward would also modulate vCA1 activity. We asked how the learned reward location shapes the dCA1 and vCA1 activity, and if the cells active at the reward location change their place fields to track the reward after translocation. Such representation could provide a stable representation for navigational goals in a malleable representation of space in the hippocampus.
We imaged calcium fluorescence from the dCA1 and vCA1 of freely-moving mice performing a spatial reward learning task using a miniaturized microscope (miniscope). Mice navigated on a circular maze with holes that were baited with rewards (cheeseboard maze) where the reward locations were initially fixed. Following the acquisition, we moved the reward location to test which spatially modulated cells in the dCA1 and vCA1 tracked the reward locations.
We present a comparison of spatial representations in the dCA1 and vCA1 and how they changed in the same cells over learning that spanned three sets of reward locations. We found that place cells accumulate at rewarded locations in the dCA1 but not in the vCA1. On individual runs towards the reward, the dCA1 cells organized their firing into a sequence that progressed faster at the reward location. We also report how the place fields remapped to follow the translocated reward.

Disclosures: P. Jarzebowski: None. **O. Paulsen:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.06

Topic: H.09. Spatial Navigation

Support: R01MH123260-01
NSF/IOS1924732
1F31EY031582-01A1

Title: Hippocampal neural representations of heading retrieval and place recognition

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Abstract: Hippocampal neural representations of heading retrieval and place recognition
Gagliardi, C.M., Normandin, M., Keinath, A.T., Lopez, M., Julian, J.B., Epstein, R.A., and Muzzio, I.A.
Epstein: Penn Psychology DepartmentJulian: PrincetonKeinath: McGill
A vast amount of research has shown that numerous species, including young children and

rodents, rely primarily on the geometric shape of navigable space to regain their sense of direction after disorientation, often ignoring non-geometric cues even when they are informative. Notably, these experiments have almost always been performed in single-chamber environments in which there is no ambiguity about place identity. In real life scenarios, lost navigators must identify their current location and recover their facing direction in order to restore their bearings. In previous work we tested the idea that these two tasks - place recognition and heading retrieval - might be mediated by distinct cognitive systems. We found that mice used non-geometric features to identify the chamber in which they were located, but failed to use those same features to recover their facing direction, instead relying exclusively on spatial geometry. These results suggested the existence of separate systems for place recognition and heading retrieval that are differentially sensitive to geometric and non-geometric cues, respectively. In this study we investigated the neuronal correlates of these processes using single unit and calcium imaging recordings in disoriented mice trained a two-chamber reorientation task. We found that the hippocampal map aligned to the geometry of each chamber and changes in firing rate served to disambiguate the contexts. These results were corroborated with calcium imaging in freely moving mice. Together these data indicated that the alignment of the hippocampal map serves to recover heading, while changes in firing rate underlie place recognition. Interestingly, while the overall spatial information content of the cells was the same in both contexts, distinct subpopulations had higher information in one context than the other, suggesting that accurate prediction of location in each environment was achieved by distinct subpopulations. These results suggest that heading retrieval and context recognition are distinctively coded at the hippocampal level.

Disclosures: C.M. Gagliardi: None. **M. Normandin:** None. **M.A. Lopez:** None. **A. Keinath:** None. **J.B. Julian:** None. **R. Epstein:** None. **I.A. Muzzio:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.07

Topic: H.09. Spatial Navigation

Support: NIH NRSA 1F32NS077840-01A1
NIH NIMH R01 2R01MH083686-06
NIH R37NS081242- 06

Title: Investigating in vivo hippocampal activity over long timescales reveals predictive nature of CA1 place cell representations in complex behavioral tasks

Authors: *K. FINKE[1], G. STRAUS[1], C. ALVARADO[1], E. HAPGOOD[1], E. KAROLCZAK[1], A. METOYER[1], C. RICHMOND[1], T. BOYD-MEREDITH[2], D. TANK[2], J. GAUTHIER[1]; [1]Swarthmore Col., Swarthmore, PA; [2]Princeton Univ., Princeton, NJ

Abstract: The hippocampus plays a vital role in navigation. It is unclear, however, how the hippocampus supports spatial learning over long timescales in complex tasks. One limitation has been the ability to track neural activity for more than a few days or weeks. Even with two-photon imaging, which allows precise anatomical localization of recorded cells, brain deformations make it difficult or impossible to repeatedly image the same plane. Here, we introduce an analysis technique to track calcium activity in large populations at cellular resolution over months of training despite substantial deformations. In our experiments, mice learned to associate reward with several locations in a virtual reality environment over the course of three calendar months. Within each cell, our analysis could follow how multiple place fields formed, drifted, and dissolved. We also identified discrete behavioral strategies that gradually evolved from the first trials through expert performance. When we compared neural activity to the trajectory of behavioral learning, unexpected patterns emerged. When a new task was introduced, place fields underwent significant changes prior to behavioral improvement. In addition, predictive representations tended to form more often in complex tasks than simple ones. Together, these findings suggest that the hippocampus does not form a general-purpose map of space, but instead that spatial representations must be suited to support the unique demands of each task. We anticipate our technique for tracking large populations could allow for recording longer timescales of activity in other brain areas.

Disclosures: K. Finke: None. **G. Straus:** None. **C. Alvarado:** None. **E. Hapgood:** None. **E. Karolczak:** None. **A. Metoyer:** None. **C. Richmond:** None. **T. Boyd-Meredith:** None. **D. Tank:** None. **J. Gauthier:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.08

Topic: H.09. Spatial Navigation

Support: NSF STC award CCF-1231216

Title: Animal-to-animal variability in hippocampal remapping

Authors: P. NILCHIAN[1], M. WILSON[2], ***H. SANDERS**[2];
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Abstract: Hippocampal place cells map an animals' environment. Remapping describes changes in place field locations and firing rates and occurs when animals' context changes. However, the variability across animals in remapping behavior is not well understood, and its characteristics have only been studied sporadically. In this work, we analyzed electrophysiological recordings from Alme et al., 2014, in which five rats were exposed to 11 different environments. To compare the hippocampal maps in two rooms, we computed average rate map correlation coefficients. We discovered that the heterogeneity in animals' remapping behavior is structured: animals' remapping behavior is consistent across a range of independent comparisons. Our

findings highlight that remapping behavior between repeated environments depends on animal-specific factors.

Disclosures: H. Sanders: None. **M. Wilson:** None. **P. Nilchian:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.09

Topic: H.09. Spatial Navigation

Support: NIH NINDS 1R01NS109362
BRAIN INITIATIVE NIH NINDS 1R01NS109994
Awards from Mathers Foundation
Klingenstein-Simons Foundation
Alfred P. Sloan Foundation
Whitehall Foundation
NYU MSTP Training Grant

Title: Behaviorally-driven divergence and stabilization of place cell maps during episodic memory formation and consolidation

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Abstract: The hippocampus is critical for the formation and recall of episodic memories which store past experience of events ('what') occurring at particular locations ('where') in time ('when'). Place cells, hippocampal pyramidal neurons which show location-specific activity during navigation, form a spatial map of the environment and are hypothesized to be the neural substrate of the 'where' of episodic memory. Previous experiments suggested that place cells should retain stable spatial tuning activity following learning to facilitate future memory recall. While long-term imaging studies in CA1 demonstrated limited reactivation of place maps upon repeated exposure to a familiar environment, these studies examined place map stability in the absence of memory and attentional demand. Thus, the relationship between episodic learning and long-term place map stability remains unanswered. To examine the relationship between long-term place map stability and episodic memory, we developed an operant, head-fixed, odor-cued, spatial navigation task and used 2-photon calcium imaging to track CA1 pyramidal neuron activity during task learning and recall. We found that existing place cells are rapidly recruited into task-dependent spatial maps and that place cells use a diverse set of remapping strategies to represent changing task goals. Furthermore, we observed the stabilization of learned place cell maps following memory consolidation and a performance dependent divergence of spatial maps between trial types. Our findings suggest that a subset of place cells is recruited by episodic

spatial learning, actively reconfigured to represent task-relevant spatial relationships, and stabilized following successful learning and consolidation.

Disclosures: **R. Zemla:** None. **J. Moore:** None. **J. Basu:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.10

Topic: H.09. Spatial Navigation

Support: NIMH 5F31MH117892
NIMH 5K08MH113036
Leon Levy Foundation
NIMH 1R01MH124047
NIND 5U19NS104590

Title: Inhibitory feedback control of behavioral time scale plasticity induction in CA1

Authors: ***S. V. ROLOTTI**, M. S. AHMED, M. SZOBOSZLAY, H. BLOCKUS, K. C. GONZALEZ, F. T. SPARKS, A. SOLIS CANALES, D. S. PETERKA, A. LOSONCZY; Dept. of Neurosci., Columbia Univ., New York, NY

Abstract: Pyramidal cells in area CA1 (CA1PCs) of the mammalian hippocampus develop spatially restricted firing fields as the organism explores an environment, forming a cognitive map. Clarifying the circuit mechanisms by which a given CA1PC forms a ‘place field' (PF) or otherwise develops de novo feature selectivity is critical to a broader understanding of memory formation, and has been a topic of considerable interest.
Recent work has revealed that PF formation occurs through a novel form of plasticity, behavioral time scale synaptic plasticity (BTSP). Importantly, BTSP can be experimentally induced in individual CA1PCs via repeated current injection at a fixed location, allowing experimenters to probe PF dynamics and representational content. To date, the limitations of intracellular electrophysiology have constrained this avenue of inquiry to single cells over short timescales, leaving the relationship of BTSP to population coding and memory behavior undetermined. The induction and subsequent longitudinal tracking of PFs in arbitrary numbers of cells would therefore represent a major advance, clarifying the circuit mechanisms regulating BTSP and the relationship of PF formation in neural ensembles to learning and behavior.
Here we argue that recurrent inhibition in CA1 represents a circuit mechanism for limiting arbitrary scaling of BTSP across the population. We present sparse-labeling and targeted-stimulation approaches that enable the first demonstrations of optogenetic PF induction in single cells. Pairing these optogenetic approaches with 2-photon calcium imaging, we characterize longer timescale dynamics of induced PFs to show that they are functionally indistinguishable from spontaneously formed PFs. We find that these approaches scale to small numbers of stimulated cells but fail to induce PFs at higher densities. Importantly, we further show that local

interneuron (IN) activity scales with stimulation density, and that manipulation of local INs to suppress recurrent inhibition raises the density limits on PF formation. Finally, we find that over-representing a location using these approaches can bias goal-directed behavior.
Overall, this study makes important advances in our understanding of circuit control of PF formation for learning and memory. We demonstrate the importance of local feedback inhibition in regulating PF formation rates, as well as demonstrating for the first time the causal role of PF over-representation in rapid spatial learning. Future work will delineate the degree to which recurrent inhibition can be manipulated to overcome endogenous limits on BTSP in CA1PC ensembles and potentially further enhance learning.

Disclosures: **S.V. Rolotti:** None. **M.S. Ahmed:** None. **M. Szoboszlay:** None. **H. Blockus:** None. **K.C. Gonzalez:** None. **F.T. Sparks:** None. **A. Solis Canales:** None. **D.S. Peterka:** None. **A. Losonczy:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.11

Topic: H.09. Spatial Navigation

Support: NIH BRAIN Initiative 1R01NS109994
Junior Investigator Award: American Epilepsy Society
Whitehall Foundation
Sloan Research Fellowship
Klingenstein-Simons Fellowship Award in Neuroscience
Flatiron Institute
McKnight Foundation

Title: Dynamic dendritic and somatic interactions underlying place field remapping in mouse hippocampal area CA3

Authors: *J. J. MOORE, M. HOPKINS, J. BASU;
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Abstract: Hippocampal “place cells” are tuned to specific locations in an environment[1]. These representations can “remap” in different environments or in the same environment in response to changes in task demand[2-4]. Remapping occurs on fast timescales, so typical plasticity mechanisms cannot completely explain this phenomenon. Dendritic spiking has been implicated in the formation of stable place fields[5]; here we explore the contributions of dendrites to the flexibility of hippocampal representations. We consider two possible remapping mechanisms: “Input remapping” in which inputs to a neuron change but the soma-dendrite correlation relationships remain the same; and “Gate remapping” in which inputs remain constant but the soma becomes more correlated with a new subset of dendritic branches. To assess these possible mechanisms of hippocampal remapping, we utilize an experimental paradigm enabling fast

switching of the physical cues defining an environment. Headfixed mice ran on a treadmill track with proximal cues defining position. The belt was then flipped over during imaging to reveal new cues and textures, enabling continuous recording from the instant the new environment is encountered. We use a 2-photon calcium imaging preparation in which the basal dendrites, cell bodies, and apical dendrites of pyramidal neurons in hippocampal area CA3 are all imaged in the same focal plane. We measured the degree of gate remapping by the change in the correlation matrix computed from the fluorescence traces of all segments (dendrite and soma) of a neuron.To process this data, we developed an automated algorithm to identify dendritic and somatic regions of interest (ROIs) from the calcium signal. The algorithm matches manually-labeled datasets, is easily applied to detecting ROIs from various neuronal morphologies, runs faster than real-time on a standard desktop computer, and has few free parameters to tune. We discovered that an entire place cell could hold on average 4 place fields when taking all dendrites into account, even though the soma expresses fewer, in line with our previous findings[6]. We find evidence for both input remapping and gate remapping, indicating a change in the internal routing of information. These results suggest that dendrites perform independent computations, and that dendritic outputs can be selectively coupled to the soma in a context-dependent manner.
1. O'Keefe & Dostrovsky, Brain Research, 1971. 2. Markus et al., J Neuroscience, 1995. 3. Dupret et al., Nat. Neurosci., 2010. 4. Gauthier & Tank, Neuron 2018. 5. Bittner et al., Science 2017. 6. Rashid et al., bioRxiv, 2020.

Disclosures: **J.J. Moore:** None. **J. Basu:** None. **M. Hopkins:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.12

Topic: H.09. Spatial Navigation

Support: NIH BRAIN Initiative R01 NS109994
NIH NINDS R01NS109362
NIH NIMH R01MH122391
McKnight Scholar Award
Klingenstein-Simons Foundation
American Epilepsy Society
Alfred P. Sloan Foundation

Title: The dendritic spatial code:branch-specific place tuning and its experience-dependent decoupling

Authors: S. K. RASHID[1], J. MOORE[2], R. DELATORRE[2], ***J. BASU**[2];
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Abstract: Dendrites of pyramidal neurons integrate different sensory inputs, and non-linear dendritic computations drive feature selective tuning and plasticity. Yet little is known about how dendrites themselves represent the environment, the degree to which they are coupled to their soma, and how that coupling is sculpted with experience. In order to answer these questions, we developed a novel preparation in which we image soma and connected dendrites in a single plane across days using in vivo two-photon microscopy. Using this preparation, we monitored spatially tuned activity in area CA3 of the hippocampus in head-fixed mice running on a linear track. We identified “place dendrites”, which can stably and precisely represent both familiar and novel spatial environments. Dendrites could display place tuning independent of their connected soma and even their sister dendritic branches, the first evidence for branch-specific tuning in the hippocampus. In a familiar environment, spatially tuned somata were more decoupled from their dendrites as compared to non-tuned somata. This relationship was absent in a novel environment, suggesting an experience-dependent selective gating of dendritic spatial inputs. We then built a data-driven multicompartment computational model that could capture the experimentally observed correlations. Our model indicates that place cells exhibiting branch-specific tuning have more flexible place fields, while neurons with homogenous or co-tuned dendritic branches have higher place field stability. We found evidence for this prediction in our experimental data sets. These findings demonstrate that spatial representation is organized in a branch-specific manner within dendrites of hippocampal pyramidal cells. Further, spatial inputs from dendrites to soma are selectively and dynamically gated in an experience-dependent manner, endowing both flexibility and stability to the cognitive map of space.

Disclosures: **J. Basu:** None. **S.K. Rashid:** None. **R. Delatorre:** None. **J. Moore:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.13

Topic: H.08. Learning and Memory

Support: Brain Initiative R01NS109994
Bettencourt Schueller Foundation

Title: Functional investigation of the lateral entorhinal cortex input to hippocampal area CA3

Authors: *V. ROBERT[1], R. DE LA TORRE[2], K. O'NEIL[2], J. BASU[3];
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Abstract: The hippocampus receives and processes sensory information from the entorhinal cortex to form episodic memories. Specifically, the lateral entorhinal cortex (LEC) provides contextual signals which are transferred sequentially through the dentate gyrus (DG) - CA3 - CA1 loop in the hippocampus. Amongst these regions, area CA3 displays the unique ability to

perform both pattern separation and completion depending on the degree of similarity of the environment. This likely stems from the unique CA3 circuitry which involves direct LEC inputs on distal dendrites, processed feedforward DG inputs on proximal dendrites, and internally-generated feedback CA3 recurrent inputs on medial dendrites. This organization may enable non-linear integration of LEC inputs in CA3 PN dendrites, which could dictate whether the CA3 network undergoes pattern separation or completion. Indeed, LEC has been shown to send excitatory and inhibitory projections to the hippocampus which allow the generation of dendritic spikes in CA1, and lesions of LEC disrupt CA3 rate remapping. However, the circuit connecting LEC to area CA3 and the consequences of its activation on CA3 output remain unknown. To address this, we expressed the light-gated opsins Chronos and ChrimsonR in LEC excitatory and inhibitory cells respectively, and performed whole-cell patch clamp recordings of CA3 interneurons and pyramidal neurons in acute slices. We found that local interneurons located in the medial apical dendritic layer received mono-synaptic connections from both glutamatergic and GABAergic projections from LEC. LEC direct glutamatergic input strongly excited these interneurons, yielding substantial feedforward inhibition of CA3 pyramidal neurons. LEC direct GABAergic input modulated the recruitment of these interneurons by LEC mono-synaptic excitation, suggesting a role for disinhibitory gating to boost overall excitation. Next, we combined selective optical stimulation of LEC excitatory and inhibitory projections with electrical stimulation of DG mossy fibers and CA3 recurrent collaterals to assess the integration of these inputs in area CA3. While CA3 pyramidal neurons did not fire action potentials in response to single stimulation of LEC excitatory input, its repeated stimulation either by itself or coincidently paired with mossy fiber and CA3 recurrent collateral input stimulations elicited spike output in the same CA3 pyramidal neurons. These results demonstrate that the LEC input can drive CA3 output when coordinated with activation of local inputs, highlighting the role of dendritic integration of LEC signals in CA3 dendrites which will be the focus of our future experiments.

Disclosures: **V. Robert:** None. **R. De La Torre:** None. **K. O'neil:** None. **J. Basu:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.14

Topic: H.09. Spatial Navigation

Support: NDSEG

Title: Differences in reward biased spatial representations in the lateral septum and hippocampus

Authors: ***H. S. WIRTSHAFTER**, M. A. WILSON;
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Abstract: The hippocampus innervates the lateral septum (LS) and provides the LS with spatial information. However, it remains unknown how the LS represents place information, and

whether this information is combined with reward signaling. To answer this question, we simultaneously recorded from rat hippocampus CA1 and caudodorsal LS during a rewarded spatial navigation task. We then compared spatial firing between the CA1 and LS. We determined that the LS contains place cells which, while less numerous than in the CA1, are similar to hippocampal place cells in field size and number of fields per cell. Place cells in the LS, however, have field shapes and center distributions that are more skewed towards reward as compared to HPC place cells. Additionally, spike cross-correlations between place cells in the CA1 and LS are greatest for cells that have reward-proximate place fields, suggesting reward-related place cells are preferentially represented in the LS. The LS may therefore play a role in relaying task-relevant hippocampal spatial information to downstream areas, such as the VTA.

Disclosures: H.S. Wirtshafter: None. **M.A. Wilson:** None.

Digital Abstract Session

P331. Modulations in Hippocampal Spatial Representations

Program #/Poster #: P331.15

Topic: H.09. Spatial Navigation

Support: GIF
ISF
Rappaport
Michigan – Israel Partnership for Research & Education
Prince

Title: Ventral Tegmental Area Stimulation Induces Hippocampal Remapping

Authors: *D. KHATIB[1], G. TOCKER[2], J. GROSS[1], G. MORRIS[1], D. DERDIKMAN[1];
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Abstract: The hippocampus and associated structures are related to the perception of space, learning, and memory. The hippocampus can represent environments via its place cells in what is known as a cognitive map. These maps are stable representations of the environment but can reorganize when the environment is changed, a phenomenon widely known as remapping.
The midbrain has gained much attention in recent years in relation to its role in learning and memory. Specifically, the dopaminergic cells in the Ventral Tegmental Area (VTA) is associated with reinforcement learning and novelty.
In the current study, we looked at the relationship between the VTA and the hippocampus, as some works suggest a role for the VTA in stabilizing hippocampal maps. The VTA itself contains mainly dopaminergic neurons but contains also GABAergic and glutamatergic populations, with the latter being suggested to have a direct effect on the hippocampus. We investigated the effects of stimulating different cell types in the VTA on the hippocampal map and the place cells constituting it.
We used three Cre-driver mouse lines, TH-Cre, DAT-Cre to target the dopaminergic population

and Vglut2-Cre to target the glutamatergic population within the VTA. We stimulated these neurons using excitatory DREADDs while recording the activity in CA1 using one-photon calcium imaging and extracellular electrophysiological tetrode recordings. We found that stimulating the dopaminergic subpopulation did not affect the representation of the environment in the hippocampus. On the other hand, stimulating the glutamatergic subpopulation induced remapping of place cells while the animal was traversing the same known environment. This suggests a role for the glutamatergic VTA population in hippocampal remapping and novelty.

Disclosures: D. Khatib: None. **G. Tocker:** None. **J. Gross:** None. **G. Morris:** None. **D. Derdikman:** None.

Digital Abstract Session

P332. Rhythmic Coordination of Hippocampal Networks: Rodent Studies

Program #/Poster #: P332.01

Topic: H.09. Spatial Navigation

Support: NIH Training Grant 5R90DA033463-09

Title: Communication subspaces for local-field potential defined network states

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Abstract: Distributed neural circuits across multiple brain regions coordinate activity and communicate with each other to process information and promote our understanding of the world. The hippocampus (HPC) and prefrontal cortex (PFC) are essential to learning and are thought to work in an integrated fashion to support cognition and memory-guided behavior (e.g., Tamura et al., 2017; Zielinski et al., 2020). The rhythmic fluctuations in the local field potentials of HPC and PFC have been shown to reveal broad behavioral states (e.g., movement) and periods of inter- and intra-regional communication but lack the full information carried by spiking activity of cellular ensembles. To incorporate individual neuronal spiking in analyses of network communication, we applied tools developed by Semedo et al. (2019) to gain insights into the co-fluctuations of neuronal populations in HPC and PFC. Ensembles of co-fluctuating neurons in the HPC and PFC reveal a substructure of interaction during unique hippocampal activity patterns such as theta and delta rhythms and sharp-wave ripples corresponding to network states crucial to memory. Importantly we find these three local-field patterns have lower dimensional neuronal interaction between as opposed to within brain regions. Overall we compare and contrast pattern-specific subspaces linking HPC-PFC neuronal activity across rhythmic states, finding separable but related states with a possible role in memory-guided behavior.

Disclosures: Z. Guo: None. **R. Young:** None. **S.P. Jadhav:** None.

Digital Abstract Session

P332. Rhythmic Coordination of Hippocampal Networks: Rodent Studies

Program #/Poster #: P332.02

Topic: H.09. Spatial Navigation

Support: R56AGO62762

Title: Alzheimer's disease-like network disruptions in hippocampus and anterior cingulate cortex in a model of diabetes mellitus

Authors: *L. A. CREW[1], R. A. WIRT[2], A. A. ORTIZ[5], A. M. MCNEELA[5], E. FLORES[5], B. N. UDIN[5], J. W. KINNEY[3], J. M. HYMAN[4];
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Abstract: Network function in the hippocampus (HC) and anterior cingulate cortex (ACC) is critical for efficient working memory function and network impairments play a significant role in the cognitive deficits seen in Alzheimer's disease (AD). While historically there has been a great concentration of amyloid pathology, recently theoretical and experimental work has linked the other prominent AD pathologies, neuroinflammation and tau hyperphosphorylation to changes in working memory network functioning. Chronic hyperglycemia leads to neuroinflammation and tau hyperphosphorylation in the HC, similar to what occurs in AD, though with no amyloidosis Interestingly, chronic hyperglycemia also leads to mild cognitive impairments, as observed in diabetes mellitus, a risk factor to the development of AD. We hypothesize that hyperglycemia is impairing HC network activity and cognitive performance, perhaps due to neuroinflammation and tau hyperphosphorylation. To test this hypothesis, we injected rats with a series of acute, low doses of streptozotocin (STZ) that damaged pancreatic beta cells and increased blood glucose levels. We then tested animals on a spatial delayed alternation task while simultaneously recording from the HC and ACC, two areas where network activity is essential for effective performance. We found that hyperglycemic animals had no differences in performance accuracy for shorter delay trials but a decrease in accuracy to chance levels for longer delays. Spectral changes observed in STZ animals showed a decrease in HC theta power and an increase in delta power in both areas. This increase in delta power resulted in an overall change in the theta/delta ratio that was more noticeable during the end of the delay period, where theta power should be more pronounced. Interestingly, STZ animals showed strong hypersynchrony, with an increase in cross-frequency coupling, in several different frequency bands, and HC-ACC coherence. The elevated coherence significantly decreased during correct trials in STZ animals compared to the opposite effect seen in controls and previous research. Finally, we found a considerable amount of hyperphosphorylated tau within the HC and ACC of STZ animals, which may be related to the oscillatory and behavioral changes seen here. Collectively, these findings display striking changes in HC and ACC network activity during a working memory task in moderately hyperglycemic animals, suggesting an association with the physiological and cognitive pathologies of AD.

Disclosures: **L.A. Crew:** None. **R.A. Wirt:** None. **A.A. Ortiz:** None. **A.M. McNeela:** None. **E. Flores:** None. **B.N. Udin:** None. **J.W. Kinney:** None. **J.M. Hyman:** None.

Digital Abstract Session

P332. Rhythmic Coordination of Hippocampal Networks: Rodent Studies

Program #/Poster #: P332.03

Topic: H.09. Spatial Navigation

Support: R56AG062762

Title: Remote Recall Deficits and Altered Hippocampal Oscillations in Hyperglycemia

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Abstract: It is estimated that by 2050 the global prevalence of Alzheimer's disease (AD) will increase to 13.8 million cases and 95% of these will be the sporadic form of AD (sAD). While the cause of sAD is unknown, several risk factors have been identified. Notably, abnormal insulin signaling in diabetes mellitus (DM) patients increases the likelihood of developing sAD by 65% and is thought to trigger metabolic dysfunction, neuroinflammation, and neurodegeneration similar to that found in AD. Administration of a diabetogenic drug (streptozotocin; STZ) in rodents can mimic sAD-like memory defects and enhanced hippocampal tau expression (Murtishaw et al., 2018). Although the HC is required for recent memory recall, theta-dependent prefrontal-hippocampal synchrony driven by the anterior cingulate cortex (ACC) supports remote recall (Wirt & Hyman, 2019). To explore differences in memory mechanisms related to hyperglycemia, we administered staggered low-dose IP injections (20mg/kg) of either STZ or a control solution. We recorded network activity from ACC and HC throughout sequential exposure to either novel or familiar environments. Consistent with the findings of (Wirt & Hyman, 2019), control animals displayed decreased thigmotaxis during both recent and remote re-exposures, suggesting functional recall for both retention intervals. Although no recall differences were found between STZ and controls during recent exposures, STZ thigmotaxis during remote recall reverted to levels comparable to their initial exposure, corroborating hyperglycemia-induced memory defects (Murtishaw et al., 2018). Electrophysiological data revealed significant disproportions in theta and delta band signal, where STZ animals exhibited decreased theta-delta ratios in the HC. Since hippocampal functionality relies on alternation between behaviorally-relevant theta and delta states to achieve different memory processes, these results suggest that ACC-HC network dysregulation may account for impaired contextual remote recall in hyperglycemic animals. Since EEG slowing is a characteristic hallmark of AD progression, examining similar network disruptions may also assist our understanding of sporadic AD development.

Disclosures: **A.M. McNeela:** None. **R.A. Wirt:** None. **J.M. Hyman:** None. **E. Flores:** None. **J.W. Kinney:** None. **A.A. Ortiz:** None.

Digital Abstract Session

P332. Rhythmic Coordination of Hippocampal Networks: Rodent Studies

Program #/Poster #: P332.04

Topic: H.08. Learning and Memory

Support: R01 NS102915
R01 NS097772
R01 MH119179

Title: Accelerating and decelerating hippocampal theta oscillations has opposing effects on spatial working memory performance

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Abstract: Brain oscillations are not only an indication of the precise timing of neural activity within a brain region, but are also thought to organize the coordination of activity across multiple brain regions to support cognitive function. For example, the coordination of hippocampal neural activity with other brain regions by oscillations in the theta range is thought to be essential for spatial working memory. To test this possibility, we took advantage of the finding that the rhythmic optogenetic activation of medial septal GABAergic neurons is sufficient for generating hippocampal oscillation frequencies that supersede endogenous theta oscillations (~8 Hz). By using this technique in a spatial alternation task we previously found that 12 Hz or 20 Hz oscillations impaired working memory performance (i.e., in trials with 10 s delays) but did not alter performance during trials that do not require a hippocampal contribution to memory (i.e., with no delay). Also, optogenetic pacing of oscillations within the endogenous theta range (i.e., at 8 Hz) did not result in any effects on working memory performance. These findings raise the question whether manipulations that slow down theta frequency also result in a memory impairment. We therefore tested mice with different delay conditions (no delay, 2 s delay, 10 s delay) while pacing oscillations across a range of frequencies (4 Hz, 6 Hz, 8 Hz, 10 Hz, and 12 Hz). The stimulation frequency and the order of delays were pseudo-randomized across days, and trials without stimulation were interleaved between trials with stimulation. As before, oscillations were paced with an optogenetic approach by first expressing channelrhodopsin in parvalbumin+ cells of the medial septal area (MSA) and by then delivering rhythmic light stimulation to the MSA. In each mouse, the effectiveness of the pacing was monitored by LFP recordings from the hippocampus, and mice with GFP expression were used to confirm that light delivery alone did not alter the behavior. As before, we found that the pacing of oscillations at 12 Hz impaired spatial working memory performance in trials with 10 s delays (n = 15 mice, p =

0.045, Wilcoxon test followed by Holm-Bonferroni correction for multiple comparisons), while pacing either at or close to the endogenous theta frequency (6 Hz, 8 Hz, and 10 Hz) did not have any effects on memory (n = 15, 15 and 14, all p values > 0.1). In contrast, pacing at 4 Hz resulted in an improvement of working memory (n = 15, p = 0.017) which raises the possibility that slower oscillation patterns may increase the coupling of the entorhino-hippocampal system with other brain regions where these oscillations are more prominent, such as VTA and prefrontal areas.

Disclosures: **N. Devico Marciano:** None. **M.L. Fu:** None. **R.E. Siretskiy:** None. **M.A. Jaramillo:** None. **T.L. Huynh:** None. **J.K. Leutgeb:** None. **S. Leutgeb:** None.

Digital Abstract Session

P332. Rhythmic Coordination of Hippocampal Networks: Rodent Studies

Program #/Poster #: P332.05

Topic: H.08. Learning and Memory

Support: R01 NS102915
R01 NS097772
R01 MH119179

Title: Type I and Type II theta oscillations in the limbic circuit are uncoupled from respiration-entrained oscillations in the olfactory bulb during an odor-cued working memory task

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Abstract: Type I theta oscillations (6-10 Hz) are movement-related and are generated in the septo-hippocampal network. They are thought to synchronize activity in the prefrontal-hippocampal network and mediate cognitive functions such as working memory (WM) and spatial navigation. On the other hand, Type II theta oscillations in the hippocampus are unrelated to movement, and are elicited when the animal is exposed to arousing sensory stimuli. Recently, respiration related oscillations (RROs) have been reported to propagate to several neocortical and subcortical areas including the prefrontal cortex and the hippocampus. We asked whether RROs in the limbic circuit couple to canonical theta oscillations during an odor-cued working memory task, and thus potentially integrate sensory processing with working memory retention. We trained mice to perform an odor-cued working memory task on a Figure-8 maze while simultaneously recording LFP signals from the olfactory bulb (OB), medial prefrontal cortex, dorsal and ventral hippocampus. LFP signals were analyzed from these four brain regions during different task phases, the odor sampling period, the central arm (where animals leave the odor port and run towards the choice point), the reward location, and the return arm (where animals run from the reward location back to the odor port). Peak frequencies of the OB oscillations ranged from 3-12 Hz in all task phases (~1200 trials; 7 mice). In addition, type I theta

oscillations were observed in the limbic regions during periods of high running speed (central and return arms), as expected. Type I theta oscillations in the limbic regions were more coherent with each other at the central arm where WM persisted compared to the return arm where mice ran at similar speeds but without a WM demand. Type II theta oscillations were observed in the limbic regions during odor sampling, but not during reward consumption, which are both periods of immobility. In addition to theta oscillations, RROs were observed in the limbic regions for all task phases. These RROs were coherent with the OB oscillations, except when RROs and theta (Types I and II) overlapped in frequency, suggesting that neither type of theta becomes coupled to RROs, even at matching frequencies. Therefore, OB oscillations - although observed at limbic regions do not appear to become coupled to theta oscillations, including when type II theta is generated during odor sampling. These results suggest that entrainment of hippocampal oscillations to sensory-coupled oscillations is not necessary for WM, and that oscillations that are intrinsically paced rather than by sensory inputs are central to information processing by limbic circuits.

Disclosures: S. Srikanth: None. **D. Le:** None. **Y. Hu:** None. **J. Leutgeb:** None. **S. Leutgeb:** None.

Digital Abstract Session

P332. Rhythmic Coordination of Hippocampal Networks: Rodent Studies

Program #/Poster #: P332.06

Topic: H.08. Learning and Memory

Support: NIH Grant 5R01MH117149-03

Title: Investigating the role of KIBRA-dependent synaptic function on hippocampal and cortical network mechanisms underlying complex cognition

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Abstract: Revealing how molecular interactions within single cells contribute to experience-dependent changes in circuit-level neural activity across large-scale brain networks is an urgent challenge in neuroscience. Disorders of complex cognition have strong genetic contributions and ultimately manifest via emergent properties of dysfunctional neural networks. However, a clear picture of how heritable factors contribute functionally to pathological symptoms remains elusive. Here I focus on investigating *in vivo* network dysfunction following removal of the synaptic scaffolding protein KIBRA. KIBRA gene variants are linked to normal variation in human memory performance and mice lacking Kibra show substantial impairment in learning and memory. KIBRA also regulates hippocampal AMPA receptor trafficking and synaptic plasticity, a cellular correlate of learning and memory. In addition, KIBRA and the protein complexes it organizes are associated with multiple neuropsychiatric disorders known to have synaptic/circuit etiologies. However, the role of KIBRA in regulating circuit dynamics is

unknown. To determine whether KIBRA-dependent plasticity mechanisms regulate behaviorally relevant circuit dynamics, I monitored simultaneous neural activity in both the hippocampus (HC) and frontal cortex (ACC) of mice with forebrain-specific deletion of KIBRA (KIBRA cKO) using *in vivo* electrophysiology in freely behaving mice. My findings indicate a failure of hippocampal circuits to properly synchronize in response to novel experience in the absence of KIBRA. I first examined whether there were any gross alterations in baseline hippocampal network function and found minimal differences between WT and KIBRA cKO mice. Next, I examined behaviorally regulated changes in oscillatory dynamics in KIBRA cKO mice. I found that KIBRA cKO mice fail to exhibit experience-dependent alterations in theta and gamma oscillatory power within the hippocampus. This finding suggests a failure of hippocampal circuits to adapt to experience in the absence of KIBRA and may impact memory encoding and consolidation. Our results further show that while most fundamental properties of remote sharp wave-ripple events and associated oscillatory dynamics are intact, KIBRA cKO mice fail to show experience-dependent modification of remote ripple properties. This indicates that experience-dependent changes in circuit dynamics that support memory consolidation are impaired in the KIBRA cKO. Together, these data reveal network-level mechanisms by which KIBRA may regulate systems-level memory consolidation in the adult brain.

Disclosures: **L. Quigley:** None. **R. Pendry:** None. **M. Mendoza:** None. **L. Volk:** None. **B. Pfeiffer:** None.

Digital Abstract Session

P333. The Role of Oscillations

Program #/Poster #: P333.01

Topic: H.08. Learning and Memory

Support: Research Foundation Flanders Grant 1102519N
Research Foundation Flanders Grant 1153418N
Grant BOF17/GOA/004

Title: Learning to synchronize: Midfrontal theta dynamics during rule switching

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Abstract: In recent years, several hierarchical extensions of well-known learning algorithms have been proposed. For example, when stimulus-action mappings vary across time or context, the brain may learn two or more stimulus-action mappings in separate modules, and additionally (at a hierarchically higher level) learn to appropriately switch between those modules. However, how the brain mechanistically coordinates neural communication to implement such hierarchical learning, remains unknown. Therefore, the current study tests a recent computational model (Verbeke & Verguts, 2019) that proposed how midfrontal theta oscillations implement such hierarchical learning via the principle of binding by synchrony (Sync model). More specifically,

the Sync model employs bursts at theta frequency to flexibly bind appropriate task modules by synchrony. 64-channel EEG signal was recorded while 27 healthy human subjects (Female: 21, Male: 6) performed a probabilistic reversal learning task. We fitted the Sync model on behavioral data and compared the fit (via AIC) with two other state-of-the-art models that not learn to switch between modules. Here, the Rescorla-wagner (RW) model learns stimulus-action mappings with the standard RW learning rule. The adaptive learning rate (ALR) model implements RW learning with an adaptable learning rate which increases on task switches. Important individual differences were observed. While some subjects fitted best with the Sync model, other subjects fitted better with the RW model. No evidence was found for the ALR model. Crucially, the Sync model also provides testable predictions for EEG data which we tested. In line with the Sync model, post-feedback theta power showed a linear relationship with negative prediction errors, but not with positive prediction errors. This relationship was especially pronounced for subjects with better behavioral fit for the Sync model. Also consistent with Sync model simulations, theta phase-coupling between midfrontal electrodes and temporo-parietal electrodes was stronger after negative feedback. Our data suggest that the brain uses theta power and synchronization for flexibly switching between task rule modules, as is useful for example when multiple stimulus-action mappings must be retained and used.

Disclosures: **P. Verbeke:** None. **K. Ergo:** None. **E. De Loof:** None. **T. Verguts:** None.

Digital Abstract Session

P333. The Role of Oscillations

Program #/Poster #: P333.02

Topic: H.08. Learning and Memory

Support: ANID Nº 21171047
ICN09_015

Title: Allocentric episodic memory onset along postnatal development during sleep

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Abstract: Allocentric episodic memory onset along postnatal development during sleepGarcía-Pérez M.A [1,2,5]; Irani M[3], Tiznado V[4], Maldonado P[2,5], Valdés JL[1,5]. *1. Laboratorio de Aprendizaje Memoria y Neuromodulación, Departamento de Neurociencia, Facultad de Medicina, Universidad de Chile.2. Laboratorio de Neurosistemas, Departamento de Neurociencia, Facultad de Medicina, Universidad de Chile.3. Laboratorio de Neurodinámica de la Cognición, Departamento de Psiquiatría, Centro Interdisciplinario de Neurociencias UC, Pontificia Universidad Católica de Chile.4. Laboratory for Brain Machine Interfaces,*

Departamento de Psiquiatría, Centro Interdisciplinario de Neurociencias UC, Pontificia Universidad Católica de Chile.5. Biomedical Neuroscience Institute (BNI), Facultad de Medicina, Universidad de Chile.
Episodic memory relies on the ability of the hippocampus to process spatial information. This type of memory emerges late in postnatal lifetime, in correlation with hippocampal development. When this memory is build-up by using external/distal environmental cues became known as allocentric memory, and it is the latest form of episodic memory to emerge. It has recently been shown that allocentric memory is observed since postnatal day 18 (P18), but it is not fully developed until P38, when it reaches the adult-like form. However, it has been proposed that early reinforcement by allocentric task training could accelerate its adult-like expression.Although the mechanisms of memory formation remain unclear, research has pointed out sleep as a memory promoter and oscillatory electrical rhythms during sleep, such as slow cortical waves (< 1Hz) and hippocampal sharp-wave ripples (SWRs,100-250 Hz) as correlates of memory consolidation. To determine whether allocentric reinforcement could anticipate episodic memory's maturation in parallel with changes in oscillatory activity, we performed an object-in-place task with or without reinforcement and in vivo LFP recordings, throughout animal development, in the somatosensory and hippocampal cortices. Our results show that allocentric memory emerges around P32 (n=11, $p< 0.042$) independently of early reinforcement and, changes in the power and density of sleep SWRs accompany the emergence of memory consolidation.
Keywords: Neurosciences, Electrophysiology, Behavior, Development.Contact: magarcia13@uc.clSupported in part by grant number ANID Nº 21171047 and ICN09_015

Disclosures: **M.A. García Pérez:** Other; Laboratorio de Aprendizaje Memoria y Neuromodulación, Departamento de Neurociencia, Facultad de Medicina, Universidad de Chile., Laboratorio de Neurosistemas, Departamento de Neurociencia, Facultad de Medicina, Universidad de Chile., Biomedical Neuroscience Institute (BNI), Facultad de Medicina, Universidad de Chile. **M. Irani:** A. Employment/Salary (full or part-time):; Laboratorio de Neurodinámica de la Cognición, Departamento de Psiquiatría, Centro Interdisciplinario de Neurociencias UC, Pontificia Universidad Católica de Chile. **V. Tiznado:** A. Employment/Salary (full or part-time):; 4. Laboratory for Brain Machine Interfaces, Departamento de Psiquiatría, Centro Interdisciplinario de Neurociencias UC, Pontificia Universidad Católica de Chile. **P.E. Maldonado:** Other; Laboratorio de Neurosistemas, Departamento de Neurociencia, Facultad de Medicina, Universidad de Chile. **J. Valdés:** Other; Laboratorio de Aprendizaje Memoria y Neuromodulación, Departamento de Neurociencia, Facultad de Medicina, Universidad de Chile., Biomedical Neuroscience Institute (BNI), Facultad de Medicina, Universidad de Chile..

Digital Abstract Session

P333. The Role of Oscillations

Program #/Poster #: P333.03

Topic: H.08. Learning and Memory

Support: NSF Grant IIS-1724405
BMBF Grant 01GQ1706

Title: Auditory closed-loop stimulation during sleep in mice modulates the retention of spatial memory

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Abstract: In humans, auditory closed-loop stimulation (ACLS) in phase with the ongoing SOs enhanced overnight memory retention (Ngo et. al., 2013, Neuron). ACLS is hypothesized to enhance the interaction between slow oscillations (SOs), hippocampal sharp-wave ripples, and thalamocortical sleep spindles presumed to underly memory consolidation. Our aim was to determine the stimulation parameters for a corresponding mouse model, a prerequisite for selective interrogation of brain regions/activity underlying sleep rhythms to determine their role in the facilitation of memory consolidation by ACLS. After a sample run of the object place recognition (OPR) task (30 minutes after lights on; 12:12 h, L/D, lights on at 8 a.m.) subjects (C57BL/6N-Rj, male, 8-10 weeks) were placed in a recording box for 3 hours to sleep. Wire arrays in the cingulate cortex and dorsal hippocampus recorded local field potentials. Upon online detection of the negative SO half-wave a white noise burst was delivered by two loudspeakers (58 dB) at one of four phases of the SO, in different sessions: close to the downstate (DS), the down-to-up transition (D2US), the up-state (UpS) and the up-to-down transition (U2DS). Stimulation was omitted in a fifth session (sham). The OPR test phase took place approximately 3 hours after the sample run. Analyses measured amplitude, density, and length of offline-detected SOs, spindle and ripple events, event-event correlations, and the preference index of the OPR task (PI = time spent with the displaced object/ time spent with both objects). ACLS entrained SOs, sleep spindle, and ripple activity as measured by the RMS (root-mean-square) signals. Preliminary analyses reveal a tendency toward increased SO density (SOs/min) in the UpS-condition compared to the sham condition (paired t-test, $p=0.05$, $n=5$). A biophysical model of the closed loop thalamo-cortico-hippocampal network revealed an increase in power in SOs, spindle, and ripple bands; and an increase in density of ripples occurring at the time of stimulation and a shift in the density of ripples toward the D2US, suggesting an increase in the coupling of ripples and SOs. Blinded rating of the OPR task found that mice performed above chance levels for ACLS delivered at the DS, D2US, and UpS whereas sham performance failed to surpass the chance level (one-sample t-test, sham: $p=0.059$, $n=7$; DS: $p=0.004$, $n=7$; UpS: $p=0.034$, $n=4$; U2DS: $p=0.027$, $n=7$). Together, despite measuring only a minor impact on electrophysiological activity, preliminary results in a small subject sample indicate, that our ACLS protocol enhances retention of spatial memory in mice.

Disclosures: S. Aksamaz: None. **M. Mölle:** None. **R. Golden:** None. **M. Bazhenov:** None. **L. Marshall:** None.

Digital Abstract Session

P333. The Role of Oscillations

Program #/Poster #: P333.04

Topic: H.08. Learning and Memory

Title: Human cortical ripples reflect a spectrum of synchronous spiking activity across spatial scale

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Abstract: Engagement of the medial temporal lobe (MTL) and neocortex in episodic memory is strongly associated with increases in high frequency activity (HFA). Recent evidence suggests that discrete HF oscillations, termed ripples, are linked to memory processes that are coupled between human MTL and neocortex. While changes in HFA during successful memory retrieval has been widely observed, dynamic changes that occur during individual trials are less clear. We hypothesized that resolving HFA changes in time can reveal discrete ripple events that reflect a spectrum of dynamic spiking activity modulated by low frequency (LF) oscillations. Twenty-one participants completed a paired-associates verbal memory task in which they studied word pairs and were later cued with a word from each pair while we recorded intracranial EEG (iEEG). Six of these participants also had simultaneous microelectrode array (MEA) recording of local field potentials (LFP) and single-unit spiking activity. We extracted spectral power from the iEEG and LFP signals between 1-200 Hz and defined discrete HFA events as ripples when 80-120 Hz spectral power exceeded two SDs for a minimum of 25 ms. Ripples were detected from simultaneous macroscale iEEG and microscale local field potential (LFP) recordings. Synchrony of ripples and spikes were measured using pairwise phase consistency (PPC). We found that HFA changes during successful memory retrieval were correlated with iEEG ripples and the amplitude of these iEEG ripples was correlated with number and synchrony of LFP ripples across MEA electrodes. Furthermore, amplitude of LFP ripples was correlated with number and synchrony of spiking units. Both LFP ripples and spikes were more strongly coordinated by low frequency oscillations within compared to outside of ripples and during correct compared to incorrect memory retrieval. Overall, our data suggest that successful memory retrieval exhibits discrete ripples across spatial scale that reflect synchronous spiking activity modulated by low frequency oscillations.

Disclosures: A.S. Tong: None. **A.P. Vaz:** None. **S.K. Inati:** None. **K.A. Zaghloul:** None.

Digital Abstract Session

P333. The Role of Oscillations

Program #/Poster #: P333.05

Topic: H.08. Learning and Memory

Support: R25 NS080685
U01 NS099577
R01 DC00566
R01 NS081248

Title: Camkii α expression levels determine the coherence of oscillations between prefrontal cortex and hippocampus

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Abstract: The alpha-isoform of calcium/calmodulin-dependent protein kinase II (CaMKIIα) is highly expressed in the brain including the hippocampus and prefrontal cortex. Mutations of CaMKIIα are implicated in a range of diseases including schizophrenia, addiction, depression, heart disease, epilepsy, Alzheimer, Parkinson's, and neurodevelopmental disorders. Important functions of CaMKIIα are participation in long term potentiation and long-term depression (synaptic plasticity). In addition, heterozygous CaMKIIα knockout mice (Het) have problems with development leading to complications with behaviors including immature dentate gyrus, hyperactivity, deficits in working memory, and social withdrawal. To further investigate the role of CaMKIIα in communication between brain regions, we tested mice deficient for CaMKIIα in an go-no go olfactory discrimination task to assess cognitive learning deficits and underlying problems in neuronal oscillatory processing in the hippocampus and prefrontal cortex. Mice learned to associate one of two odorants with a water reward. Subsequently, wild type (WT), Het, and knockout (KO) mice received double tetrode implants aimed at the CA1 region of the hippocampus and medial prefrontal cortex. We find that the strength of phase amplitude coupling to theta oscillations (tPAC) differs between the genotypes. In addition, the variability of the peak angle for tPAC increases for the unrewarded odor. Furthermore, we calculated theta phase-related power (tPRP). tPRP increases for S+ and decreases for S- as the animal becomes proficient and is significantly different between the genotypes. Finally, we analyzed oscillatory coherence between prefrontal cortex and hippocampus. Our results indicate that coherence increases for S+ and decreases for S- as the animal becomes proficient and is lowest for KO. These observations suggest an essential role for CaMKIIα in coherence between prefrontal cortex and hippocampus in associative odorant learning.

Disclosures: D. Ramirez-Gordillo: None. **D. Restrepo:** None. **K. Bayer:** None. **A. Parra:** None. **L. Mediavillo:** None.

Digital Abstract Session

P333. The Role of Oscillations

Program #/Poster #: P333.06

Topic: H.08. Learning and Memory

Support: NIH Grant R01NS105472

Title: Dentate spikes and external control of hippocampal function

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Abstract: Mouse hippocampus CA1 place-cell discharge typically encodes current location but during slow gamma dominance (SG_{dom}), when slow gamma oscillations (30-60 Hz) dominate mid-frequency gamma oscillations (60-90 Hz) in CA1 local field potentials, CA1 discharge switches to represent distant recollected locations. We now report that dentate spike type 2 (DS_M) events initiated by MECII-DG inputs promote SG_{dom} and change CA1 discharge, whereas type 1 (DS_L) events initiated by LECII-DG inputs do not. Just before SG_{dom}, LECII-originating slow gamma oscillations in dentate gyrus and CA3-originating slow gamma oscillations in CA1 become optimally phase and frequency synchronized at the DS_M peak when the firing rates of DG, CA3, and CA1 principal cells increase to promote DG-CA3-CA1 cofiring optimized for the 5-10 ms DG-to-CA1 neuro-transmission that coincides with SG_{dom}. Several properties and consequences of DS_M demonstrate extrahippocampal control of SG_{dom}, identifying a cortico-hippocampal mechanism that switches between memory-related hippocampal information processing modes.

Disclosures: D. Dvorak: None. **A. Chung:** None. **E. Park:** None. **A.A. Fenton:** None.

Digital Abstract Session

P333. The Role of Oscillations

Program #/Poster #: P333.07

Topic: H.08. Learning and Memory

Support: R01 AG012609, F31 AG055263, McKnight Brain Research Foundation

Title: Decreased dynamic range of hippocampal CA1 gamma in aged rats

Authors: *L. CROWN[1,2], D. T. GRAY[1,2], L. A. SCHIMANSKI[1], C. A. BARNES[1,2,3], S. L. COWEN[1,2];
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Abstract: Decreased episodic memory is a common age-associated cognitive change that significantly impairs quality of life in the elderly. Changes within the hippocampus and surrounding regions including the medial entorhinal cortex (MEC) likely contribute to these impairments in humans and animal models of brain aging. One mechanism through which these regions are thought to communicate is through synchronized gamma oscillations. Gamma oscillations (30 Hz-120 Hz) are implicated in numerous cognitive processes including sensory

binding, memory, and attentional selection. Within the rodent hippocampus, gamma has been shown to be modulated by running speed (Ahmed & Mehta, 2012), suggesting that gamma frequency reflects the speed of sensory or cognitive processing. In this study we examine gamma oscillations in the CA1 region of the hippocampus in 6 middle aged (9-12 months) rats and 5 old (25-28 months) rats as they ran back and forth on a horseshoe shaped maze while performing a spatial eye-blink conditioning task. We hypothesized that cognitive slowing in aged animals would result in impaired CA1 gamma frequency modulation by running speed. To assess how gamma frequency changed as a function of running speed, normalized power spectral densities were acquired and binned by running speed and averaged for each animal. After obtaining the frequency of maximum gamma power for each speed bin, a robust regression was performed to obtain the slope and intercept of the relationship. We found that aged animals had significantly shallower slopes than younger animals ($t(5.6)=3.92$, $p=0.009$) as well as a higher intercept ($t(8.9)=-3.45$, $p=0.007$). This suggests that aged animals have a narrower dynamic range for gamma frequencies which may be indicative of early age-related cognitive slowing.

Disclosures: L. Crown: None. **D.T. Gray:** None. **L.A. Schimanski:** None. **C.A. Barnes:** None. **S.L. Cowen:** None.

Digital Abstract Session

P334. Hippocampal Mechanisms Underlying Avoidance Behaviors

Program #/Poster #: P334.01

Topic: H.08. Learning and Memory

Support: NIH R01MH065961

Title: Ventral, but not dorsal, hippocampus mediates the context-dependence of signaled active avoidance

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Abstract: Avoidance is a common coping technique for fear disorders like PTSD because the relief experienced with reduced exposure to the trauma reminder reinforces the behavior. Although the effect of context on other common PTSD symptoms such as re-experiencing (represented by freezing in rats) has been well-characterized, the role of context in active avoidance has not been established. Because avoidance involves learning sequential Pavlovian and instrumental associations, we hypothesized that it is context-dependent and depends on the hippocampus. To assess these hypotheses, male rats were trained in a two-way signaled active avoidance task, in which they shuttled across a divided chamber during a tone CS in order to prevent a footshock US. Training entailed 30 trials per day for 4 or 8 days. After training,

avoidance responding was tested in the conditioning context or a novel context in a counterbalanced fashion (10 CS-alone presentations in each test). Rats (n=31) performed significantly more avoidance responses in the same context than in the different context, demonstrating the context-dependence of avoidance. To examine the role of the hippocampus in the context-dependence of avoidance, we reversibly inhibited either the dorsal (DH) or ventral hippocampus (VH) with muscimol before test session. VH inactivation(n=13) increased avoidance responses in the different context compared to controls injected with vehicle(n=12) while having no effect on responding in the same context. In contrast, inactivation of DH(n=14) had no effect on avoidance compared to controls injected with vehicle(n=12) in either context. Therefore, VH, but not DH, mediates the context-dependence of avoidance behavior. This is congruent with earlier work (Burhans and Gabriel, 2007) suggesting a role for the ventral subiculum in the contextual modulation of avoidance behavior. Further studies will examine ventral hippocampal outputs that are necessary for the context-shift deficit.

Disclosures: C.R. Oleksiak: None. **K. Ramanathan:** None. **O. Miles:** None. **S. Maren:** None. **J.M. Moscarello:** None.

Digital Abstract Session

P334. Hippocampal Mechanisms Underlying Avoidance Behaviors

Program #/Poster #: P334.02

Topic: H.08. Learning and Memory

Support: Memory RO1MH115304
Info-processing RO1NS105472

Title: Mapping hippocampal synaptic circuits changes in remote place avoidance memory.

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Abstract: Several input-specific populations of synapses in the hippocampus can undergo synaptic changes, potentially to store memory, but which synaptic populations change with experience is unknown. The recurrent connectivity amongst CA3 collaterals has motivated hypotheses that CA3 functions as a storage site. Place cell dynamics and their discharge relationships with gamma oscillations in the LFP identified a specific role for CA3-CA1 stratum radiatum (sr) synapses in memory recollection, and an information encoding role for EC3-CA1 stratum lacunosum moleculare (slm) synapses. Inputs from spatially-tuned medial entorhinal (MEC) cells and less-spatially tuned lateral entorhinal (LEC) cells, differentially target dendritic compartments in the supra- and infra-pyramidal blades of dentate gyrus (DG), such that LEC inputs are hypothesized to provide contextual inputs and MEC spatial signals. The atypical protein kinase C isoform PKMζ is both necessary and sufficient for the maintenance of LTP at hippocampal synapses and is crucial for the persistence of a long-term hippocampus-dependent active place avoidance memory. We used PKMζ immunostaining to test hypotheses for which

input-specific synaptic populations might change in long-term memory storage. To investigate the subcellular distribution of PKMζ we used ArcCreER^{T2} x ChR2-EYFP mice to label memory-associated cells. Trained (n=4) mice had 30-min trials to learn a conditioned place avoidance, and untrained (n=4) control mice only explored the environment. Prior to the last training trial when trained mice expressed strong avoidance memory, all mice received 4-OH tamoxifen to tag memory activated Arc-expressing cells with EYFP. A month later, the persistence of the conditioned avoidance was confirmed prior to sacrifice followed by PKMζ and EYFP immunohistochemistry. EYFP memory-tagging identified that CA1 more than CA3 pyramidal cells are activated, and also more DG granule cells in the suprapyramidal blade than the infrapyramidal blade. Memory-induced increases of PKMζ also followed this pattern, with increases in CA1 > CA3 and increases in supra-DG > infra-DG, which did not differ from untrained controls. PKMζ expression in EYFP memory-tagged cells identified input-specific patterns of memory-related synaptic populations. In CA1, memory-training caused increased PKMζ co-expression with EYFP at sr synapses, but not at slm. These observations putatively identify memory-related sites of synaptic storage and support hypotheses that predict input-specific changes in the storage of specific long-term memories.

Disclosures: A.B. Grau-Perales: None. **A.A. Fenton:** None.

Digital Abstract Session

P335. Manipulations Affecting Mnemonic Behaviors in Rodents

Program #/Poster #: P335.01

Topic: H.08. Learning and Memory

Support: University of Michigan Office of Research Award to NCT

Title: Sex-specific memory deficits and hippocampal activation following neuroimmune activation

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Abstract: The neuroimmune system is critical for carrying out normal neural processes and cognition, and as such, it is not surprising that neuroimmune dysfunction induces significant cognitive deficits including with learning and memory. Despite evidence that memory processes and the neuroimmune system are intricately linked, and that there are sex differences in immune and neuroimmune cells and responses, we do not know precisely how neuroimmune activation interacts with mechanisms of learning and memory and how this differs in males and females. This is particularly relevant for the current pandemic. Coronavirus disease 19 (COVID-19) caused by the virus SARS-CoV-2 presents with significant cognitive impairments that have been linked to neuroimmune activation, and men typically have worse outcomes than women. We used central administration of a synthetic viral mimic, polyinosinic:polycytidylic acid (poly I:C) in male and female C57Bl/6 mice to determine how activation of the neuroimmune system

disrupted hippocampal-dependent memory in both sexes. We found that poly I:C induced significant acute cytokine and chemokine responses in the hippocampus in both sexes, but males showed a greater magnitude response than females. This was evident for IL-1α, IL-1β, IL-6, IL-10, IFNα, TNFα, CCL2, and CXCL10. Next, we tested whether neuroinflammation disrupted learning and memory consolidation of context fear conditioning. While pre-training poly I:C disrupted learning of context fear in both sexes, males were more vulnerable to post-training poly I:C disruption of memory consolidation. We measured cFos in the hippocampus after pre-training poly I:C to determine if changes in hippocampal activation played a role in the disruption of learning in both sexes. Females treated with poly I:C and trained in context fear conditioning showed exaggerated hippocampal activation, whereas poly I:C in males did not alter cFos induction following training. Taken together, these data suggest that while neuroimmune activation disrupts learning in both sexes, it is via separate cellular mechanisms. Specifically, males showed greater neuroimmune responses, and females showed more cFos+ cells in the hippocampus at the same time that both sexes showed learning deficits in context fear conditioning. Future experiments will identify intracellular signaling mechanisms impacted by both neuroimmune activation and memory, with the goal of determining novel, sex-specific target pathways that play a role in memory- and cognitive-related diseases, including Alzheimer's Disease and Post-Traumatic Stress Disorder.

Disclosures: C.K. Posillico: None. **N.C. Tronson:** None.

Digital Abstract Session

P335. Manipulations Affecting Mnemonic Behaviors in Rodents

Program #/Poster #: P335.02

Topic: H.08. Learning and Memory

Support: AFOSR Grant 20RHCOR04

Title: Influence of transcranial direct current stimulation intensity on memory performance and the hippocampal proteome

Authors: S. H. JUNG[1], S. HARSHMAN[2], J. MARTIN[3], R. JANKORD[5], ***C. N. HATCHER-SOLIS**[3,4];
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Abstract: Transcranial direct current stimulation (tDCS) in humans and animals has been shown to produce beneficial effects on cognition and memory. We previously reported that the cognitive enhancement induced by a single 30 minute session of anodal tDCS at 250 μA is strongly associated with the modification of hippocampal proteins. However, the effects of anodal tDCS dosimetry on cognitive performance and the hippocampal proteome remain largely unknown. Thus, the purpose of this study was to investigate the effects of different current intensities of anodal tDCS on memory performance and protein regulation in hippocampal

synaptosomes. Two different current intensities (250µA and 500µA) of anodal tDCS were applied to Sprague Dawley rats (male, 8-10 weeks old) for 30 min for 2 days, just before the training and testing sessions of the passive avoidance test (PAT). Isolated hippocampal synaptosomes were profiled using the Waters Acquity UPLC M-class LC and normalized proteomic abundance datasets were analyzed by multiple bioinformatics methods and advanced statistical analyses. Our results show that anodal tDCS at both 250 µA and 500 µA enhance PAT memory performance. Moreover, both current intensities significantly modify hippocampal synaptosomes compared to sham conditions. Although similar modifications in hippocampal synaptosomes were detected at both current intensities, a significant number of proteins were differentially regulated. In conclusion, we report that anodal tDCS at 250 µA and 500 µA both enhance memory performance in the PAT with potentially different underlying mechanisms. Future studies are warranted to further investigate the effects of tDCS dosimetry on behavior and hippocampal protein regulation.

Disclosures: C.N. Hatcher-Solis: None. **S.H. Jung:** None. **S. Harshman:** None. **J. Martin:** None. **R. Jankord:** None.

Digital Abstract Session

P336. MTL Circuits and Functions: Rodents

Program #/Poster #: P336.01

Topic: H.08. Learning and Memory

Support: NIH Grant R01NS39600
NIH Grant U01MH114829

Title: Hippocampome.org - a simulation-ready knowledge base of the hippocampal neuron-type circuit

Authors: *D. W. WHEELER[1], A. O. KOMENDANTOV[1], D. J. HAMILTON[1], K. MORADI[1], S. M. ATTILI[1], C. TECUATL[1], J. D. KOPSICK[1], N. SUTTON[1], A. SANCHEZ-AGUILERA[2], L. M. DE LA PRIDA[2], G. A. ASCOLI[1,2];
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Abstract: Hippocampome.org is an open-access knowledge base of the rodent hippocampal formation at the level of neuron types. In this knowledge base, peer-reviewed literature is used to define over 120 neuron types by their main neurotransmitter and the patterns of their axonal and dendritic presence across the parcels of the hippocampal formation: dentate gyrus, CA3, CA2, CA1, subiculum, and entorhinal cortex. For each of the neuron types in Hippocampome.org, information is included about molecular markers; intrinsic electrophysiological parameters; quantitative firing-pattern classification; parameters of single- and multi-compartmental Izhikevich models; estimated counts; known and potential connectivity, and statistical estimations of the probabilities of the potential connectivity between the various neuron types; the amplitude, kinetics and short-term plasticity of each potential connection; and the in-vivo

phase locking of neuronal firing to theta oscillations and sharp-wave ripples in the hippocampal formation. All data are cross correlated with each other and linked to extracted publication excerpts and illustrations. We are also proceeding to use the collated knowledge in a real-scale spiking-neural-network computational simulation of the complete hippocampal formation, starting with subregion CA3. As always, we endeavor to expand the foundations of Hippocampome.org by continually data mining the literature for new neuron types and neuronal properties while cross-linking and integrating new and prior knowledge.

Disclosures: D.W. Wheeler: None. **A.O. Komendantov:** None. **D.J. Hamilton:** None. **K. Moradi:** None. **S.M. Attili:** None. **C. Tecuatl:** None. **J.D. Kopsick:** None. **N. Sutton:** None. **G.A. Ascoli:** None. **A. Sanchez-Aguilera:** None. **L.M. de la Prida:** None.

Digital Abstract Session

P336. MTL Circuits and Functions: Rodents

Program #/Poster #: P336.02

Topic: H.08. Learning and Memory

Support: NWA Grant 1228 191 208

Title: The tired hippocampus: memory engrams in the sleep-deprived brain

Authors: *Y. G. BOLSIUS[1], P. R. A. HECKMAN[2,1], M. J. H. KAS[1], S. RAMIREZ[3], P. MEERLO[1], R. HAVEKES[1];
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Abstract: Sleep loss is a common problem in our modern 24/7 society due to social and economic demands. Sleep deprivation negatively impacts brain function and particularly affects cognitive processes that require the hippocampus. We previously showed in mice that a brief period of sleep deprivation following training impairs memory processes in the object-location task. It is unclear, however, whether these deficits are due to a failed storage of information or rather a result of attenuated retrievability of the stored information. To address this question, we used 8 week old male c-fos tTA mice injected with a TRE-ChR2-mCherry AAV in the dentate gyrus to specifically tag a memory engram in this region of the hippocampus. Mice were kept of doxycycline 24 hours prior to training in the object-location memory task and immediately put back on doxycycline at the end of training. All mice were then sleep deprived for 6 hours. During the retention test 24h later, we optogenetically reactivated the spatial memory engram (20Hz, 15 ms for 3 min) in half of the sleep deprived-mice. In line with our previous studies, we found that sleep deprived mice failed to detect the spatial novelty (*i.e.* detect which object was moved to a novel location). However, this impairment was overcome by optogenetic stimulation of the memory engram prior to the retrieval test. Even 5 - 8 days after the training, optogenetic stimulation of the engram resulted in proper memory retrieval in the sleep deprived mice. Altogether, these observations demonstrate that sleep deprivation does not prevent the storage of

hippocampal memory engrams. Rather, it makes the engrams stored under sleep deprivation conditions inaccessible for natural retrieval. These findings underscore the exciting possibility that more information is stored in the brain under sleep deprivation conditions than expected, and may support the development of novel therapeutic strategies to combat cognitive deficits associated with sleep deprivation.

Disclosures: **Y.G. Bolsius:** None. **P.R.A. Heckman:** None. **M.J.H. Kas:** None. **S. Ramirez:** None. **P. Meerlo:** None. **R. Havekes:** None.

Digital Abstract Session

P336. MTL Circuits and Functions: Rodents

Program #/Poster #: P336.03

Topic: H.08. Learning and Memory

Support: NIH Grant R01MH115304

Title: Do engram cells discharge like memory cells?

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Abstract: Engram cell (EC) experiments genetically tag neurons that are active during learning so that they can be optogenetically manipulated to test whether their reactivation is necessary and sufficient for the expression of a memory. Hippocampal ECs have been identified in mice using threat-conditioning paradigms, but the results challenge established notions of how the hippocampus encodes and processes information. Consider for example, that hippocampal ECs are place cells that discharge at specific locations of a conditioning environment to represent that environment. During conditioning, a pair of cells with coincident firing fields in the north of the environment may discharge together, but never when cells with firing fields in the south discharge. However, these three place/engram cells would be activated synchronously during optogenetic activation, making it difficult to imagine how the optogenetic activation could result in replaying or reactivating the representations that were stored during conditioning. We have begun to investigate this contradiction. We first established that optogenetic tagging and activation of CA1 was sufficient to express a conditioned-place avoidance known to require place cells and their dynamic temporal discharge. Next, we recorded ensembles of CA1 cells in these same mice under anesthesia after the optogenetic test and behavioral demonstration of sufficiency. Firing rates increased ~77% during 7 mW, 473 nm, 10 Hz optogenetic activation, and increased their likelihood of discharging to 1% in response to each 15 ms light pulse. Remarkably, CA1 cell pairs maintained their pre-established co-firing relationships such that pairs of cells that cofired before stimulation continued to cofire during stimulation, and cell pairs that did not cofire continued not to cofire (7mW: $r = 0.36$, compared to control: $r = 0.51$).

Ensemble CA1 recordings in awake, head-fixed mice also confirmed that the 10 Hz optogenetic stimulation produces an evoked discharge pattern in single cells that can be distinguished from control discharge. These initial observations indicate the importance of monitoring patterns of neural discharge during EC experiments in order to assess whether or not memory-related temporal discharge patterns are maintained during optogenetic stimulation in EC experiments that elicit conditioned responses.

Disclosures: C. Jou: None. **E. Park:** None. **D. Dvorak:** None. **A.A. Fenton:** None. **E. Levy:** None.

Digital Abstract Session

P336. MTL Circuits and Functions: Rodents

Program #/Poster #: P336.04

Topic: H.08. Learning and Memory

Support: #DGE1746891
R01 NS039456

Title: Individual CA1 place cells automatically encode local sensory cues via rate remapping

Authors: *W. HOCKEIMER[1,2], R.-Y. LAI[1], J. J. KNIERIM[1,2];
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Abstract: Beyond their well-described role in representing spatial location, CA1 place cells also modulate their firing in response to non-spatial cues. This dual role in spatial and non-spatial information processing led to the proposal that CA1 neurons utilize a multiplexed code for location and items via rate remapping. Significantly, this code could provide the mechanistic underpinning of a cognitive map. Further, it has been proposed that CA1 automatically encodes the content of episodic experience into a short-term buffer to support later, offline computations. To address the role of CA1 in automatic attending, and to further characterize the nature of rate remapping, tetrode recordings were taken from four Long-Evans rats (3 M / 1 F) as they completed laps around a rectangular track containing nine reward zones. Each reward zone contained one of three textured floorplates which bore no relation to the probability of reward. The plates were pseudo-randomly interchanged across trials to allow an analysis of the place cell response to each stimulus at each location. Cells were pooled across days for analysis. Approximately 8-15% of neurons displayed significantly different firing rate responses to the stimuli, according to a permutation test (8.3%, $p = 0.035$) and a linear mixed effects model (16%, $p = 2.4e-7$). A majority of days contained a greater abundance of texture-responsive cells compared to the proportion stipulated by the alpha level (alpha = 0.05) (6/9 days, permutation test, $p = 0.2$; 8/9 days LMER models, $p = 0.02$). Texture-responsive cells did not alter their firing rates throughout the place field, but rather displayed micro-fluctuations within the field. These data support the hypothesis that CA1 automatically encodes information about experience in the

framework of a stable spatial map, possibly to support an episodic buffer. Further, the data suggest that CA1 neurons are capable of representing information on a finer spatial scale than that of their place fields, suggesting the presence of under-explored computational properties of the network.

Disclosures: W. Hockeimer: None. **R. Lai:** None. **J.J. Knierim:** None.

Digital Abstract Session

P336. MTL Circuits and Functions: Rodents

Program #/Poster #: P336.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NARSAD Young Investigator Grant
National Science Foundation Graduate Research Fellowship Program under Grant No. 1650113
R01 MH108623
R01 MH111754
R01 MH117961
Weill Scholar Award
IMHRO/One Mind Rising Star Award

Title: Distinct representations of task-related variables in dorsal and ventral CA1 during associative learning

Authors: *J. S. BIANE[1], M. A. LADOW[1], F. STEFANINI[2], S. P. BODDU[1], A. FAN[1], S. T. HASSAN[1], D. L. APODACA-MONTANO[1], N. DUNDAR[1], K. M. LITKE[1], N. I. WOODS[1], M. KHEIRBEK[1];
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Abstract: The dorsal and ventral (d/v) hippocampus exhibit distinct anatomical, genetic and functional properties. Although both regions have been implicated in associative learning, their individual roles in this process are not well understood. Here, we used 2-photon imaging to track the activity of single neurons in the dorsal or ventral CA1 (dCA1, vCA1) across phases of an odor-reward learning task in which an odor and delay period preceded reward delivery. We found robust coding of odor identity in populations of neurons in dCA1 across all phases of the task (baseline, learning, extinction and reinstatement), indicating that dCA1 neurons code for stimulus identity. In contrast, encoding of odor identity in vCA1 neurons was highly dependent on the perceived value of the odorants. Analysis of the delay period between odor and reward found that both dCA1 and vCA1 showed little/no response at the onset of learning. However, following learning both regions displayed robust responsivity to the CS+ delay period, but not the CS- delay period. This signal faded following extinction of the CS+/US contingency, but was rapidly restored when the contingency was reinstated. Moreover, in well-trained animals this pre-reward signal displayed considerable overlap across sessions, suggesting a stable representation

for reward expectation. Finally, we examined the within-trial temporal dynamics of population activity. After associative learning, dCA1 exhibited distinct ensembles of neurons representing each component of the trial (odor, delay, reward). In contrast, in vCA1 we found overlapping representations to these trial epochs, and these neural representations persisted for seconds after the trial was over. This suggests that dCA1 neuronal ensembles may represent the distinct epochs of a trial while vCA1 combines these representations into a format that allows downstream areas to extract meaning from the experience. Collectively, these data indicate a shift from externally based (stimulus ID) to internally based (value) encoding of environmental variables along the d/v axis of the hippocampus.

Disclosures: **J.S. Biane:** None. **M.A. Ladow:** None. **F. Stefanini:** None. **S.P. Boddu:** None. **A. Fan:** None. **S.T. Hassan:** None. **D.L. Apodaca-Montano:** None. **N. Dundar:** None. **K.M. Litke:** None. **N.I. Woods:** None. **M. Kheirbek:** None.

Digital Abstract Session

P336. MTL Circuits and Functions: Rodents

Program #/Poster #: P336.06

Topic: H.08. Learning and Memory

Support: NIH Grant 1K99MH122226
NIH Grant R37 MH068542
NIH Grant R01 MH083862
NIH Grant R01 AG043688
NIH Grant R01 NS081203

Title: Chronic and transient suppression of adult born granule cell function reduce neural flexibility

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Abstract: The dentate gyrus (DG) exhibits a unique form of neural plasticity that results from continuous integration of newly born excitatory granule cells, termed adult hippocampal neurogenesis. Recent studies have proposed that adult neurogenesis promotes cognitive flexibility, the ability to encode new memories without interference from previously stored memories that share similar features. While recent evidence suggested that adult born neurons differentially modulate input to the mature granule cells during processing of spatial information, the manner in which adult neurogenesis contributes to DG network activity and computations is incompletely understood. Here, we employed a well-established method of focal X-ray-irradiation (IR) of the hippocampus to completely eliminate neurogenesis and examined population neural activity using *in vivo* 2-photon Ca^{2+} imaging. Granule cells are thought to implement a pattern separation computation by remapping their firing fields in response to a contextual change. To assess the degree of remapping of neural representations of space, we

compared the similarity between single-cell spatial tuning profiles by subjecting the mice to two sequential exposures of either identical or different environments in different sessions while foraging for randomly dispersed water rewards. While spatial rate maps in Sham mice were less stable across than within environments (i.e., display remapping), IR mice exhibited significantly lower levels of remapping compared to controls. To address the role of adult born neurons specifically during their critical period of maturation (4-6 weeks) we chemogenetically inhibited immature granule cells. In line with chronically suppressing adult neurogenesis, albeit to a lesser degree, transient and selective silencing of immature granule cells resulted in significantly higher levels of stability in spatial rate maps across different environments compared to control mice. Furthermore, a higher proportion of mature granule cells that are matched between sessions exhibited increased activity upon transiently silencing immature granule cells compared to controls, consistent with the prevailing model of adult born neurons' function in regulating the activity of mature granule cells. Our results highlight the long-hypothesized role of adult neurogenesis in providing flexibility for DG to rapidly generate a context specific, distributed representation of important sensory stimuli such as spatial cues, which ultimately promotes cognitive flexibility.

Disclosures: S.N. Tuncdemir: None. **R. Hen:** None. **V. Luna:** None. **C. Lacefield:** None.

Digital Abstract Session

P337. Molecular Mechanisms of Cognition

Program #/Poster #: P337.01

Topic: H.08. Learning and Memory

Support: ARC Grant DP170103952
Australian Government Research Training Fellowship

Title: The conditions under which acquisition of conditioned freezing requires NMDA receptor activation in the basolateral amygdala complex.

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Abstract: NMDA-type glutamate receptors (NMDAR) play a key role in aversive Pavlovian conditioning, including the acquisition of conditioned freezing to a context or discrete cue conditioned stimulus (CS) paired with a shock unconditioned stimulus (US). Acquiring conditioned freezing to a discrete cue CS (e.g., tone or light) requires NMDAR activity in the basolateral amygdala complex (BLA), while acquiring freezing to a context CS requires NMDAR activity in the BLA and the dorsal hippocampus. Learning in the BLA remains NMDAR-dependent when conditioning multiple cues and contexts (Lee & Kim, 1998), including reconditioning to a previously-extinguished context (Laurent & Westbrook, 2009). Interestingly, however, context-shock learning in the dorsal hippocampus is NMDAR-dependent when animals are naïve or the shock US is delivered at unexpected times (Finnie et al., 2018),

but is NMDAR-independent when rats have previously undergone the same learning in a different context (Finnie et al., 2018; Hardt et al., 2009; Sanders & Fanselow, 2003). The present series of experiments examined the conditions under which acquisition of conditioned freezing requires activation of NMDAR in the BLA. The experiments used a conditioning protocol in which rats were exposed to presentations of a serial S2-S1 compound (tone and light, counterbalanced) paired with shock, and then tested for freezing to S2. BLA infusions of the NMDAR antagonist, D-AP5, disrupted acquisition of conditioned freezing to S2 when the animals had not undergone prior training (Experiment 2), but had no effect when the animals underwent S1-shock training prior to serial-order conditioning (Experiments 1-2). However, the requirement for NMDAR activity in the BLA could be restored by omitting the shock US or the already-conditioned S1 from stage two of training (Experiments 3-4), or by conducting the two stages of training in different contexts (Experiment 5). Finally, we confirmed that that NMDAR-independent conditioned freezing to S2 requires AMPA receptor (AMPAR) signalling in the BLA (Experiment 6), including calcium-permeable AMPAR (CP-AMPAR) (Experiment 7). Our results extend NMDAR-independent findings from the dorsal hippocampus to the BLA, and suggest that the animal's prior history determines whether NMDAR activation in the BLA is required for new learning. Our results are also consistent with in vitro work from the BLA, which showed that NMDAR-independent LTP required CP-AMPAR activity (Mahanty & Sah, 1998; Polepalli et al., 2010, 2020). This plasticity took place in inhibitory interneurons, which are also necessary for the acquisition of conditioned freezing in the BLA (Krabbe et al., 2018).

Disclosures: M.J. Williams-Spooner: None. **N.M. Holmes:** None. **F. Westbrook:** None.

Digital Abstract Session

P337. Molecular Mechanisms of Cognition

Program #/Poster #: P337.02

Topic: H.08. Learning and Memory

Support: NIH Grant R01 MH104384
NIH Grant F31 NRSA MH118754

Title: The medial entorhinal cortex mediates basolateral amygdala effects on spatial memory and downstream ARC protein expression

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Abstract: The basolateral amygdala (BLA) modulates the consolidation of dorsal hippocampus (DH)-dependent spatial and dorsolateral striatum (DLS)-dependent cued-response memories, often in competition with one another. Evidence suggests that a critical mechanism for BLA

influences on memory consolidation is via effects on activity-regulated cytoskeletal-associated protein (ARC) in downstream brain regions. However, the circuitry by which the BLA modulates ARC in multiple competing memory systems remains unclear. Prior evidence indicates that optogenetic stimulation of BLA projections to the medial entorhinal cortex (mEC) enhances the consolidation of spatial learning and impairs the consolidation of cued-response learning, suggesting this pathway provides a circuit for favoring one system over another. Therefore, we hypothesized the BLA-mEC pathway mediates effects on downstream ARC-based synaptic plasticity related to these competing memory systems. To address this, male and female Sprague-Dawley rats underwent spatial or cued-response Barnes maze training and, 45 min later, were sacrificed for ARC analysis in synaptoneurosomes from the DH and DLS. Initial experiments found that spatial training alone increased ARC levels in the DH above those observed in control rats and rats that underwent a cued-response version of the task. Post-spatial training optogenetic stimulation of the BLA-mEC pathway altered the balance of ARC expression in the DH vs. DLS, specifically shifting the balance in favor of the DH-based spatial memory system, although the precise region of ARC changes differed by sex. These findings suggest that BLA-mEC pathway influences on ARC in downstream regions are a mechanism by which the BLA can favor one memory system over another.

Disclosures: K.L. Wahlstrom: None. **R. LaLumiere:** None. **C.K. McIntyre:** None. **A. Alvarez-Dieppa:** None.

Digital Abstract Session

P337. Molecular Mechanisms of Cognition

Program #/Poster #: P337.03

Topic: H.08. Learning and Memory

Support: NIH Grant R35 NS111562
NIH Grant F31 NS108506

Title: Homeostatic Synaptic Scaling Establishes the Specificity of Aversive Taste Memory

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Abstract: Hebbian plasticity mechanisms such as long-term potentiation (LTP) are widely considered critical for learning and memory. Nonetheless, LTP can initiate a positive feedback process that, if left uncontrolled, will pervasively increase synaptic strengths, and result in a failure to achieve memory specificity. Synaptic scaling, a form of homeostatic plasticity, has been theorized to constrain this run-away LTP by globally adjusting post-synaptic strengths and thus ensure faithful encoding of memory. While compelling on theoretical grounds, it has not been tested whether synaptic scaling partners with LTP *in vivo* during memory formation. Furthermore, the impact of disrupted synaptic scaling on memory fidelity remains unknown. Here we directly examined how synaptic scaling shaped memory specificity in conditioned taste

aversion (CTA), a form of associative learning that relies on Hebbian plasticity within the gustatory cortex (GC). We hypothesize that perturbation of synaptic scaling in the GC, the brain region involved in both acquisition and maintenance of CTA, will degrade the stimulus-specificity of CTA. We first demonstrated that after CTA conditioning, young rats transitioned from a generalized to a taste-specific aversion over a timescale of ~24 hours. Furthermore, the duration of this transient general aversion correlates with the strength of conditioning. To elucidate whether synaptic scaling established the specificity of CTA, we perturbed homeostatic synaptic scaling *in vivo* using viral vectors to introduce either the C-terminus fragment of GluA2 or a mutant GluA2, both known to block synaptic scaling *in vitro*. Notably, we found that blocking synaptic scaling in the GC prolonged the phase of CTA-induced general aversion without impairing the acquisition of CTA. This result was further corroborated by a third viral manipulation known to specifically block synaptic downscaling. Next, we found that GC neuronal ensembles active during conditioning were robustly reactivated by the generalized tastant when animals manifested general aversion. Abolishing synaptic scaling in the GC conditioning-active ensembles led to a striking and persistent increase in postsynaptic strengths that correlated with the prolonged general aversion. Taken together, our work establishes that synaptic scaling is important for sculpting the transition from a generalized to a specific state of associative memory, and that homeostatic regulation of synaptic strengths contributes to precise and stable memory formation.

Disclosures: C. Wu: None. **R.A. Ramos:** None. **D.B. Katz:** None. **G. Turrigiano:** None.

Digital Abstract Session

P337. Molecular Mechanisms of Cognition

Program #/Poster #: P337.04

Topic: H.08. Learning and Memory

Support: ASOs provided by Ionis pharmaceuticals
Funding for rat experiments and RNA-seq provided by Vanderbilt University
Pharmacology Chair start-up funds

Title: Evidence that an HDAC2-targeted ASO persistently upregulates cortical acetylcholine and dopamine signaling via a CREB-Gs positive feedback loop

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Abstract: Previous studies have shown that modulation of chromatin accessibility through histone deacetylation can facilitate learning and memory. In this study, we examined the effects of a single injection of histone deacetylase 2 (HDAC2)-targeted anti-sense oligonucleotides (ASOs) on cognitive behavior and gene expression. HDAC2 ASO-injected rats displayed increased novelty preference, decreased cortical and hippocampal HDAC2 mRNA and protein, and upregulated gene expression that persisted 1-month post-injection. Cortical RNA-seq

analysis revealed strongly increased transcription of a subset of cyclic adenosine monophosphate (cAMP)-response element binding (CREB) genes known to influence synaptic plasticity, along with dopamine (DRD1, DRD2) and adenosine (ADORA2A) G-protein-coupled receptors (GPCRs). Our analysis identified evidence of a positive-feedback loop that amplified expression of CREB-regulated Gs GPCRs and genes in cAMP/Gs/Gi signaling pathways. Additionally, we found differential expression of enzymes that shift neurotransmitter biosynthesis away from norepinephrine and toward dopamine and acetylcholine (DBH, CHAT). We also observed increased expression of genes important for neurotransmitter packaging (SV2C, VMAT) and release (SYT9). The data indicate that persistent inhibition of HDAC2 expression enables long-lived enhancement of aspects of cognition through increased cortical transcription of a subset of CREB-regulated genes amplified by a positive-feedback mechanism that increases synaptic plasticity and shifts neurotransmitter balance toward increased dopaminergic and cholinergic signaling.

Disclosures: T.H. Sanders: None.

Digital Abstract Session

P337. Molecular Mechanisms of Cognition

Program #/Poster #: P337.05

Topic: H.08. Learning and Memory

Support: Canadian Institutes of Health Research (CIHR)
Heart and Stroke Foundation (HSF)
Natural Sciences and Engineering Research Council of Canada (NSERC)
Canadian Foundation for Innovation (CFI)
Michael Smith Foundation for Health Research.

Title: Impact of type 1 diabetes on cerebral microcirculation and cognitive function

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Abstract: Introduction Recent work from our lab has shown that the brain capillaries routinely get "stuck," clogged by cells and debris even under healthy conditions. We reported that about one third of these clogged capillaries were eliminated from the blood vessel network and never get replaced. The present study was undertaken to determine how diabetes affect the capillary obstruction in the brain. **Methods** C57BL/6 male and female mice were injected with streptozotocin to induce type 1 diabetes. To assess rates of capillary stalling and the composition of plugged capillaries, mice were implanted with cranial windows and cortical volumes were repeatedly imaged *in vivo* from 4-8 weeks after induction of diabetes. Furthermore we intravenously injected 5μm diameter fluorescent microspheres in diabetic (treated with or without insulin) and control mice to assess group differences in susceptibility to capillary stalling. The density of microsphere obstructed capillaries was quantified across 15 different

brain regions at 30min and 3 days after injection. To determine the impact of diabetes on cognitive and sensorimotor activity, mice were subjected to a battery of behavioural tests. **Results** Longitudinal 2-photon imaging indicated that diabetic mice displayed higher rates of capillary stalling that became more pronounced with increased duration of diabetes. Diabetic mice also showed a trend towards more vessel pruning events over time. Preliminary imaging of Rhod6G labelled leukocytes and platelets suggests that the majority of stalled capillaries are plugged by leukocytes. Our fluorescent microsphere obstruction assay yielded similar results as both male and female diabetic mice showed significantly higher density of capillary obstructions at both 30 minutes and 3 day time intervals. These effects were most apparent in cortical (frontal, motor, somatosensory, retrosplenial, visual), hippocampal and white matter regions while no differences were detected in the amygdala, striatum and substantia nigra. Behaviourally, diabetic mice showed significantly higher mean escape latency than the control mice in the Morris water maze task. No significant effect of diabetes was observed for the other tests of sensorimotor activity, anxiety, vision or locomotion. **Conclusions** These pilot studies suggests that diabetes is associated with greater risk for capillary obstructions in the brain as well as spatial learning deficits. Our future aims will provide insight into the mechanistic understanding of how diabetes leads to increased capillary obstructions and the ways to clear these obstructions in both the healthy and diabetic brains.

Disclosures: S. Sharma: None. **C.E. Brown:** None.

Digital Abstract Session

P337. Molecular Mechanisms of Cognition

Program #/Poster #: P337.06

Topic: H.08. Learning and Memory

Support: NIMH R00MH109626

Title: Visualizing and quantifying mitochondria in the hippocampus with protein-retention expansion microscopy

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Abstract: In neurons, mitochondria are critical organelles that produce energy and buffer calcium to support synaptic transmission, maintenance and plasticity. There is increasing evidence of diversity in mitochondrial morphology and function across, and even within, cell types. Our lab found that the expression of mitochondrial genes differs regionally in the hippocampus. Specifically, genes encoding mitochondrial proteins that are involved in bioenergetics and calcium handling are selectively higher in area CA2 compared with neighboring areas CA1 and CA3. Because larger mitochondria tend to be more metabolically active, we looked at the number and size of mitochondria labeled with mitochondrial marker COX4 in the three hippocampal areas. We saw both regional and subcellular differences in

number and size of mitochondria, suggesting that mitochondria can be regulated differently based on their local environment. Unfortunately, subcellular organelles are hard to visualize within a single neuron in dense tissue. To overcome this, we used protein-retention expansion microscopy (Pro-ExM) in combination with immunolabeling of mitochondria in transgenic reporter mice to physically expand and optically clear tissue to better resolve mitochondrial morphology and number in the adult mouse hippocampus. A limitation of Pro-ExM is that florescent proteins and antibodies are digested and diluted during the process. To optimize the pro-ExM protocol for co-visualizing immunolabelled mitochondria and reporter proteins, we varied the length of digestion (2, 4, 8 hours or overnight) and compared reporter florescence retention and expansion factors from individual neurons across conditions. We also assessed the quality of labeling under different digestion conditions (Proteinase K or autoclave) and varied the order of immunostaining, either before or after Pro-ExM. We demonstrate that we can visualize and segment mitochondria best with IHC pre-ExM and digestion in Proteinase K for 8 hours. We quantified COX4-labeled mitochondria in two male genetic reporter mice, one which was expanded with the optimized Pro-ExM protocol and one which was similarly immunostained but left unexpanded. After correcting for expansion, expanded mitochondria were on average smaller, more numerous and less variable in size than unexpanded mitochondria. This suggests that mitochondria, which are too close to be resolved in unexpanded tissue, can be properly segmented in expanded tissue. Moving forward with this optimized Pro-ExM protocol, we can further probe how regional mitochondrial diversity impacts hippocampal neuron function.

Disclosures: K.E. Pannoni: None. **L. Campbell:** None. **N. Savory:** None. **S. Farris:** None.

Digital Abstract Session

P337. Molecular Mechanisms of Cognition

Program #/Poster #: P337.07

Topic: H.08. Learning and Memory

Support: RO1 AG003376, McKnight Brain Research Foundation

Title: The relationship between microglia density and age-associated perineuronal net alterations in the retrosplenial cortex of rhesus macaques

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Abstract: Perineuronal nets (PNNs) are extracellular matrix (ECM) structures around certain neuron types and provide a support structure to maintain synapse function (Sorg et al., 2016). In early adulthood, the emergence of PNNs has been linked to reductions in neuroplasticity that define the closing of the critical period. The characteristics of PNNs later in life and their

potential roles in declining cognition are less clear. Microglia-derived proteases have also been shown to play a role in regulating synaptic plasticity by modifying ECM composition (Crapser et al., 2020). These cells are vulnerable to age-related changes and show increased activity with normative and pathological aging (Conde & Streit, 2006; Spittau, 2017). The present study sought to further understand 1) the possible contributions of PNN and microglia densities to age-associated cognitive decline, and 2) the role of microglia in regulating PNNs in older brains. To these ends, we used fluorescence labeling and unbiased quantification of the PNN marker *Wisteria floribunda agglutinin* (WFA) and the microglial marker IBA1 on the brains from a colony of 30 rhesus macaque monkeys ranging in age from 7 to 32 years (human equivalent ~21 – 96 years). All monkeys underwent tests of spatial short-term memory, object recognition memory, and object discrimination, which allowed relationships between PNN density and microglia expression and cognition to be investigated. Preliminary results indicate that aged animals had fewer PNNs in the retrosplenial cortex (RSC) compared to younger animals, and this decrease correlated with lower performance specifically on object recognition tasks. Microglia density was higher in the RSC of the aged monkeys compared to adults. Notably, this increase was also significantly associated with poorer performance on object recognition tasks. Importantly, a weak yet significant negative relationship emerged such that animals with greater microglia density had lower PNN densities. Together these results suggest that microglial networks may contribute to an age-associated degradation of the ECM that has circuit-specific impacts on cognition in older monkeys, likely through a destabilization in synapse function.

Disclosures: **S.O. Khattab:** None. **D.T. Gray:** None. **K. McDermott:** None. **I. Sinakevitch:** None. **R. Schwyhart:** None. **A.C. Smith:** None. **W. Härtig:** None. **C.A. Barnes:** None.

Digital Abstract Session

P337. Molecular Mechanisms of Cognition

Program #/Poster #: P337.08

Topic: H.08. Learning and Memory

Support: AFOSR Grant 20RHCOR04
AFOSR Grant 16RHCOR362

Title: Anodal and cathodal tDCS regulates synaptosomes in cerebral cortex in rats

Authors: *S. H. JUNG, R. MOORE, N. BECHMANN, A. V. QUALLEY, J. MARTIN, S. HARSHMAN, R. JANKORD, C. HATCHER-SOLIS;
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Abstract: Transcranial direct stimulation (tDCS) in humans and animals has been shown to produce beneficial effects on cognition and memory. Previously, we reported how anodal tDCS at 250μA and 500μA modified transcriptomic levels in the cerebral cortex without cellular/tissue damage. However, it is still unclear how tDCS polarity and intensity affect protein expression in the cortex and to our knowledge no previous study has investigated the effects of a one-time

tDCS application on proteomic regulation in the cerebral cortex. In this study, we employed anodal and cathodal tDCS at the 250uA and 500uA current intensities to Sprague Dawley rats (male, 8-10 weeks old) for 30 min, immediately followed by collection of the cerebral cortex. Cortex synaptosomes were profiled. Normalized proteomic abundance datasets were analyzed by multiple bioinformatics methods and advanced statistical analysis. Total number of 2966 proteins were detected. Only 2763 proteins were analyzed because they were detected from more than 90% of samples. IPA comparison analysis between 2 current intensities of anode and cathode detected 2 (scores ≥ 20) and 3 (scores ≥ 17) networks, respectively. Further network analysis detected significant functions, including cell-to-cell signaling and interaction (anodal network-1: 11 nodes; p = 1.06E-3 – 2.01E-2), nervous system development and function (anodal network-2: 15 nodes; p = 7.86E-7 – 1.74E-3), RNA damage and repair (cathodal network-1: 29 nodes; p = 1. 27E-63 – 1.27E-63), memory (cathodal network-2: 6 nodes; p = 0.0106), and post-translational modification (cathodal network-3: 6 nodes, p = 1.79E-4 – 4.75E-3). Comparison analysis across all groups detected only 3 intersection proteins: ActL7A, Gpt & Nubp1. Protein-protein interaction (PPI) network for 3 proteins (confidence cutoff = 0.4; max. additional interactors = 10; species = Rattus norvegicus) was constructed and analyzed (PPI enrichments p = 1.0E-16; enrichments clustering coefficient = 0.555; enrichments average degree = 4.31). PPI network analysis resulted in significant associations with metabolism (rno00250, FDR q = 3.88E-10; RNO-1430728, FDR q = 0.00045; rno01100, FDR 1 = 0.00097). In conclusion, we provided evidence for the safety use of both anodal and cathodal tDCS with the max current intensity of 500uA and beneficial effects of tDCS based on our synaptosomes proteomics data. However, a further study is needed to investigate whether these beneficial modifications in the synaptosomes are associated with positive changes in behaviors, especially cognitive performance.

Disclosures: **S.H. Jung:** None. **R. Moore:** None. **N. Bechmann:** None. **A.V. Qualley:** None. **J. Martin:** None. **S. Harshman:** None. **R. Jankord:** None. **C. Hatcher-Solis:** None.

Digital Abstract Session

P337. Molecular Mechanisms of Cognition

Program #/Poster #: P337.09

Topic: H.08. Learning and Memory

Support: NIGMS Grant R01GM123157
NIH CBI fellowship T32GM008515
NIGMS P30 GM124169
High-end instrumentation grant S10OD018483

Title: Heterogeneity in human hippocampal CaMKII transcripts reveals allosteric hub-dependent regulation

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Abstract: Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) plays important roles in processes crucial to learning and long-term memory formation. Each subunit of CaMKII is comprised of a kinase domain, hub domain, variable linker region and regulatory segment. A fully assembled CaMKII complex is comprised of an oligomer of 12-14 subunits. CaMKII in humans is expressed from 4 genes (α,β,δ,γ) and each of these are alternatively spliced. All of these variants are highly conserved in all domains except for the variable linker region. From 3 human hippocampal samples, we sequenced over 70 different CaMKII variants. We are trying to understand why all these variants are necessary for function. Previously, it has been shown that there is a relationship between the variable linker length and kinase activity. We measured the activity of a wide range of CaMKII variants expressed in the human hippocampus to better understand the role of these variants in memory. We measured the activity of human CaMKII (expressed recombinantly) using a coupled-kinase assay. The three variants of alpha tested show that linker-containing variants are much more easily activated compared to the zero-linker variant. When β variants were tested, surprisingly the length/composition of the variable linker did not make a difference in Ca^{2+}/CaM sensitivity, with a similar EC_{50} value in variants that ranged from 0 to 217 residues in linker length. Further study revealed an activity regulation role for the hub domain; this has not been previously characterized before. This opens up the possibility as a potential drug target for this protein in human neurological diseases where mutations in CaMKII cause disruptions in functionality.

Disclosures: **N. Dziedzic:** None. **R. Sloutsky:** None. **M.J. Dunn:** None. **R.M. Bates:** None. **A.P. Torres-Ocampo:** None. **S. Boopathy:** None. **B. Page:** None. **J.G. Weeks:** None. **L.H. Chao:** None. **M.M. Stratton:** None.

Digital Abstract Session

P338. Signaling and Genes in Cognition

Program #/Poster #: P338.01

Topic: H.08. Learning and Memory

Support: NIA T32 AG000096
NIA AG050787
NIA AG051807
NIA AG054349
NIMH MH101491
NIDA DA025922
NIDA DA036984

Title: Exercise opens a 'molecular memory window' to facilitate memory and synaptic plasticity

Authors: A. A. KEISER[1,1], T. DONG[1], E. KRAMÁR[1], C. BUTLER[2], D. MATHEOS[1], N. BERCHTOLD[2], S. CHEN[3], M. SAMAD[3], C. MAGNAN[3], S. SHANUR[1], P. BALDI[3], C. COTMAN[2], M. WOOD[1];
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Abstract: Our labs have previously demonstrated that in males, exercise enables hippocampal-dependent learning in conditions that are normally subthreshold for encoding and memory formation and depends on hippocampal induction of brain-derived neurotrophic factor (BDNF) as a key mechanism. In male mice with prior exercise experience, only a brief exercise period is required to reactivate the increase of BDNF in the hippocampus, suggesting that there exists a 'molecular memory' for the prior exercise experience that facilitates subsequent learning. However, the underlying mechanisms that mediate the 'molecular memory' are currently unknown. We hypothesize that an epigenetic molecular memory of exercise as a previous experience primes specific genes for subsequent activation upon new learning, resulting in facilitated memory formation. In this study, we used RNA-sequencing to begin to define the molecular and epigenetic signature underlying exercise-enhanced learning in the hippocampus. Adult male mice underwent different periods of initial exercise (0-3 weeks), a sedentary delay period (0-2 weeks), and a brief 2-day period of reactivating exercise, followed by 3 min subthreshold training in an object location memory task. This allowed us to identify exercise parameters that enable the formation of robust long-term memory for object location in conditions that are normally subthreshold for encoding. Those parameters were then used to examine hippocampal long-term potentiation (LTP). Our results indicate that 2 weeks of initial exercise is sufficient to engage robust memory and enhance LTP compared to sedentary controls. Additionally, we found that a brief 2-day period of reactivating exercise was sufficient to re-gain elevated levels of LTP after they had returned to baseline following a 2-week sedentary delay. Using the same parameters, we also examined gene expression using RNA sequencing during the memory consolidation period, one hour after training. We found that different exercise parameters led to distinct transcriptional profiles in dorsal hippocampus. Of particular interest, was a gene coding for a novel plasticity protein, ACVR1C, which was found to be up-regulated only in mice that underwent exercise parameters that were capable of facilitating LTM formation. Sequencing data has also suggests a link between exercise and cellular metabolism. Together, these data provide a number of interesting target genes and mechanisms to explore that may be key contributors in the maintenance of the 'molecular memory window' for exercise and suggests that changes in the transcriptional profile of the hippocampus contribute to exercise-enhanced learning.

Disclosures: A.A. Keiser: None. **T. Dong:** None. **E. Kramár:** None. **C. Butler:** None. **D. Matheos:** None. **N. Berchtold:** None. **S. Chen:** None. **M. Samad:** None. **C. Magnan:** None. **S. Shanur:** None. **P. Baldi:** None. **C. Cotman:** None. **M. Wood:** None.

Digital Abstract Session

P338. Signaling and Genes in Cognition

Program #/Poster #: P338.02

Topic: H.08. Learning and Memory

Support: NIA T32 AG000096
NIA AG050787
NIA AG051807
NIA AG054349
NIMH MH101491
NIDA DA025922
NIDA DA036984

Title: Examining the 'molecular memory' of exercise enhanced learning

Authors: *T. N. DONG[1], E. KRAMÁR[1], C. BUTLER[2], D. MATHEOS[1], N. BERCHTOLD[2], S. CHEN[3], M. SAMAD[3], C. MAGNAN[3], S. SHANUR[1], P. BALDI[3], C. COTMAN[2], M. WOOD[1], A. KEISER[1];
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Abstract: Our labs have demonstrated that exercise engages epigenetic mechanisms that prime brain-derived neurotrophic factor (BDNF) induction in the hippocampus to allow for stable changes in neuronal plasticity to improve learning. Particularly, our studies have demonstrated that in male mice, exercise enables hippocampal-dependent learning in conditions that are normally subthreshold for long-term memory (LTM) in sedentary animals. A previous study by the Cotman lab demonstrates that following a break from exercise (during which elevated BDNF levels in the hippocampus decline to baseline), only a brief exercise period can reactivate the increase in BDNF, suggesting a 'molecular memory' of the initial exercise experience that enables the subsequent brief exercise to facilitate learning. We have recently identified specific exercise parameters that establish and maintain this 'molecular memory' of exercise to facilitate synaptic plasticity and LTM formation. Here, we investigate the molecular and epigenetic mechanisms underlying this 'molecular memory'. We hypothesize that the previous exercise experience establishes an epigenetic molecular memory of exercise that primes specific genes for subsequent activation upon new learning to facilitate LTM formation. To understand the extent of this 'molecular memory', RNA-sequencing was used to examine the gene expression profiles in the hippocampus within the memory consolidation window, 1h following a 3-minute subthreshold object location memory acquisition session in mice that underwent initial exercise (2 weeks), a sedentary break (1-2 weeks) followed by brief reactivating exercise (2 days). We found exercise parameters that facilitated learning, engaged distinct transcriptional profiles compared to those that did not. A particular gene target, *ACVR1C*, was one of the two genes found to be upregulated only in conditions in which exercise facilitated learning. We chose to further investigate this target as a previous study has shown *ACVR1C* to be necessary for LTP

maintenance and synaptic tagging. This upregulation of *ACVR1C* was confirmed via RT-qPCR. In addition, we found a number of genes associated with cellular metabolism to be upregulated in conditions where exercise facilitated learning. Together, these data suggest that *ACVR1C* and metabolic processes might contribute to the maintenance of the "molecular memory window" associated with exercise. Currently, we are investigating the causal role for identified targets such as the novel plasticity protein, ACVR1C, and metabolic mechanisms in the maintenance of a 'molecular memory' for exercise and how this may be different in females and aging mice.

Disclosures: **T.N. Dong:** None. **E. Kramár:** None. **C. Butler:** None. **D. Matheos:** None. **N. Berchtold:** None. **S. Chen:** None. **M. Samad:** None. **C. Magnan:** None. **S. Shanur:** None. **P. Baldi:** None. **C. Cotman:** None. **M. Wood:** None. **A. Keiser:** None.

Digital Abstract Session

P338. Signaling and Genes in Cognition

Program #/Poster #: P338.03

Topic: H.08. Learning and Memory

Support: Korea NRF-2019R1A2C1006040
BK21 FOUR Program

Title: Arhgef4 (Rho guanine nucleotide exchange factor 4) deficiency enhances long-term memory

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Abstract: Guanine nucleotide exchange factors (GEFs) play multiple functional roles in neurons. In our previous study, *Arhgef4* (Rho guanine nucleotide exchange factor 4, also known Asef1) functioned as a negative regulator of the excitatory synaptic function by sequestering postsynaptic density protein 95 (PSD-95). However, much of *Arhgef4* role in behavioral level has not been examined. Thus we performed comprehensive behavioral tests in knockout (KO) mice to investigate of the effects of *Arhgef4* deficiency. In consistent with our previous *in vitro* findings, the size of PSD-95 particle was significantly increased in hippocampal neuronal cultures from *Arhgef4* KO mice. *Arhgef4* KO mice exhibited general motor activity and anxiety-like behavior comparable to those of the wild type littermates. However, spatial memory and object recognition memory were significantly enhanced in the *Arhgef4* KO mice, whereas depression-like behavior was decreased. Taken together, these data confirm the role of *Arhgef4* as a negative synaptic regulator at the behavioral level.

Disclosures: **K. Yoo:** None. **H. Kim:** None. **K. Lee:** None.

Digital Abstract Session

P338. Signaling and Genes in Cognition

Program #/Poster #: P338.04

Topic: H.08. Learning and Memory

Support: NIH Grant DA041781
NIH Grant DA042581
NIH Grant DA042499
NIH Grant DA041883
NIH Grant DA045463
NIH Grant DA046436
NIH Grant DA042620

Title: Sex differences in the role of cornichon homolog-3 on spatial memory & synaptic plasticity

Authors: *H. E. FRYE[1,2,3], Y. IZUMI[4,5,6], C. R. TROUSDALE[2,3], A. N. HARRIS[7], M.-Y. SUN[4], S. WILLIAMS[8], A. D. SAUERBECK[9], T. T. KUMMER[9], S. J. MENNERICK[4,5,6], C. F. ZORUMSKI[4,5,6], E. C. NELSON[4], J. D. DOUGHERTY[10,5], J. MORON-CONCEPCION[2,3,5]; [1]Neurosci. Program, [2]Anesthesiol., [3]Pain Ctr., [4]Psychiatry, [5]Neurosci., [6]Taylor Family Inst. for Innovative Psychiatric Res., [7]Genet., Washington Univ. Sch. of Med., Saint Louis, MO; [8]Washington Univ. Sch. of Med., St. Louis, MO; [9]Neurol., [10]Genet. and Psychiatry, Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Cornichon homolog-3 (CNIH3) is an AMPA receptor (AMPAR) auxiliary protein highly expressed in the dorsal hippocampus (dHPC), a region where AMPARs are critical for spatial memory and synaptic plasticity. However, the role of CNIH3 on synaptic plasticity and behavior is currently unknown. In this study, *Cnih3*$^{-/-}$ female C57BL/6 adult mice displayed significantly attenuated short-term memory in a Barnes maze spatial memory task compared to *Cnih3*$^{+/+}$ females, reduced synaptic density in the dHPC, altered levels of post-synaptic proteins in the dHPC, and attenuated synaptic plasticity compared to controls, whereas *Cnih3*$^{-/-}$ males were unaffected. Further study revealed that impairments in spatial memory were most pronounced during the metestrus phase of the estrous cycle only in *Cnih3*$^{-/-}$ female mice. In addition, viral overexpression of *Cnih3* in the dHPC was sufficient to improve short-term spatial memory in females compared to females injected with a control virus, but viral overexpression of *Cnih3* had no effect in males. To ensure scientific rigor, multiple cohorts of samples and animals were tested in each experiment to confirm reproducibility of the results, sample sizes were consistent with prior literature for each experiment, and experimenters were blinded to sample group when analyzing estrous cycle data and in synaptic density experiments. This study identifies a previously unknown role for CNIH3, as manipulation of this gene unmasks sexually dimorphic and estrous-specific effects in spatial memory and hippocampal plasticity.

Disclosures: **H.E. Frye:** None. **Y. Izumi:** None. **C.R. Trousdale:** None. **A.N. Harris:** None. **M. Sun:** None. **S. Williams:** None. **A.D. Sauerbeck:** None. **T.T. Kummer:** None. **S.J. Mennerick:** None. **C.F. Zorumski:** None. **E.C. Nelson:** None. **J.D. Dougherty:** None. **J. Moron-Concepcion:** None.

Digital Abstract Session

P338. Signaling and Genes in Cognition

Program #/Poster #: P338.05

Topic: H.08. Learning and Memory

Title: Genetic variants in TP53BP1 are associated with superior memory in human SuperAgers

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Abstract: SuperAgers (SA) are adults aged 80 or older with episodic memory performance that is at least as good as that of cognitively average individuals in their 50's and 60's. In this study, we performed whole-exome sequencing (WES) to identify genetic variants that are associated with the SA phenotype. WES was conducted on 72 SA, and as a control group, we used genotypes from 22 cognitively-average controls (CTLs) from the Alzheimer's Disease Neuroimaging Initiative (ADNI). DNA was extracted from whole blood, and libraries were sequenced on the HiSeq 2500 System (Illumina) using 150 basepair paired-end read chemistry. Preprocessing was conducted using BWA and GATK, and association analysis was conducted using the multi-marker SKAT test accounting for both common and rare variants.The final dataset included 45,275 single nucleotide variants (SNVs). Total genotyping rate across these SNVs was 98.7%. SKAT analysis identified a significant signal in *TP53BP1* (adj p = 2.2E-04), including three common (rs2230451-T, rs689647-T, rs560191-C) and one rare SNV (rs201906661-A). The frequency of the three common variants was higher in the SAs, whereas the frequency of the rare variant was higher in the CTLs. Allele frequencies in CTLs were in the same range of those reported in the *gnomAD v2.1.1* database (non-Finnish European cohort) for rs560191-T and rs201906661, but not for rs2230451 and rs689647-T (higher and lower in *gnomAD*, respectively). rs689647-T and rs560191-C are missense variants, with CADD scores 17 and 15, respectively.We found new common and rare genetic associations with the SA phenotype in the coding sequence of the *TP53BP1* (Tumor Protein P53 Binding Protein 1) gene. The protein encoded by this gene is involved in the DNA double-strand break repair pathway. A polymorphism in *TP53*, a binding partner of TP53BP1, was previously associated with human longevity, and TP53BP1 itself was linked to healthy aging through increased response against stress-induced DNA damage. These findings suggest that genetic variation in DNA repair may be linked to cognitive resilience during aging and not simply human longevity. Validation of these findings is needed in additional SAs.

Disclosures: **I.S. Piras:** None. **A. Siniard:** None. **A. Bonfitto:** None. **M. De Both:** None. **R. Richholt:** None. **C. Balak:** None. **J. Pouya:** None. **E. Bigio:** None. **S. Weintraub:** None. **B. Makoski-Woidan:** None. **M. Huentelman:** None. **M. Mesulam:** None. **C. Geula:** None. **E. Rogalski:** None.

Digital Abstract Session

P338. Signaling and Genes in Cognition

Program #/Poster #: P338.06

Topic: H.08. Learning and Memory

Support: CPRIT RTA RP170345
CPRIT RP180055
NCI R01 CA224675
Quincy and Estine Lee Endowment Fund

Title: Investigating the underlying mechanisms by which vortioxetine reverses cognitive impairment mediated by the medial prefrontal cortex and hippocampus after androgen deprivation therapy for prostate cancer treatment

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Abstract: Androgen deprivation therapy (ADT) is a mainstay treatment for prostate cancer but is accompanied by profound cognitive impairment in areas of executive function, visuospatial learning, memory, and attention. Imaging studies of prostate cancer patients given ADT have found reduced functional connectivity within the medial prefrontal cortex (mPFC). Further, decline on cognitive tasks mediated by the hippocampus (Hipp) are reported across multiple studies. Vortioxetine (VTX) is a novel multimodal antidepressant that has been shown to improve cognition in depressed patients, and we hypothesized that VTX may be efficacious in improving cognition after ADT. In a rodent model of ADT, rats display cognitive impairments mediated by the mPFC and the Hipp. Chronic treatment with VTX (28 mg/kg/day) administered through the diet reversed these deficits. VTX also reversed the attenuated response of the mPFC to input from the Hipp after ADT. The androgen receptor is a transcription factor, so ADT-induced impairment is likely to be related to changes in gene expression. VTX has been reported to induce changes in gene expression related to neuroplasticity in healthy rats. Therefore, we further hypothesized that VTX may overcome changes in plasticity-related gene expression induced in mPFC and Hipp by ADT. To test this, gene expression was analyzed in tissue collected from the mPFC, and the dorsal and ventral Hipp using a whole genome microarray

(Agilent Technologies, Inc.). Castration significantly altered over 1,000 genes in each of the three brain regions (adjusted p value<0.05). VTX on its own, and within castrated animals, had no significant effects on gene expression, but pathway analysis revealed significant changes in processes involved in synaptic plasticity, such as synapse formation, axonogenesis, and the MAPK signaling cascade. As castration was found to affect gene expression, we investigated genes that were similarly affected in all three brain regions to serve as candidate genes for further exploration. There were 145 genes that overlapped between all three regions and included a number of factors involved in the MAPK and mTOR/PI3K pathways, such as Akt, ERK, and eIF4B, as well as a number of glutamate receptors, ATM, Rac, RhoB, and CREB. Analysis is still underway to determine the full effects of VTX to compensate for or reverse changes in mechanisms disrupted by ADT, including alterations in gene expression which were implicated in plasticity-related processes. In conclusion, VTX may be a potential therapeutic option for patients that experience cognitive changes with ADT, as it is already FDA-approved for depression and is relatively well-tolerated.

Disclosures: **A. Vaiana:** None. **A. Dornbusch:** None. **R. Fernandez:** None. **J. Gelfond:** None. **T. Johnson-Pais:** None. **R. Leach:** None. **C. Ramamurthy:** None. **I. Thompson:** None. **D.A. Morilak:** None.

Digital Abstract Session

P339. Consolidation and Reconsolidation: Physiology and Mechanisms

Program #/Poster #: P339.01

Topic: H.07. Long-Term Memory

Support: NSERC

Title: Changes in levels of NMDA receptor subunits in perirhinal cortex relate to their dynamic roles in object memory destabilization and reconsolidation.

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Abstract: The storage of long-term memory is dynamic. This is reflected by the fact that the presentation of reminder cues can destabilize consolidated memories, rendering them modifiable before they return to a stable state through the protein-synthesis dependent process of reconsolidation. However, not all memories destabilize following reactivation. Older and stronger memories resist this process and require the presentation of reminders along with salient novel information in order to destabilize. We have previously demonstrated in rats that novelty-induced object memory destabilization requires acetylcholine (ACh) activity at the M_1 receptor. Other research predominantly focuses on glutamate, which modulates both fear memory destabilization and reconsolidation, through GluN2B- and GluN2A-containing NMDARs, respectively. In the current study, we demonstrate the same dissociable role of GluN2B/N2A-containing NMDARs in perirhinal cortex (PRh) for object memory destabilization and

reconsolidation when boundary conditions are absent. However, neither GluN2 receptor subunit was required for novelty-induced destabilization of remote, resistant object memories. Furthermore, GluN2B and GluN2A subunits were upregulated in PRh 24h following learning, but returned to baseline by 48h, suggesting that NMDARs, unlike muscarinic receptors, have only a temporary role in object memory destabilization. Indeed, activation of M_1-receptors in PRh at the time of reactivation effectively destabilized remote memories despite inhibition of GluN2B-containing NMDARs. These findings suggest that cholinergic activity at M_1 receptors can overcome boundary conditions to destabilize resistant memories when other established mechanisms are insufficient.

Disclosures: C. Wideman: None. **J. Nguyen:** None. **S. Jeffries:** None. **B.D. Winters:** None.

Digital Abstract Session

P339. Consolidation and Reconsolidation: Physiology and Mechanisms

Program #/Poster #: P339.02

Topic: H.07. Long-Term Memory

Support: NSERC

Title: Reactivation-based object memory updating in rodents is reliant on M1 muscarinic acetylcholine receptor signaling in perirhinal cortex

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Abstract: Previously consolidated memory traces can be reactivated with reminder cues, and this reactivation (RA) can destabilize memories for modification. Reactivated memory traces can be “erased” or strengthened, but little is known about mechanisms facilitating constructive memory changes. Our recent work has implicated an M1 muscarinic ACh receptor (mAChR) cascade in object memory destabilization; here, we assessed whether this cascade similarly gates RA-based object memory updating. To test this, we developed the post-RA object memory modification (PROMM) task for rodents. In this task, animals study two identical copies of an object, then 24h later receive a brief re-exposure to the same objects. Immediately post-RA, the rodent is placed into a separate empty context to explore for 5min. In the test phase, another 24h later, the rodent explores the objects in either the same alternate context that was seen post-RA, or a different alternate context. We found that male rats and mice tend to explore the objects less in the ‘same alternate context’ condition compared to the ‘different alternate context’ condition. This suggests that the object-context combination in the ‘same alternate context’ condition seems more familiar, despite the fact that the objects have never been directly explored in that context before the test phase. Thus, context information introduced post-RA appears to incorporate into the original object memory. Subsequently, we found that pre-RA blockade of M1 mAChRs, their downstream signaling molecules, or proteasome functioning within perirhinal cortex prevented

this behavioural effect in male rats, supporting our hypothesis that M1 mAChR functioning is critical for RA-induced object memory updating. Furthermore, young male mice displayed the object memory updating effect, but aged male mice were impaired. Age-related deficits in the PROMM task are consistent with the notion that M1 mAChR functioning is critical for object memory updating because cholinergic dysfunction and cognitive inflexibility are characteristic of the aging brain. This research therefore has implications for understanding and treating disorders characterized by extreme cognitive inflexibility, such as PTSD, phobias, and dementia.

Disclosures: K. Jardine: None. **C. Wideman:** None. **C. Sgarbossa:** None. **C. MacGregor:** None. **S. McGraw:** None. **D. Orr:** None. **K. Mitchnick:** None. **B.D. Winters:** None.

Digital Abstract Session

P339. Consolidation and Reconsolidation: Physiology and Mechanisms

Program #/Poster #: P339.03

Topic: H.07. Long-Term Memory

Support: NSERC Discovery Grant
NSERC CGSD
OGS

Title: Unlocking persistent memories: Hippocampal M1 muscarinic cholinergic receptor activation drives destabilization of strongly encoded object location memories

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Abstract: Following exposure to cues associated with the original learning experience, previously consolidated memories can become destabilized, rendering them vulnerable to disruption, strengthening or other modifications. This necessitates a protein-synthesis dependent process of reconsolidation in order to re-stabilize the memory trace to ensure its survival within long-term memory. Stronger or older memories are destabilization-resistant, but destabilization can be promoted by exposure to contextual novelty during memory reactivation. Our recent work has demonstrated that acetylcholine acting at muscarinic cholinergic receptors (mAChRs) within perirhinal cortex, a brain region important for object memory storage, is critical for both the destabilization of weaker object memories, as well as novelty-induced destabilization of strongly encoded object memories. We hypothesized that this mechanism is not specific to perirhinal cortex and would generalize to the dorsal hippocampus (dHPC), a brain region that is important for the storage of spatial memories. Using the object location (OL) task, which is dHPC dependent, we conducted six within-subject experiments in male Long-Evans rats showing that 1) protein synthesis is required within the dHPC for reactivation-dependent reconsolidation of OL memories; 2) strongly encoded OL memories require exposure to novelty during memory reactivation to promote destabilization; 3) dHPC mAChR activation is required for standard and

novelty-induced OL memory destabilization; and 4) activation of M1 mAChRs within the dHPC promotes destabilization of strongly encoded OL memories, even without the presence of explicit contextual novelty during memory reactivation. These findings suggest that this mAChR-dependent process could be a general neurobiological mechanism for memory destabilization, and suggests implications for the understanding and treatment of disorders characterized by maladaptive strongly encoded memories such as those that drive post-traumatic stress disorder and phobias. This also suggests implications for the understanding of memory flexibility deficits in populations with cholinergic deficiencies, such as the aging population and those with Alzheimer's Disease. Supported by NSERC Discovery Grant, NSERC CGSD and OGS.

Disclosures: A.E. Huff: None. **S.D. McGraw:** None. **W.S. Messer:** None. **B.D. Winters:** None.

Digital Abstract Session

P339. Consolidation and Reconsolidation: Physiology and Mechanisms

Program #/Poster #: P339.04

Topic: H.07. Long-Term Memory

Support: NSERC

Title: Muscarinic cholinergic receptor antagonism blocks reconsolidation-based object memory updating in mice

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Abstract: For behavior to remain adaptive in changing environments, memories require updating to incorporate new relevant information. The inability to update memories likely underlies an array of psychiatric disorders and the cognitive impairments seen in aging and Alzheimer's Disease (AD). Memory updating occurs during a labile window after a consolidated memory trace has been reactivated and destabilized by presentation of a reminder. Novel information presented during the labile period can be integrated with the existing memory, which is then reconsolidated into long term memory. While much work has focused on memory weakening or strengthening during the reconsolidation process, the mechanisms driving destabilization preceding integrative memory updating remain unclear. We hypothesize that memory destabilization is mediated by muscarinic acetylcholine receptor (mAChR) activity. To test this hypothesis, we developed a novel object memory updating task for rodents. The post-reactivation object memory modification (PROMM) task exposes mice to novel contextual information immediately after a previously consolidated object memory is reactivated. Total object exploration is measured on test day in the same or different alternate context seen post-reactivation. Consistent with our previous findings with rats, young adult male mice tested in the same alternate context displayed decreased object exploration levels compared to those tested in

the different alternate context. Based on innate rodent preference for novelty, this decreased exploration suggests that the object-context configuration is familiar due to the integration of the contextual information presented during the labile period. Next, we antagonized mAChRs with systemic scopolamine (0.4mg/kg, ip) prior to reactivation to assess the role of mAChR activation on post-reactivation information integration. Scopolamine negated the difference between same and different alternate context exploration, suggesting that mAChR blockade prevented the memory destabilization required for updating. These results are consistent with our recent findings using the PROMM task in rats, further supporting a role for mAChRs in integrative memory updating, and could have implications for cognitive impairment in aging and AD, conditions characterized by compromised cholinergic system function.

Disclosures: S.D. McGraw: None. **K.H. Jardine:** None. **B.D. Winters:** None.

Digital Abstract Session

P339. Consolidation and Reconsolidation: Physiology and Mechanisms

Program #/Poster #: P339.05

Topic: H.07. Long-Term Memory

Support: NIH grant AA025380
NIH grant AA021260

Title: A straightforward and sensitive homecage-based novel object recognition task for rodents

Authors: *J. LEASURE[1], J. WOODEN[2], M. SPINETTA[3];
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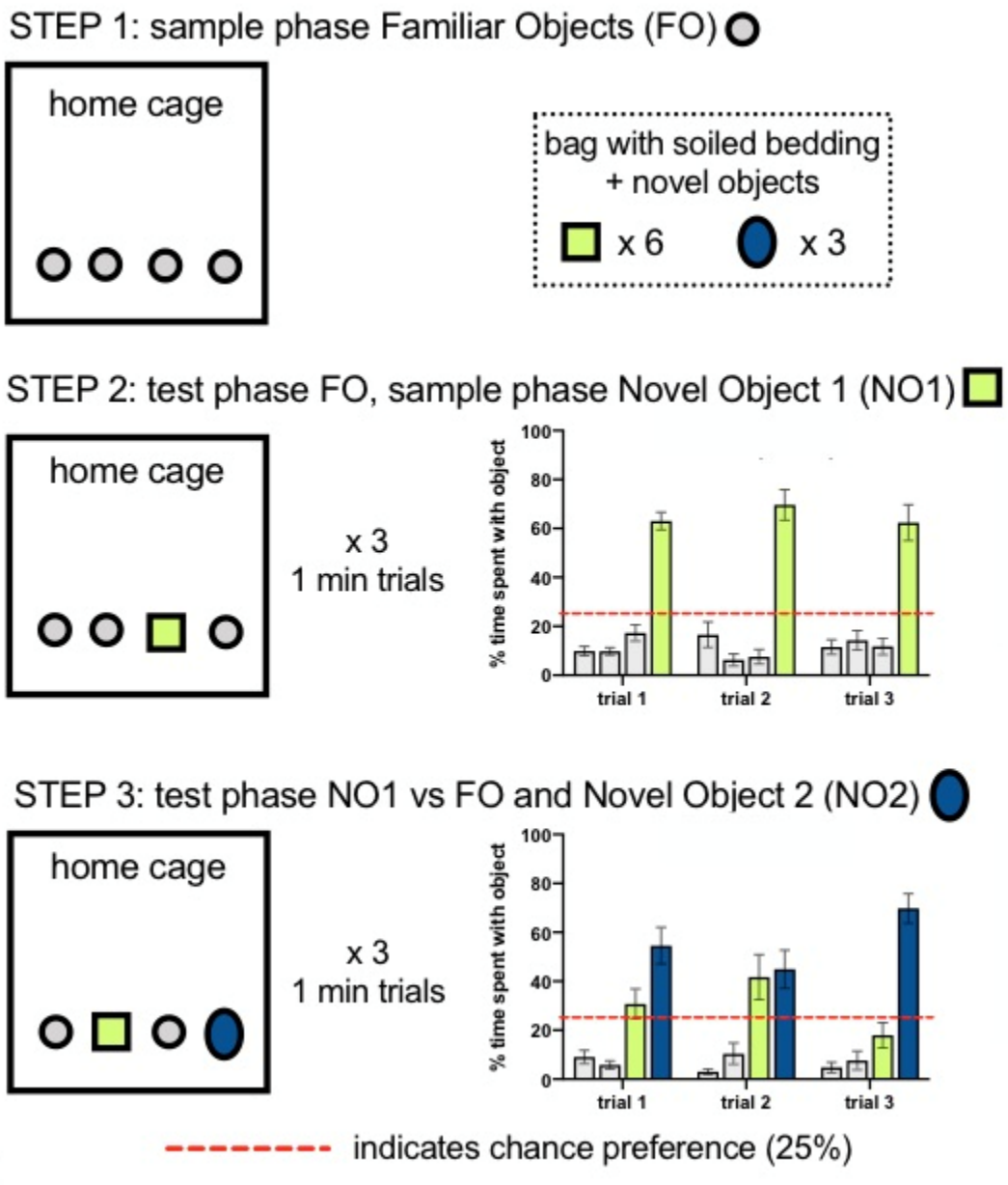
Abstract: The detection of novel objects is a common cognitive test for rodents, but current paradigms have shortcomings that limit their utility, such as low sensitivity, potential for odor confounds and increased stress due to being performed outside of the homecage. We have developed a paradigm that takes place in the homecage to reduce stress and utilizes 4 stimuli per trial, to increase sensitivity. Odor confounds are eliminated because stimuli consist of inexpensive, machined wooden beads purchased in bulk, so each experimental animal has its own set of beads. In Step 1, animals freely explore Familiar Objects (FO) overnight. Novel Objects (NO1 and NO2) are soiled with bedding from the homecage, to acquire odor cues identical to those of the FO. Steps 2 and 3 serve as tests of novelty preference (see graphical representation). Herein we report results of this paradigm from neurologically intact adult rats and mice of both sexes (results shown for rats). In this iteration of the paradigm, delay between steps was 24-hr. Identical procedures were used for both species, except that the stimuli used for the mice were smaller. As expected in Step 2, male and female rats and mice explored NO1 significantly more than FO. Interestingly, in Step 3, rats of both sexes demonstrated a preference for NO2, while this preference for NO2 was seen only in female mice. Male mice preferred both novel stimuli equally over FO. These results indicate robust novelty detection and preference

during Steps 2 and 3 in rats. In mice, this was reliably seen only in Step 2, suggesting that Step 3 is difficult for mice under the given parameters. Thus, it is important to adjust the length of the sampling phase, and the delay between test and sampling phases, to fit the model being tested. In sum, this paradigm is simple to perform, requires no expensive supplies or equipment, is conducted in the homecage (reducing stress), eliminates odor confounds, is sensitive to novelty preference, can be performed in both rats and mice, and is highly flexible, as the delay between steps can be adjusted to tailor task difficulty.



Disclosures: **J. Leasure:** None. **M. Spinetta:** None. **J. Wooden:** None.

Digital Abstract Session

P339. Consolidation and Reconsolidation: Physiology and Mechanisms

Program #/Poster #: P339.06

Topic: H.07. Long-Term Memory

Support: VA BLR&D 5IK2BX004105-02

Title: Maintenance and consolidation of memory using repetitive transcranial magnetic stimulation

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Abstract: Brain stimulation is known to affect canonical pathways and proteins involved in memory. However, literature has shown conflicting results on the ability of brain stimulation to improve to memory due to variation in protocols. Time dependent protein synthesis is important for long term memory consolidation and periodic reactivation of consolidation pathways maintains hippocampus dependent memories. Research has shown the potential to promote increased activation of these pathways after the learning event to maintain consolidation. Therefore, we hypothesized that repetitive transcranial magnetic stimulation (rTMS) given following a learning task and within the time period before retrieval could help consolidate and maintain the memory. We first implanted male C57/Bl6J mice (n=32) with a cranial attachment to secure the rTMS coil so that the mice could be given consistent stimulation to the frontal area whilst freely moving. Mice then underwent object recognition test (ORT) familiarization for ten minutes and then given treatment +3, +24, +48 hours following the test. Treatment consisted of ten minutes 10Hz rTMS stimulation (TMS, n=10) or sham treatment (SHAM, n=11). At +72 hours mice were tested for their exploration of the novel vs familiar object. Only TMS mice distinguished between the two objects, indicated by their greater exploration of the novel object than the SHAM mice. We also included a group of naïve mice which did not do the ORT or receive treatment (NAIVE, n=11). Following the behavior test, the phosphorylation of specific proteins was analyzed through Western Blot. In the hippocampus, TMS mice had significantly greater ERK phosphorylation than NAIVE and TMS also increased from SHAM but statistically significant. Whereas in the frontal cortex there was an overall increase in GLUR1 phosphorylation at S831 with TMS, but contrarily a decrease in synaptic GLUR1 S845 phosphorylation. Using immunohistochemistry, we showed region specific changes to co-localized PSD95 and SV2A staining, indicating active synaptic connections. Overall, in the frontal cortex and entorhinal cortex we saw an increase in synaptic connections in both TMS and SHAM mice compared to NAIVE. Whereas, in the perirhinal cortex and CA1 only the mice which had TMS had increased synaptic connections. We have shown rTMS can promote the consolidation and maintenance of a specific memory if given within the time between encoding and retrieval. This effect was supported by changes to synaptic connections in the perirhinal

cortex and CA1, elevated activation of immediate response proteins and a dynamic shift in activation of receptors relating to synaptic plasticity.

Disclosures: **A.M. Heath:** None. **M. Brewer:** None. **J. Yesavage:** None. **M. McNerney:** None.

Digital Abstract Session

P340. Human Long-Term Memory: Medial Temporal Lobe - Hippocampal and Cortical Circuitry

Program #/Poster #: P340.01

Topic: H.07. Long-Term Memory

Support: ERC-2016-STG-715714 (STREAM)
ERC-2015-647954

Title: Theta-phase separates object features in human hippocampus during an associative memory task

Authors: ***M. TER WAL**[1], J. LINDE DOMINGO[1,2], F. ROUX[1], L. KOLIBIUS[1], S. GOLLWITZER[3], J. LANG[3], H. HAMER[3], D. ROLLINGS[4], V. SAWLANI[4], R. CHELVARAJAH[4], B. STARESINA[1], S. HANSLMAYR[1,5], M. WIMBER[1,5];
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Abstract: Theta oscillations have been shown to clock memory processing in hippocampus. Recent studies using spatial memory tasks have shown that when the task contains opposing alternatives, e.g. left versus right, the hippocampus represents these alternatives at different theta cycles (Kay et al., Cell, 2020) or theta phases (Kunz et al., Sci. Adv., 2019). It is unclear whether this theta-locked separation extends to non-spatial tasks.
Here, we explore the signatures of encoding and retrieval of associations and how these are impacted by the presence of opposing answer alternatives. We recorded intracranial EEG from the hippocampi of 9 epilepsy patients using Behnke-Fried electrodes, while the patients learned and later recalled cue-object associations. The patients were asked to remember two features about each object, namely whether they were photographs or drawings, and animate or inanimate objects. We analysed the representational similarity (RSA) between objects and features using local field potentials (LFPs) and single unit activity from hippocampus.
We report an increased separation during encoding of cue-object association patterns for both LFPs and spikes. The cue-object binding patterns were reinstated after the cue was shown in the retrieval phase of the task. Intriguingly, pattern similarity oscillated with frequencies in the low theta range (1-5 Hz). Opposing object features, e.g. photo versus drawing, were more prominent at opposite phases of this theta rhythm, suggesting task demand influences the representation of

objects in hippocampus. Our data suggest that representations in hippocampus not only reflect perceptual information, but also capture the mnemonic decision.

Disclosures: **M. Ter Wal:** None. **J. Linde Domingo:** None. **F. Roux:** None. **L. Kolibius:** None. **S. Gollwitzer:** None. **J. Lang:** None. **H. Hamer:** None. **D. Rollings:** None. **V. Sawlani:** None. **R. Chelvarajah:** None. **B. Staresina:** None. **S. Hanslmayr:** None. **M. Wimber:** None.

Digital Abstract Session

P340. Human Long-Term Memory: Medial Temporal Lobe - Hippocampal and Cortical Circuitry

Program #/Poster #: P340.02

Topic: H.07. Long-Term Memory

Support: HBP SGA3 945539
HBP SGA2 785907

Title: Preferential functional connectivity of anterior-lateral entorhinal cortex with proximal subiculum and distal CA1 in humans

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Abstract: While a hallmark of hippocampal function is the integration of different types of information, anatomical and functional research in rodents and monkeys indicate segregated functional pathways in the transversal axis of the entorhinal-hippocampal (EC-HC) circuitry. Accordingly, distinct functional subregions within the EC and the subiculum were identified in humans (Maass, Berron, et al., 2015; Navarro Schröder et al., 2015). Here, we replicate and advance these findings in an independent ultra-high resolution 7 Tesla fMRI dataset (N = 32) and provide striking insights into the organizational principles of the human EC-HC circuitry. The results show a preferred functional connectivity of the anterior-lateral EC seed with proximal subiculum and distal CA1 in the HC body. In addition, our explorative investigation of the HC head indicate a highly specific connectivity profile, notably expressing features that resemble ex vivo atlases of the human HC head. The identified functional connectivity profile along the transversal axis of the EC-HC circuitry bridges findings from animal and ex vivo human research towards in vivo fMRI. Clinically, the connectivity preference between distal CA1 and proximal subiculum and anterior-lateral EC is highly relevant as tau pathology can be observed early on in those subregions in Alzheimer's disease. Our findings replicate recent studies and fundamentally advance insight into the architecture of the human EC-HC circuitry that help to understand its central role in memory function and decline.

Disclosures: **X. Grande:** None. **M. Sauvage:** None. **A. Becke:** None. **E. Duezel:** None. **D. Berron:** None.

Digital Abstract Session

P340. Human Long-Term Memory: Medial Temporal Lobe - Hippocampal and Cortical Circuitry

Program #/Poster #: P340.03

Topic: H.07. Long-Term Memory

Support: Max Planck Society
Kavli Foundation
European Research Council: ERC-CoG GEOCOG 724836
Centre of Excellence scheme of the Research Council of Norway – Centre for Neural Computation (223262/F50)
The Egil and Pauline Braathen and Fred Kavli Centre for Cortical Microcircuits, and the National Infrastructure scheme of the Research Council of Norway – NORBRAIN (197467/F50)

Title: Interval-scale sequence memories in the hippocampus generalize temporal structure across sequences

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Abstract: Time is a fundamental dimension along which our experience unfolds. Sequences of events shape episodic memory. The hippocampal-entorhinal region forms cognitive maps of learned relations such as the temporal relationships of events in a sequence. However, it is unclear whether representations of sequence structure reflect the order of events, elapsing time, or whether they are flexibly scaled to experimentally-defined temporal reference frames. Furthermore, how the formation of specific sequence memories relates to the extraction of regularities from sequences with shared temporal structure remains elusive. Here, we combined a sequence learning task with fMRI. Participants encountered four event sequences and inferred the times of individual events based on infrequent umaskings of a hidden clock. Importantly, we manipulated the clock's speed between sequences to partially dissociate event times from sequence order and objectively elapsing time. Memory tests revealed that participants successfully learned which events belonged to which sequence. They accurately inferred the times of individual events relative to the hidden clock. After learning, multi-voxel pattern similarity in the anterior hippocampus correlated with temporal distances between event pairs. These sequence representations reflected temporal relations relative to the hidden clock beyond event order and elapsed time. Our findings further revealed that temporal relations shape representational similarity in the hippocampus differently for event pairs from the same or from

two different sequences. In contrast, the anterior-lateral entorhinal cortex employed a common representational format within and across sequences. Our findings suggest that hippocampal sequence representations can be scaled to incorporate interval-level knowledge of temporal relations that does not exclusively depend on order and passively elapsing time. Consistent with the cognitive mapping of structural knowledge, our findings demonstrate that temporal structure organizes memories across multiple sequences.

Disclosures: J.L.S. Bellmund: None. **L. Deuker:** None. **N.D. Montijn:** None. **C.F. Doeller:** None.

Digital Abstract Session

P340. Human Long-Term Memory: Medial Temporal Lobe - Hippocampal and Cortical Circuitry

Program #/Poster #: P340.04

Topic: H.07. Long-Term Memory

Support: ERC Consolidator Grant 647954
ESRC Grant ES/R010072/1

Title: Unifying STDP and theta-phase-dependent synaptic plasticity in a computational model to explain theta-dependent memory effects in humans and animals

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Abstract: Long-lasting synaptic modifications found in the hippocampus make the region ideal to support binding rich multisensory information into unitary episodes. Rodent studies suggest that the timing of spikes relative to ongoing hippocampal theta activity determines whether potentiation or depression of synapses arise. Such changes also depend on the timing between spikes of pre- and post-synaptic neurons. This form of synaptic plasticity, known as spike-timing-dependent plasticity (STDP), together with theta-phase-dependent learning, has inspired several computational models of learning and memory. However, evidence to elucidate how these mechanisms directly link to human episodic memory is lacking. We build on a previous model, the Sync/deSync model, to explore the interaction between these mechanisms and their impact on episodic memory. As such, we modulate the two components of STDP, long-term potentiation (LTP) and long-term depression (LTD), by opposing phases of a simulated theta rhythm. With the addition of hetero-synaptic plasticity on non-stimulated pathways, alongside thresholds for potentiation and depression, we are able to reproduce findings from a popular hippocampal cell culture study. In this study, synaptic potentiation and depression were observed to occur during burst-like activity in opposing phases of a theta rhythm. Further, we successfully simulate several results obtained in humans, which used a novel multisensory entrainment

paradigm to provide causal evidence that relative timing between stimulus inputs and theta activity are critical determinants of human episodic memory. Two stimulus inputs were modulated by cosine waves with synchronous and asynchronous phase offset conditions. Our model successfully replicates the key empirical findings in that learning for the synchronous condition was more efficient than for the asynchronous conditions. This learning advantage was specific to theta modulated stimulus inputs and was not observed for alpha or delta modulated stimulus inputs. Interestingly, this learning advantage is particularly reliant on the presence of theta-modulated LTD, indicating the importance of phase-dependent synaptic depression in increasing the specificity of encoded episodic memories. Moreover, simulations with and without each mechanism suggest that both STDP and theta-phase-dependent LTP and LTD are necessary to replicate the findings from these human episodic memory experiments. Together, the results indicate a functional role of the circuit-level mechanisms in human memory, which bridges the gap between slice preparation studies to human memory dependent behaviour.

Disclosures: D. Wang: None. **G. Parish:** None. **K.L. Shapiro:** None. **S. Hanslmayr:** None.

Digital Abstract Session

P340. Human Long-Term Memory: Medial Temporal Lobe - Hippocampal and Cortical Circuitry

Program #/Poster #: P340.05

Topic: H.07. Long-Term Memory

Support: Medical Research Council UK
National Institute of Mental Health, Besthesda, USA
University College London Child Health Research Trust

Title: Increased atrophy of hippocampal subregions is associated with improved recall and visuospatial perception in developmental amnesia

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Abstract: BACKGROUND: Early-life episodes of hypoxia-ischemia can damage the hippocampus and lead to developmental amnesia (DA). Patients with DA exhibit severe episodic memory impairment while their semantic memory is largely preserved. It is not clear however whether the residual hippocampus plays a role in encoding and/or retrieval of new information, or the surrounding neocortical areas compensate to rescue hippocampal function after early injury.

AIMS: We aimed to investigate anatomo-functional correlations between cognitive performance and the volumes of residual hippocampal subregions in patients with DA. We also aimed to evaluate the structural integrity of the parahippocampal gyrus in these patients.
METHODS: We used manual segmentation on MRI acquisitions to estimate the volume of three hippocampal subregions: uncus, CA-DG (including CA fields and dentate gyrus) and subicular complex; and the volume of three surrounding cortical areas: entorhinal, perirhinal and parahippocampal cortices, in an exceptionally large cohort of 21 patients with DA and 26 controls of comparable age. The groups were assessed on: i) the Four Mountains Test, which provides measures of spatial perception and immediate spatial memory, and ii) the Doors and People Test, which provides equated measures of recognition and recall.
RESULTS: In patients with DA, the level of atrophy was greater in the CA-DG than in the Subicular complex and greater in the Subicular complex than in the Uncus which showed only mild atrophy in most of the patients. Patients' spatial perception and spatial memory performance correlated negatively with the volume of the Subicular complex. Also, patients' recall, but not recognition memory performance, correlated negatively with the volume of the Uncus. The entorhinal, perirhinal and parahippocampal cortices did not show evidence of volume reduction in patients with DA. Also, we did not observe correlations between the volume of the rhinal cortices and patients' performance.
CONCLUSIONS: In patients with DA, cognitive processing is compromised as a function of extent of atrophy in hippocampal subregions, such that the greater the damage, the more likely that preserved surrounding cortical areas will be recruited to rescue the putative functions of the damaged subregions. This causes the paradoxical finding that greater atrophy in specific hippocampal subregions leads to improved task-dependent mnemonic or spatial function.

Disclosures: L.J. Chareyron: None. **W.K.K. Chong:** None. **M. Mishkin:** None. **N. Burgess:** None. **R.C. Saunders:** None. **F. Vargha-Khadem:** None.

Digital Abstract Session

P340. Human Long-Term Memory: Medial Temporal Lobe - Hippocampal and Cortical Circuitry

Program #/Poster #: P340.06

Topic: H.07. Long-Term Memory

Support: ERC CoG 647954
ESRC Grant ES/R010072/1

Title: Theta and gamma oscillations facilitate neural signal transmission in the service of human memory formation

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Abstract: Episodic memory relies on the efficient encoding of memory associations, which are often complex in nature. Animal research suggests that brain oscillations in the theta and gamma frequency range in the hippocampus regulate memory encoding via synaptic plasticity mechanisms, which reflect fine-grained timing differences in firing between neurons. One such mechanism is spike timing dependent plasticity (STDP), which states that the time delay between the firing of pairs of cells dictates the change in synaptic connection (i.e. shorter latencies resulting in stronger weight changes). We explored this question by recording single and multi-unit activity alongside local field potentials in the human medial temporal lobe during an associative episodic memory task. Results show that the complete encoding of memory associations correlates with increased spike-field coherence in a fast gamma (65-70 Hz) and a fast theta (8-10 Hz) frequency band. Conversely, synchronization to slower gamma and theta oscillations resulted in an incomplete or no memory. Importantly, memory formation correlated with a short latency (~20 ms) of co-incident spikes between pairs of theta/gamma coupled neurons. A computational model exploring the consequences of spike-field coupling in theta/gamma oscillations demonstrates that synchronization to fast gamma/theta oscillations efficiently encodes memory associations via STDP. These results demonstrate that theta and gamma oscillations play a crucial role in memory via establishing efficient neuron-to-neuron communication. The difference in gamma and theta frequencies, and the time windows of co-firing are consistent with a well-known synaptic plasticity mechanism (STDP) and implicate this mechanism for the first time in humans during an episodic memory task.

Disclosures: F. Roux: None. **G. Parish:** None. **M. Self:** None. **D. Rollings:** None. **R. Chelvarajah:** None. **V. Sawlani:** None. **M. ter Wal:** None. **H. Hamer:** None. **S. Gollwitzer:** None. **G. Kreiselmeyer:** None. **P. Roelfsema:** None. **M. Wimber:** None. **B. Staresina:** None. **S. Hanslmayr:** None.

Digital Abstract Session

P340. Human Long-Term Memory: Medial Temporal Lobe - Hippocampal and Cortical Circuitry

Program #/Poster #: P340.07

Topic: H.07. Long-Term Memory

Support: ERC CoG 647954

Title: Exploration of human cortico-hippocampal communication in humans through intracranial single pulse stimulation

Authors: *M. VAN DER PLAS[1], F. ROUX[2], R. CHELVARAJAH[3], D. ROLLINGS[3], V. SAWLANI[3], G. PARISH[2], L. KOLIBIUS[1], M. TER WAL[2], B. STARESINA[2], M. WIMBER[1], S.

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Abstract: The hippocampus (HIPP) is tightly connected to other areas within the brain. It's connectivity to neocortical structures (NC) has been deemed important for a wide variety of cognitive functions, such as the formation and retrieval of episodic memories. While there is an abundance of studies investigating both areas individually, the connectivity between the HIPP and the NC is still underexplored. Most research into the HIPP and its connectivity to NC is observational/correlational, drawing only indirect links between the areas. Furthermore, the majority of the research is based on animal models, due to the relative inaccessibility of the HIPP. How the properties determined in these animal models carry over to humans is therefore still an open question. In this exploratory study, epileptic patients (N=6) under clinical observation, were implanted with stereotactic 'Behnke-Fried' depth electrodes in the hippocampus, which can record iEEG signal at eight channels along their shafts and are even sensitive enough to detect single and multi-unit spiking activity at the tips. These electrodes allowed us to directly record electrophysiological data from the human HIPP and the NC, while repeatedly and systematically administering single pulse electrical stimulation (SPES) throughout several sites within the HIPP and the NC. By recording data while administering electrical pulses, we were able to causally investigate properties of cortico-hippocampal connectivity. The results show that SPES leads to increased neural activity following a pulse, both locally in the HIPP and in connected NC regions. These pulses allowed us to gauge the cortico-hippocampal conduction delay and compare it to existing animal models as well as correlational findings in humans. Furthermore, we investigated oscillatory patterns elicited by SPES and the role of phase as possible functional signatures in cortico-hippocampal communication. We relate these findings to a computational model describing cortico-hippocampal interactions [1], which ascribes special importance to the relationship for specific frequency bands in the HIPP (Theta power, 4-8 Hz) and NC (alpha/beta power, 8-20 Hz) for episodic memory processes. This study is one of few studies causally investigating the electrophysiological mechanisms underlying cortico-hippocampal communication in humans by directly altering the states of the investigated areas.
[1] Parish, G., Hanslmayr, S., & Bowman, H. (2018). The Sync/deSync Model: How a Synchronized Hippocampus and a Desynchronized Neocortex Code Memories. Journal of Neuroscience, 38(14), 3428-3440.

Disclosures: M. Van Der Plas: None. **F. Roux:** None. **R. Chelvarajah:** None. **D. Rollings:** None. **V. Sawlani:** None. **G. Parish:** None. **L. Kolibius:** None. **M. ter Wal:** None. **B. Staresina:** None. **M. Wimber:** None. **S. Hanslmayr:** None.

Digital Abstract Session

P340. Human Long-Term Memory: Medial Temporal Lobe - Hippocampal and Cortical Circuitry

Program #/Poster #: P340.08

Topic: H.07. Long-Term Memory

Support: PSI2017-84933-P
PSI2017-91955-EXP
Agencia Canaria de Investigación, Innovación y Sociedad de la Información de la Consejería de Economía, Industria, Comercio y Conocimiento.
Fondo Social Europeo (FSE) Programa Operativo Integrado de Canarias 2014-2020, Eje 3 Tema Prioritario 74 (85%).

Title: Connectivity in the hippocampal subfields during resting-state fMRI

Authors: *L. EZAMA[1,2,3], J. A. HERNÁNDEZ-CABRERA[1,3,4], S. L. SEOANE[1,2,3], E. PEREDA[5,3], N. JANSSEN[1,2,3];
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Abstract: The hippocampus is a central brain structure that plays a role in many cognitive functions as well in various neurological diseases. Neuro-imaging studies have revealed that when a person is at rest, different sets of brain regions become activated at different moments in time. The relationship between the hippocampus and these so-called resting-state networks remains poorly understood. Previous studies in resting-state functional magnetic resonance imaging (rs-fMRI) have revealed that the hippocampus participates in a resting state network called the default mode network (DMN). However, it is still not known: 1) What section of the hippocampus connects to the DMN, 2) whether the hippocampus also connects to other types of resting-state networks, and 3) how the different subfields of the hippocampus participate in the resting state networks. Here we examined these issues using the high spatial-resolution rs-fMRI 7T dataset from the Human Connectome Project (HCP). Specifically, we analyzed the functional connectivity of the hippocampus and its subfields with the rest of the brain by means of data-driven techniques that relied on spatially restricted Independent Component Analysis (srICA). The srICA technique was used to observe hippocampal activation areas and to determine clusters of voxels within the hippocampus that become activated during the resting-state. In addition, connectivity between these hotspots and the rest of the brain was derived using the Dual Regression technique. Group-based analyses relied on linear mixed-effect group-analyses that took into account participant-specific brain morphology. Consistent with other studies, our results revealed two main activity hotspots inside the hippocampus. The first hotspot was placed in the anterior part of the hippocampus, and it was correlated with the somatomotor network. The second hotspot of activity was located from middle to posterior sections along the hippocampal long-axis, and was correlated with the DMN. In addition, we found that whereas the anterior/somatomotor network relied on activity in the CA4, DG, CA1 and CA3 subfields (and less on the subiculum), the middle-posterior/DMN network relied on activity in the CA4, DG and subiculum subfields (and less on CA1 and CA3). These results therefore clarify the precise section of the hippocampus that participates in the DMN, that the hippocampus participates in at

least two different resting-state networks, and that there are dissociations in connectivity between the CA1 and CA3 subfields on the one hand, and the subiculum on the other. Overall these results further our understanding of hippocampal functional connectivity.

Disclosures: L. Ezama: None. **J.A. Hernández-Cabrera:** None. **S.L. Seoane:** None. **E. Pereda:** None. **N. Janssen:** None.

Digital Abstract Session

P340. Human Long-Term Memory: Medial Temporal Lobe - Hippocampal and Cortical Circuitry

Program #/Poster #: P340.09

Topic: H.07. Long-Term Memory

Support: NIH Grant R01MH107512
NIH Grant K99NS115918
NIH Grant R01NS64033

Title: Frontotemporal theta multiplexing underlies memory formation in the developing brain

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Abstract: Neuronal oscillations are ubiquitous and associated with processes ranging from physiological to cognitive to pathological. These periodic rhythms change considerably during development. However, typical scalp EEG does not access medial brain areas directly and offers low spatial resolution of lateral areas it does access. Noninvasive EEG therefore offers a limited window into the development of cognitive processes such as declarative memory which rely on the medial temporal lobe (MTL) and its connections to specific lateral prefrontal (PFC) areas. To circumvent this issue, we capitalized on a unique opportunity to record EEG directly from the brains of pediatric neurosurgical patients (i.e., ECoG). Twenty-one children and adolescents (5.9-20.5 years) attended to visual scenes in preparation for a memory recognition test. We analyzed theta rhythms, which are consistently linked to memory processes in the MTL of adults as well as animal models, in 80 MTL channels and 357 PFC channels. After disentangling oscillatory components from aperiodic 1/f activity, we detected a faster rhythm ~7 Hz which sped up with age and a slower rhythm ~3 Hz which slowed down with age. These results reveal that: (1) recent findings of fast and slow theta rhythms in the adult MTL extend to children; (2) both rhythms are also evident in PFC; and (3) development is associated with the functional differentiation of theta oscillations. Further analyses showed that both theta oscillations support MTL-PFC functional connectivity patterns predictive of subsequent memory formation. Specifically, fast theta supported inter-regional phase coupling and slow theta supported inter-regional amplitude coupling, demonstrating that MTL and PFC interact via distinct oscillatory mechanisms during successful memory formation. These results link the functional differentiation of theta oscillations to the development of declarative memory and suggest that

multiplexed interactions between MTL and PFC underlie memory formation in the developing brain.

Disclosures: **E.L. Johnson:** None. **Q. Yin:** None. **L. Tang:** None. **E. Asano:** None. **N. Ofen:** None.

Digital Abstract Session

P340. Human Long-Term Memory: Medial Temporal Lobe - Hippocampal and Cortical Circuitry

Program #/Poster #: P340.10

Topic: H.07. Long-Term Memory

Support: NINDS Intramural Funding
NINDS F31 NS113400

Title: Spiking sequences as a general organizational principle for neural activity in the human cortex

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Abstract: The temporal dynamics of spiking activity in cortical networks are of great importance in understanding behavior, cognition, and disease states. An organizational motif, the spiking sequence, has recently been shown to be associated with memory formation and retrieval in the human cortex. However, it remains unknown how sequences may manifest in other behavioral scenarios and if spiking sequences are a general organizational principle for network level activity in the human brain. We recorded intracranial micro-LFP and single unit data in 6 participants with medically refractory epilepsy during monitoring for potential resective surgery. To accomplish this, we implanted Utah micro-electrode arrays under the subdural macro-iEEG contacts in the anterior temporal lobe of each patient. In order to examine the presence of spiking sequences across many behavioral states, we examined spiking activity from the participants during rest, memory formation and retrieval, and during distractor periods. We found that spiking sequences are present across all epochs of our data. In fact, we were able to extract an average 'backbone' sequence that was loosely adhered to across all behavioral states, and individual time periods exhibited variation around this sequence. To explicitly demonstrate the temporal and behavioral invariance of this pattern of activity, we show that the average sequence from the 5 minute resting period persists through memory tasks, distractor periods, and subsequent rest periods for up to an hour later. Our findings demonstrate that spiking sequences are a general motif for the organization of spiking activity in the human cortex. In broader terms, the existence of an average 'backbone' spiking sequence likely indicates a constrained set of neural dynamics that arise inherently from the network level synaptic structure of a given cortical column.

Disclosures: **A.P. Vaz:** None. **J.H. Wittig:** None. **S.I. Inati:** None. **K.A. Zaghloul:** None.

Digital Abstract Session

P341. Human Studies of Memory

Program #/Poster #: P341.01

Topic: H.04. Executive Functions

Support: LMU Faculty Research Grants

Title: Localized gamma EEG activity differences during cognitive testing in young adults

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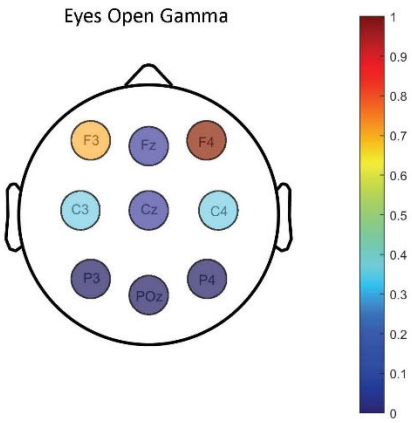
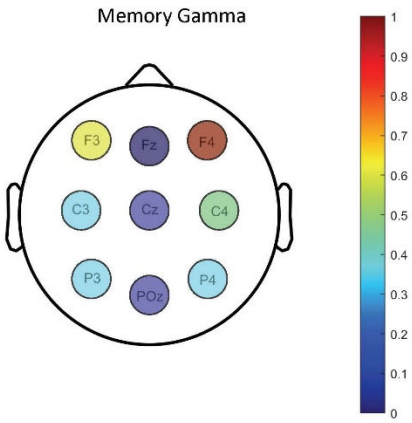
Abstract: Electroencephalograph (EEG) shows distinct patterns of activity in engaged neural networks, and may be a useful clinical biomarker. For example, the power spectral density (PSD) of the gamma frequency band is elevated during learning and working memory, and lower in patients with Alzheimer's disease. While differences in EEG activity at different scalp locations may be clinically important, an understanding of EEG activity in normal populations can also be useful. The purpose of this study was to describe spatial patterns of EEG PSD while normal young adults (n = 29) completed cognitive tests of clinical relevance (National Institutes of Health-Toolbox: NIHTB). Wireless EEG recordings from 9 scalp sensor locations (3 frontal, 3 central, 3 posterior) were collected during an eyes open resting state (RS), and three NIHTB cognitive tests. PSD for delta, theta, alpha, beta and gamma was computed following artifact decontamination and data smoothing. Here, only the statistically significant (p<.05 or less) EEG findings for PSD gamma activity (30-40 Hz) during the NIHTB picture sequence memory (PSM) test, a standardized episodic working memory task, are reported. During RS, gamma PSD was greater at the central right location (C4) compared to the parietal right site (P4) and to Cz. In both the RS and PSM test, gamma PSD at Fz was smaller compared to F3 and F4. During the PSM test, gamma PSD was greater at the three most posterior sites (P3, POz, P4) compared to RS gamma PSD at these sites (see Figure). In summary, in normal young adults, we found a discrete localization of higher gamma PSD during a cognitive test to the more posterior (parietal) scalp locations. Figure Legend: Illustrations of eyes open gamma (RS) and memory gamma (PSM) identify relative gamma PSD standardized for each event across 9 sensory sites, with proportional differences in activity highlighted in the color spectrum (1 refers to highest gamma PSD activity level; 0 to lowest gamma PSD activity level).





Disclosures: **M.R. Foy:** None. **J.G. Foy:** None.

Digital Abstract Session

P341. Human Studies of Memory

Program #/Poster #: P341.02

Topic: H.04. Executive Functions

Support: European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH –CREATE – INNOVATE T1EDK-02890 acronym: e-Prevention

Title: Can a smartwatch help cognitive neuroscience? the relation between physical activity and cognitive performance in healthy young adults.

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Abstract: The association between physiological/physical activity levels and cognitive performance was examined in 23healthy young adults (12 women/11men) with age range between 21-35 years (SD=2,7). Participants carried the Samsung Gear S3 smartwatch with multiple sensors of biometric data for a long period of 3 consecutive months. Cardiovascular activity was measured with photoplethysmogram, physical activity levels were measured with

pedometer, accelerometer and gyroscope and sleep/ wake activity with sleep tracker. Physical activity levels during waking as well as during sleep were assessed using short term energy extracted from the accelerometer and gyroscope and number of steps and distance covered using the pedometer (during waking hours only). Physiological activity was measured with indexes of long-and short term diurnal changes of heart rate variability. Finally an extensive neuropsychological battery was administered covering domains of cognition (speed of processing, working memory, sustained attention, executive function, verbal learning, verbal fluency, vocabulary). Our results showed a specific relation between physical activity measured using the accelerometer short energy during waking hours and performance in Verbal Fluency tests (Category Fluency Test and Controlled Oral Word Association Test COWAT) (General Linear Model GLM, $F_{1,15}$ =9.96, p = 0.006, η^2 = 0.39) . The same correlation was observed by using short term energy extracted from the gyroscope (GLM, $F_{1,15}$ = 8.83, p = 0.009, η^2 = 0.37), total steps using the pedometer (GLM, $F_{1,15}$ = 12.3, p = 0.003, η^2 = 0.44) and distance covered using the pedometer (GLM, $F_{1,15}$ = 12.07, p = 0.003 , η^2 = 0.44). There was a significant correlation of physiological activity measured during waking hours but not during sleep using the low and high frequency power of heart rate variability and working memory (GLM low freq, $F_{1,15}$ = 8 p = 0.012, η^2 = 0.35; high freq. $F_{1,15}$ = 8, p = 0.01, η^2 = 0.35), executive function (GLM low freq. $F_{1,15}$ = 8, p = 0.01 , η^2 =0.36, high freq. , $F_{1,15}$ = 8 p = 0.01, η^2 = 0.36) and verbal fluency (GLM, low freq. $F_{1,15}$ = 5, p = 0.038, η^2 = 0.26; high freq. $F_{1,15}$ = 5, p = 0.038, η^2 = 0.26) cognitive function. These results suggest a common underlying neurophysiological mechanism connecting physical activity and physiological reactivity and cognitive ability, especially the motor component of verbal fluency, namely the oral-motor features of word production.

Disclosures: **M. Lazaridi:** None. **P. Filntisis:** None. **E. Kalisperakis:** None. **A. Zlatintsi:** None. **N. Efthymiou:** None. **P. Maragos:** None. **N. Smyrnis:** None. **T. Karantinos:** None. **A. Mantas:** None.

Digital Abstract Session

P342. MTL Circuits and Functions: Humans

Program #/Poster #: P342.01

Topic: H.08. Learning and Memory

Support: ERC CoG 647954

Title: Index neurons retrieve unique episodic memory events in the human hippocampus

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Abstract: Retrieval of episodic memory hinges on the reinstatement of neural activity at initial encoding in the hippocampus. According to the indexing theory the hippocampus does not store episodic content itself but instead acts as a pointer to the information that is stored in the neocortex [1]. This pointer is thought to be represented by a sparse, distributed assembly of single units.
In search of the index, we leveraged intracranial EEG recordings to investigate the firing pattern of single neurons in the human hippocampus during encoding and retrieval of episodic memories. In total, we recorded 631 putative single neurons during a self-paced free association paradigm.
We computed the firing frequency for every single neuron during each individual trial and then determined the firing reinstatement by multiplying the firing rate for each trial during encoding and retrieval. Using a shuffling procedure, we then calculated the reinstatement expected under the null. In a second level shuffling procedure we showed that a significant number (N = 173) of single units exhibited a memory specific reinstatement of neural firing for individual episodes.
How single neurons are initially allocated as index neurons remains an open question. Here we find that indexed episodes were characterized by a decreased delta power preceding the encoding period. This is in line with literature on engram allocation in animals that suggest higher excitability being the key factor responsible for single neuron recruitment. Another potential mechanism for index formation is ripple activity. Ripples are transient high-frequency oscillations that have been linked to memory formation in humans. Here we showed an increase in both the number and length of hippocampal ripples during the encoding period.
Although proposed over three decades ago, this is the first empirical evidence of index cells in humans. We propose two candidate mechanisms through which single neurons might be allocated as index units: (1) a decrease in pre-stimulus delta activity and (2) an increased ripple activity in the hippocampal local field potential.
[1] Teyler, T. J., & DiScenna, P. (1986). The hippocampal memory indexing theory. Behavioral Neuroscience, 100(2), 147.

Disclosures: L.D. Kolibius: None. **F. Roux:** None. **G. Parish:** None. **M. Ter Wal:** None. **M. Van Der Plas:** None. **R. Chelvarajah:** None. **V. Sawlani:** None. **D. Rollings:** None. **J. Lang:** None. **S. Gollwitzer:** None. **R. Hopfengärtner:** None. **G. Kreiselmeyer:** None. **H. Hamer:** None. **B. Staresina:** None. **M. Wimber:** None. **H. Bowman:** None. **S. Hanslmayr:** None.

Digital Abstract Session

P342. MTL Circuits and Functions: Humans

Program #/Poster #: P342.02

Topic: H.10. Human Learning and Cognition

Support: NIH Grant R01NS084017
MSCA Fellowship 750955

Title: Neurons in the human medial temporal lobe dynamically represent multiple implicit associations between objects in a map-like manner

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Abstract: Events and objects that we encounter often follow certain rules, for example, they occur cyclically, or they are organized into hierarchies. Humans have a remarkable ability to extract these sometimes complex patterns rapidly and implicitly. This type of learning plays an important adaptive role in our everyday life but the underlying brain mechanisms remain largely unknown. Here, we recorded the activity of 1009 neurons from twelve pre-surgical epilepsy patients (8 females; age: 38 ± 15 y. o.) to test the hypothesis that neurons in the human medial temporal lobe (MTL) represent an associative structure among multiple objects in a dynamic and map-like manner. For each participant, we selected six images of people that elicited selective neuronal responses in the preceding screening task and, unbeknownst to the patients, we assigned these images to different locations on an arbitrary associative pyramid graph. During learning (~ 40 min), the order of stimuli presentation was determined by the graph's structure, so that only images that shared a direct link were shown immediately one after another. Before learning (baseline) and after learning (read-out) the sequence of images was fully randomized thus in those sessions no implicit temporal structure was present. In all parts of the study, the participants performed behavioral tasks unrelated to the main research question. We found that over the course of learning, MTL neurons that initially responded to only one preferred image started responding more strongly to images that were directly linked to the preferred image on the pyramid graph. Control analyses showed that this pattern of responses was not present for neurons located outside of the MTL. Furthermore, we were able to reconstruct the structure of the associative pyramid graph from the population activity of MTL neurons. This reconstruction became more accurate with learning and the effect persisted after learning, when no implicit structure was present. Together, these findings demonstrate that the human MTL neurons dynamically change their spiking activity to represent implicit associations between multiple objects in a map-like manner.

Disclosures: P. Tacikowski: None. **G. Kalender:** None. **D. Ciliberti:** None. **I. Fried:** None.

Digital Abstract Session

P342. MTL Circuits and Functions: Humans

Program #/Poster #: P342.03

Topic: G.06. Anxiety Disorders

Support: NIH 1UH3NS107673-01A1

Title: Intracranial Neurophysiology of Valence Encoding in Humans

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Abstract: Critical to the survival of all animals is the detection of appetitive, neutral and aversive stimuli in the environment. Pathological valence processing, particularly altered threat evaluation and response, represents the core etiology of anxiety disorders and post-traumatic stress disorder (PTSD)]. Thus, characterizing the intracranial neural mechanisms of valence attribution is clinically relevant to the development of strategies directly targeting core anxiety-related symptoms. Here, we provide the first characterization of medial temporal lobe iEEG oscillatory dynamics across a range of emotional stimuli (negative/neutral/positive) in humans (8) with and without anxiety-related disorders (PTSD/Generalized Anxiety Disorder (GAD)). Based on prior reports in rodents and humans, we hypothesized that (i) amygdala theta and gamma power will be increased during viewing of aversive stimuli compared to positive and neutral stimuli and (ii) patients with anxiety-related disorders will have reduced oscillatory discrimination between valence categories. Results confirm these hypotheses and highlight a key role of amygdala theta power in tagging negatively charged stimuli.

Disclosures: J. Langevin: None. **J. Gill:** None. **Z. Aghajan:** None. **D. Villaroman:** None. **J. Kao:** None. **A. Bari:** None. **R. Koek:** None. **A. Adhikari:** None. **J. Chen:** None. **S.E. Krahl:** None. **M.S. Fanselow:** None. **N. Suthana:** None.

Digital Abstract Session

P342. MTL Circuits and Functions: Humans

Program #/Poster #: P342.04

Topic: H.08. Learning and Memory

Support: ERC 819814 - RememberEx

Title: Hippocampal theta and gamma oscillations differentially signal surprise and uncertainty

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Abstract: To adaptively encode information into memory, the hippocampus should be able to extract statistical regularities in the environment in order to form predictions about upcoming sensory inputs. Indeed, previous fMRI findings show that the anterior hippocampus tracks the expected uncertainty of an event in a given context, such that it is maximally engaged in highly uncertain conditions (Strange et al., 2005). Here we used intracranial EEG to examine the pre-

and post-stimulus temporal dynamics underlying hippocampal sensitivity to contextual uncertainty (Shannon's entropy) and surprise (a given event's improbability). Ten medication-resistant epilepsy patients, with depth electrodes implanted in the anterior hippocampus, participated in the experiment. On each trial, patients were presented with a coloured shape, and performed a match-to-sample task. Two colours and two shapes were combined to form four possible stimuli per block. In each block, there are likely and unlikely stimuli, varying surprise within-block; the entropy of stimuli varied across blocks (different probability distribution of the four stimuli in each block). Time-frequency analysis of the electrophysiological data utilized a trial-wise GLM approach, with two predictors of interest: entropy and surprise. Mean entropy over blocks was included as a covariate to model non-specific time effects. Cluster-based permutation t-tests were used for statistical inference at the group level. Consistent with previous findings, we found a positive association between surprise and hippocampal theta power (2.5-7.5 Hz) from stimulus onset until 300 ms post-stimulus. We also found a negative association between entropy and gamma power (47.5-82.5 Hz) in the pre-stimulus time window (-980 to -520 ms). Overall, these results suggest hippocampal theta oscillations signal the improbability of a given event, whereas gamma oscillations track anticipation of upcoming stimuli, with decreased synchronization in uncertain contexts.

Disclosures: D. Frank: None. **B. Strange:** None. **J. Sarnthein:** None. **R. Toledano:** None. **A. Gil-Nagel:** None.

Digital Abstract Session

P342. MTL Circuits and Functions: Humans

Program #/Poster #: P342.05

Topic: H.08. Learning and Memory

Support: JSPS KAKENHI Grant 16K18367
JSPS KAKENHI Grant 18K07348
Takeda Science Foundation

Title: Elucidation of functional connectivity patterns of the lateral/medial mammillary body using resting-state fMRI in the human brain

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Abstract: The mammillary body (MB) has been thought to implement mnemonic functions. Although recent animal studies have revealed dissociable roles of the lateral and medial parts of the MB, the dissociable roles of the lateral/medial MB in the human brain is still unclear. Functional connectivity using resting-state functional magnetic resonance imaging (fMRI) provides a unique opportunity to noninvasively inspect the intricate functional organization of the human MB with a high degree of spatial resolution. The present study aimed to reveal the

dissociation between the two systems consisting of the lateral/medial MB and target regions of interest (ROIs) connected with the MB, such as the hippocampus, tegmental nuclei (TN), and anterior thalamus (AT).
Ten right-handed healthy young subjects (six men and four women;mean age, 27.0 ± 7.7 years (mean ± SD), age range, 20–39 years) participated in the experiments. Resting-state fMRI (RSfMRI) data were acquired using multiband gradient-echo echo-planar sequences with a 3-T MRI scanner (Siemens Skyra).To attain a sufficient signal-to-noise ratio in functional images with a higher spatial resolution ($1.25 \times 1.25 \times 1.25$ mm^3), each subject was highly sampled: 1,000 volumes in each of the 10 daily sessions. RSfMRI data were preprocessed using SPM8, and the temporal correlation between time-series signals of MB and those of target ROIs was calculated. After the whole MB was manually delineated using normalized functional images, the whole MB was divided into lateral and medial parts according to the anatomical structure of the past studies. The target ROIs were manually delineated using normalized functional and structural images.
RSfMRI analysis revealed a significant difference of connectivity between medial/lateral MB. The medial MB was functionally connected with the subiculum, ventral TN, and AT. On the other hand, the lateral MB was functionally connected with the pre/parasubiculum and dorsal TN. A three-way analysis of variance (ANOVA) was conducted with the region, lateral/medial MB, and left/right sides as factors; a significant interaction (subiculum or pre/parasubiculum × lateral/medial MB) was observed ($F_{(1,9)} = 27.9$, $P = 0.0005$), and a significant interaction (ventral or dorsal TN× lateral/medial MB) was observed ($F_{(1,9)} = 35.9$, $P = 0.0002$). A two-way ANOVA of AT revealed a significant main effect of the lateral/medial MB ($F_{(1,9)} = 16.3$, $P = 0.003$). The present study provides the human analog of the two extended systems that implement different functions whose underlying biological mechanism is centered in the lateral/medial MBs.

Disclosures: **M. Tanaka:** None. **T. Osada:** None. **A. Ogawa:** None. **S. Han:** None. **K. Kamagata:** None. **S. Aoki:** None. **S. Konishi:** None.

Digital Abstract Session

P342. MTL Circuits and Functions: Humans

Program #/Poster #: P342.06

Topic: H.09. Spatial Navigation

Support: MOP125958

Title: Does pattern separation/ magnitude estimation operate on information recovered from remote long-term memory? Modulation of activation along the long axis of the hippocampus, parahippocampal and retrosplenial cortex during spatial distance and object size judgements.

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Abstract: It has been proposed that pattern separation processes, mediated by the hippocampus (HC), operate on information encoded into memory to produce orthogonal memory representations for stimuli that are similar to one another (overlapping features). *Does pattern separation in HC, especially along its longitudinal axis, operate similarly on both object and spatial information retrieved from remote long-term memory?* To address this question, 24 participants were asked to make both spatial distance judgements among well-known Toronto landmarks and size judgements among well-known animals in the MRI scanner. For the spatial distance task, participants need to decide whether a cue landmark (A) was closer to 1 of 2 target landmarks (B or C). In the animal size task, participants needed to judge whether a cue animal (a) was larger in size than 1 of 2 target animals (b or c). All stimuli were displayed in words. The differential distance or size (i.e., ΔAB-ΔAC or Δab - Δac; Δ: distance/size difference between cue A/a and targets B/b or C/c) was varied from small to large across trials. This allowed us to examine not only the brain activation triggered by spatial distance and object size processing, but also how the activation can be modulated by processing different magnitudes (refineness) of the distance and size difference. We found spatial distance processing engaged HC more strongly than animal size processing. In the spatial task, the posterior HC (pHC) showed stronger activation than the anterior HC (aHC). Importantly, in the animal size processing task, the aHC and pHC activation were independent of the magnitude of the differential size differences (i.e., whether Δab and Δac are similar). However, both aHC and pHC, as well as other spatial processing network regions such as the parahippocampal and retrosplenial cortex, were activated more strongly when more refined differential distances were processed (i.e., when ΔAB is closer to ΔAC). Interestingly, aHC activation showed stronger distance dependence than pHC. We conclude (1) that pattern separation processes operate on information retrieved from remote long -term memory, more so on space than on objects ;(2) that regions of the long-axis of the hippocampus, and spatial network, are modulated differently by judgements of object size and spatial distance.

Disclosures: M. Moscovitch: None. **M. Ziegler:** None. **Z. Liu:** None. **J. Arsenault:** None. **C. Dang:** None. **C. Grady:** None. **S. Rosenbaum:** None.

Digital Abstract Session

P342. MTL Circuits and Functions: Humans

Program #/Poster #: P342.07

Topic: H.06. Social Cognition

Support: NIH Grant R01MH110483

Title: Sub-acute differences in right inferior frontal gyrus activation following a mild traumatic brain injury and correlations with sleep quality

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Abstract: Sub-acute differences in right inferior frontal gyrus activation following a mild traumatic brain injury and correlations with sleep quality Sleep deficits after mild Traumatic Brain Injury (mTBI) are common, but the underlying neurobiological mechanisms are still unclear. Increasingly, functional Magnetic Resonance Imaging (fMRI) studies suggest that mTBImayalterthe activation of brain regions associated withattention and emotionregulation. However,the relationship betweensuch changesand sleep impairments after mTBI has not been studied.To explorethat relationship, subjects (n=90, female=50)were recruited from Emergency Departments (EDs) after experiencing traumas such as motor vehicle accidents, physical assaults, or sexual assaults. Theywere assigned to either the mTBI group (n=22) or non-mTBIgroup (n=68) based on the American Congress of Rehabilitation mTBI diagnostic criteria.Not all subjects experienced a head injury.Approximately two weeks after their traumas, subjectswere administered the Pittsburg Sleep Quality Index (PSQI) questionnaire and completed the Shifted-attention Emotion Appraisal Task (SEAT) during fMRI scanning. For SEAT, subjects viewed fearful or neutral faces superimposed on indoor or outdoor scenesin three sub-tasks designed to assessimplicit emotion processing (identifying male/female faces), attentionshifting (identifying indoor/outdoor scenes), and appraisal (like/dislike the faces).The non-mTBI group had significantly greater activation associated with the appraisal of fearful and neutral faces in the right inferior frontal gyrus (rIFG;peak voxel: x=50, y=34, z=0, Z-max=4.33;cluster size=553 voxels; FWE corrected in FSL). The mean cortical activation of the rIFGcluster negatively correlated with the PSQI (r(19)=0.338, p=0.134)in the non-mTBI group, suggesting that greater activation of that region was associated with lower PSQI scores, or better sleep quality. In contrast, for the mTBI group, the correlation between rIFG activation and the PSQI was not statistically significant (r(61)=-0.265, p=0.036).The PSQI scores also did not differ significantly between the two groups (t(82)=-0.771, p=0.443). These findings suggest that mTBI may alter rIFG activation associated with implicit emotion modulation during the appraisal of fearful and neutral faces. Moreover, the fact that rIFG activation only correlated with better sleep quality in trauma survivors without mTBI suggests that mTBI may disrupt the association between the two.

Disclosures: O.Y. Raevskaya: None. **A.S. Cotton:** None. **J. Mathew:** None. **R. Boddapati:** None. **A. Grau:** None. **E. Gibson:** None. **S. Grider:** None. **C. Shih:** None. **T. Lewis:** None. **H. Xie:** None. **X. Wang:** None.

Digital Abstract Session

P343. Prefrontal Circuit Mechanisms

Program #/Poster #: P343.01

Topic: H.04. Executive Functions

Support: NIMH (R00MH115082)

Whitehall Foundation

Title: Frequency-specific driving of frontal cortical feedback differentially engages V1 microcircuits.

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Abstract: Temporal synchronization among distributed regions and cell types in the cortex tends to be rhythmic or oscillatory in nature. Given the diverse intrinsic properties of individual neurons and synapses, distinct neuro-oscillatory frequency bands may serve to selectively route information processing in cortical networks. That is, in cortical circuits, it may matter not only which brain regions innervate each other, but in what frequency this innervation occurs at a given time. Feedback inputs from higher level brain regions are known to modulate sensory processing in lower sensory regions in accord with context and behavioral goals. Observational work during a visual attention task suggests that this feedback may typically occupy the beta frequency range (20Hz), but it is unclear whether activity of these circuits in other frequencies (alpha or gamma) may have functional relevance. Further, given the distinct intrinsic firing rates and synaptic properties of local interneuron populations, top-down feedback may serve to differentially engage these cells depending on the frequency of activation. We aimed to test whether frequency-specific activation of prefrontal cortical (PFC) feedback inputs leads to neuron-type specific activation in primary visual cortex (V1). Here, we used simultaneous wide-field optogenetics (AAV1-viral expression of ChR2 in PFC) and fast two-photon microscopy (GCaMP6s; 28-Hz) to activate PFC axonal segments in V1 (i.e. feedback inputs) at specific frequencies (0-40Hz) while recording activation of V1 cells of awake mice (n=8) at rest. We focused on genetically targeted cell types, such as somatostatin (SST+) interneurons and pyramidal (VGlut+; PYR) cells, in layers 2-4 of cortex. Interestingly, SSTs were strongly activated when PFC inputs were driven at gamma range (40Hz), while decreasing its activation when PFC inputs were probed at theta (6Hz) through beta (20Hz). Notably, there were a subset of SST cells that did not follow these trends, suggesting variability within the SST class of interneurons. Alternatively, we found that PYRs increased their activation when PFC inputs were driven at frequencies from delta (2Hz) to beta (20Hz). This study demonstrates that the microcircuit dynamics of V1 is determined by the oscillatory profile of its feedback connections, and the cells it innervate. Given that SST interneurons and PYR cells showed a unique preferred activation bandwidth, our results indicate a frequency-dependence in the ability of PFC to route information across regions. Future work is needed to understand the effects of frequency-specific feedback input during task-relevant processing and behavior.

Disclosures: G. Bastos: None. **C.G. Gallimore:** None. **J.P. Hamm:** None.

Digital Abstract Session

P343. Prefrontal Circuit Mechanisms

Program #/Poster #: P343.02

Topic: H.04. Executive Functions

Title: Synchronous oscillatory neural ensembles flexibly encode inference of abstract rules in prefrontal and posterior parietal cortices

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Abstract: To act intelligently in a situation, we must learn what rules to follow for that context. Then, as the situation changes, we must learn new rules. To understand the neural mechanisms of learning rules, we trained monkeys on a task that required them to learn a new attentional 'rule' on each block of trials. On each trial, monkeys chose between three random colors. They were rewarded as a function of how close their chosen color was to the current 'template color'. Importantly, the animals were not instructed as to the template color. Instead, they had to learn it through trial and error. Once the animals learned the template color, it changed abruptly (and without cuing), requiring the animal to repeatedly learn new attentional templates throughout the day. Recordings in prefrontal and parietal cortex found oscillatory synchronization of local field potentials (LFPs) between frontal and parietal areas change with learning. Immediately after a switch, there was a sharp decrease in beta-band synchrony between prefrontal and parietal cortex. This was followed by an increase in beta-band activity, as the animal discovered the new rule. These results are consistent with beta-band oscillations carrying top-down attentional signals. In contrast, synchrony in the gamma band had a sharp increase immediately after the switch, suggesting an increase in bottom-up drive. Our results build on previous work showing oscillations support rule representations and argue that these representations can develop during learning.

Disclosures: S. Tafazoli: None. **C.I. Jahn:** None. **N.T. Markov:** None. **C.J. MacDowell:** None. **T.J. Buschman:** None.

Digital Abstract Session

P343. Prefrontal Circuit Mechanisms

Program #/Poster #: P343.03

Topic: H.04. Executive Functions

Support: NICDS Grant P01HD080679

Title: A neural circuit mediating rat category learning

Authors: *M. B. BROSCHARD[1], J. KIM[1], B. C. LOVE[2], J. H. FREEMAN[1];
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Abstract: Category learning is a critical cognitive process that organizes our memories and environments into a meaningful structure that can be generalized to novel situations. Despite its

importance, the neural mechanisms mediating category learning are largely unknown. We recently adapted classic human learning paradigms to investigate categorization in rats, a model organism well-suited for leveraging key neural mechanisms. Using a touchscreen apparatus, rats are trained to categorize distributions of circular stimuli containing black and white gratings that across exemplars change in their spatial frequency and orientation. For some rats, the stimuli must be categorized according to a single stimulus dimension (1D tasks; either spatial frequency or orientation). For other rats, the stimuli must be categorized according to both dimensions (2D tasks; spatial frequency and orientation). In the current experiments, we used NMDA lesions and inhibitory DREADDs to test the necessity of the prelimbic prefrontal cortex (PL), dorsal hippocampus (HPC), dorsomedial striatum (DMS), and dorsolateral striatum (DLS) as rats learned to categorize 1D and 2D tasks. The PL was critical for learning the 1D tasks, but not the 2D tasks. The HPC and DMS were critical to learn both task types. The DLS was not necessary to learn either task type. Then, we used the neural network model SUSTAIN to simulate the inactivation data and examine the function of each target region. Simulations suggest that the PL directs attention to relevant stimulus information, the HPC stores previous training experiences, and the DMS maps exemplars to appropriate behavioral responses.

Disclosures: **M.B. Broschard:** None. **J. Kim:** None. **B.C. Love:** None. **J.H. Freeman:** None.

Digital Abstract Session

P343. Prefrontal Circuit Mechanisms

Program #/Poster #: P343.04

Topic: H.04. Executive Functions

Support: NIH Grant P01-HD080679

Title: Neural dynamics among the prefrontal cortex, dorsal striatum, and hippocampus during rule-based rat visual categorization

Authors: *J. KIM, M. B. BROSCHARD, S. J. FARLEY, N. S. CREMERS, J. H. FREEMAN; Univ. of Iowa, Iowa City, IA

Abstract: Visual categorization is a critical cognitive function. Quick but reliable categorization either reduces danger or increases the benefits of new stimuli and experiences. The prefrontal cortex (PFC), dorsal striatum (DS), and hippocampus (HPC) are thought to be involved in successful categorization, but how those sites interact during category learning has not been determined. Here, we conducted simultaneous multi-tetrode recordings from the PFC, DS, and HPC while rats learned a rule-based categorization task in which Gabor patches differ in spatial frequency and orientation. Both single unit activity and local field potentials (LFP) were recorded throughout training. Single cell spiking activity from all three sites showed stronger category-specific modulation after rats learned the task. The category-specific firing, however, differed in time when the rat processed the category stimuli: PFC cells showed the earliest category-specificity while the other two regions tended to lag behind. Spectral power analyses

showed a similar category-specificity in specific power frequencies of LFPs. Category selectivity in theta power (4 - 10 Hz) in the three regions became differentiated once the rat learned the task. In the HPC, beta (15 - 20 Hz) power also showed learning-driven category-specificity. More direct comparisons of firing patterns of single units or oscillatory patterns of the LFPs were conducted to build similarity matrices by categories. After category learning, similarity matrices became more similar within-categories, while they become more dissimilar between-categories, indicating that all three sites show category-specific neural modulations. Functional connectivity analyses showed that coherence patterns elongated in both time and frequency became very well localized after the learning. Complimentary computational modeling and machine learning also supported learning-driven category-specific neural modulations of three sites. The findings indicate that interactions among the PFC, DS, and HPC strengthen as rats learn categories.

Disclosures: J. Kim: None. **M.B. Broschard:** None. **S.J. Farley:** None. **N.S. Cremers:** None. **J.H. Freeman:** None.

Digital Abstract Session

P343. Prefrontal Circuit Mechanisms

Program #/Poster #: P343.05

Topic: H.13. Schizophrenia

Support: NIH Grant 5R01DA044761-05

Title: Disynaptic, VTA-mediated, cerebellar modulation of the prefrontal cortex

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Abstract: The cerebellum (Cb) has been associated with cognitive disorders that potentially affect the medial prefrontal cortex (mPFC), such as schizophrenia and autism. However, how the cerebellum affects the mPFC remains to be established. The mPFC is thought to be involved in decision-making processes that guide behavior based on predicted outcomes. The ventral tegmental area (VTA) is a key region of the brain reward system that provides reward-related signals to the mPFC via dopaminergic projections. We have recently shown that the Cb sends direct excitatory projections to the VTA, raising the possibility that there might be a disynaptic pathway from the Cb to the mPFC via the VTA. Here we describe experiments aimed at delineate the anatomical and functional properties of the Cb->VTA->mPFC circuit in the mouse brain. Using intersectional tracing, we found that ~50% of VTA neurons that receive inputs from the Cb also send direct synaptic projections to the mPFC, confirming the presence of this disynaptic circuit. Using in vivo recordings in head-fixed awake mice, we found that optogenetic stimulation of Cb axons in the VTA excites mPFC cells within a fast time scale (average latency of 31.9 ± 13 ms). Moreover, by using fiber photometry we show that optogenetic activation of

Cb axons in the VTA releases dopamine in the mPFC, supporting the functional connectivity of the proposed circuit. These results show that the cerebellum might have a direct contribution to cortical dopamine levels, pointing to dopamine deregulation as a possible link between cerebellar dysfunction and mental disorders.

Disclosures: J.A. Vera: None. **M. Oñate:** None. **C. Chen:** None. **N. Cayla:** None. **V. Lovallo:** None. **K. Khodakhah:** None.

Digital Abstract Session

P343. Prefrontal Circuit Mechanisms

Program #/Poster #: P343.06

Topic: H.04. Executive Functions

Support: 1R01AA025652-01, 1P50AA022534-01 & T32AA014127

Title: Molecular and circuit level modulation of flexibility: Understanding the effects of PAE on reversal learning

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Abstract: Across species moderate prenatal alcohol exposure (PAE) is known to impair executive functioning specifically behavioral flexibility. Thus, understanding the mechanisms responsible for mediating cognitive flexibility is of critical importance in developing successful treatments for individuals with cognitive impairment. Studies in rodent models show that the dorsal striatum (dS) is heavily engaged during choice learning while the lateral orbitofrontal (OFC) cortex is important for tracking and updating changing reward values. In addition, NMDAR subunits 2A, 2B have been suggested to act as a gate for synaptic plasticity during learning experiences. While we have shown that loss of cortical 2B is sufficient to impair flexibility, it is still unknown is if the 2A/2B ratio varies during stages of learning and reversal. We therefore investigated if dynamic regulation of the GluN2A/2B ratio in the synapse is critical for efficient learning, flexibility during reversal. C57BL/6J were trained on a touchscreen based discrimination-reversal task. Using immunoblotting we quantified the levels of 2A, 2B, N1 after the criterion for various stages of learning and reversal was reached. In an attempt to rescue the inflexibility following PAE, a separate cohort was trained, micro infused with channelrhodopsin-expressing adeno-associated virus (AAV-CAMKαII-ChR2 (H134R)-mCherry), fitted with fiber stub for optogenetic stimulation. After 4 weeks, during reversal sessions 1-4 PAE/SAC mice received light pulses (10 Hz, 5mW, 5ms pulse for 1s) or no stimulation, 1 second following a correct choice. We then used retrograde expression of ChR2 in the dS to tag OFC-dS projection neurons and test if stimulation of these neurons was sufficient to restore flexibility. NMDAR subunit quantification revealed a striking trend in increase in the 2B subunit in the OFC during early reversal stage, increase in the dS when the mice have learned to discriminate, during which

plasticity changes are important in these two regions. Direct stimulation of OFC reduced average perseverative responding in PAE mice compared to controls. Stimulation of just the projection neurons not only improved the flexibility in PAE mice but also in SAC controls which received stimulation. These data provide evidence that NMDAR subunit expression is dynamically regulated, stimulation of specific populations of cortical neurons may reduce the impaired flexibility seen after PAE. Current studies, by combining in vivo electrophysiology and optogenetics, are examining if this direct stimulation of OFC-dS projection neurons was sufficient to restore behavioral flexibility and coherence within the OFC.

Disclosures: J. Chandrasekaran: None. **J. Kenton:** None. **C. Valenzuela:** None. **J.L. Brigman:** None.

Digital Abstract Session

P343. Prefrontal Circuit Mechanisms

Program #/Poster #: P343.07

Topic: H.04. Executive Functions

Support: NIH/NIMH R00 MH101234
R01 MH116008

Title: Muscarinic acetylcholine receptor localization on distinct excitatory and inhibitory neurons within the ACC and LPFC of the rhesus monkey

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Abstract: The lateral prefrontal (LPFC) and the anterior cingulate cortex (ACC) are two functionally-distinct prefrontal areas that are differentially innervated by ascending cholinergic pathways that modulate arousal and motivational signaling in higher-order functions. The nature and organization of prefrontal-cholinergic circuits in primates are not well understood although it is clear that muscarinic acetylcholine receptors (mAChRs) play a predominant role. Using multi-labeling immunohistochemistry and high-resolution microscopy, we assessed the co-localization of m1 and m2 mAChRs with distinct excitatory and inhibitory neuronal populations and pre-synaptic markers in the brains of rhesus monkeys. Our findings showed that 50-57% of either m1+ or m2+ neurons in supragranular layers 2-3 (L2-L3) of ACC and LPFC are excitatory pyramidal neurons, and the rest are non-pyramidal interneurons. However, within pyramidal neuron subpopulations expressing specific cytoskeletal proteins, MAP2+ and SMI32+, LPFC had significantly ($p<.05$) greater densities of m1+/MAP2+ and m2+/SMI32+ neurons compared to ACC. Distinct inhibitory neuron classes also showed regional diversity in mAChR expression; the density of m2+ calretinin-interneurons was significantly greater in ACC and m1+ and m2+ parvalbumin interneurons were significantly greater in LPFC, specifically in L3. The functional

effect of mAChRs strongly depend on synaptic localization. Compared to LPFC, ACC exhibited a greater proportion of excitatory (VGLUT1+) axon terminals with presynaptic m2+. The distribution of m2+ on inhibitory (VGAT+) axon terminals targeting specific pyramidal neuron compartments varied between the two areas. Compared to ACC, LPFC exhibited significantly greater m2+ colocalization on axo-axonal VGAT+ cartridges, and VGAT+ terminals targeting MAP2+ dendrites. However, the density of perisomatic m2+/VGAT+ inputs specifically on SMI32+ pyramidal neurons was greater in ACC than LPFC. To test the functional effects of muscarinic activation on excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs), we used in vitro whole-cell patch-clamp recording from L3 pyramidal neurons. The cholinergic agonist, carbachol (CCh), significantly decreased the frequency of EPSCs in ACC and LPFC pyramidal cells, but decreased the frequency and prolonged decay times of IPSCs to a greater degree in LPFC, suggesting stronger muscarinic suppression of inhibitory inputs in LPFC than in ACC. Together, these findings contribute to our understanding of cholinergic neuromodulation of specific cell types in functionally-distinct prefrontal areas that underlie executive function.

Disclosures: A. Tsolias: None. **W. Chang:** None. **T. Guillamon-Vivancos:** None. **C. Kopp:** None. **S. Busch:** None. **J.I. Luebke:** None. **M. Medalla:** None.

Digital Abstract Session

P343. Prefrontal Circuit Mechanisms

Program #/Poster #: P343.08

Topic: H.04. Executive Functions

Support: T32T32 DA 7244-30
NIH P60 AA011605-21

Title: Relationship between alcohol misuse, gaba:glutamate/glutamine ratios in dorsolateral prefrontal cortex, and habitual behavior in adulthood

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Abstract: Purpose: Excessive alcohol intake blunts gamma-aminobutyric acid (GABA) signaling and upregulates excitatory glutamate signaling, disrupting excitatory/inhibitory (E/I) balance. This misuse of alcohol by excessive consumption puts individuals at increased risk of adverse health consequences. Both acute and chronic alcohol use in humans has been shown to cause deficits in executive functioning that requires the prefrontal cortex. Moreover, data from rodent models show that binge ethanol exposure results in persistent loss of GABAergic neurons in prefrontal cortex. However, it is unknown how adult alcohol misuse impacts E/I balance in the human prefrontal cortex and what effect this has on habitual behavior. We addressed this question using a behavioral flexibility task and single-voxel proton magnetic resonance spectroscopy (^{1}H-MRS) to detect GABA and glutamate/glutamine (Glx) within the left

dorsolateral prefrontal cortex (DLPFC) of adults. Methods: We recruited healthy adult participants (22-38 years; mean=27) who reported either a history of adolescent binge drinking (ABD; n=10, 6 females) or no adolescent binge drinking (CON; n=15, 9 females). We used perseverative errors as a proxy of habitual behavior, which was measured using the Hidden Association Between Images Task (HABIT; described in McKim 2016). Adult alcohol misuse was calculated based on pattern of drinking, adapted from Townshend & Duka's binge score (2005). We conducted ^{1}H-MRS on a Siemens 3T MRI system using water-suppression with J-difference spectral editing to detect GABA and glutamate/glutamine (Glx). A T1-weighted structural image was used to correct for gray matter fraction within the voxel. We used hierarchical regression analysis to assess whether current adult alcohol use predicts levels of GABA, Glx, or GABA/Glx ratio, and whether any of these levels predict habitual behavior. Results: Controlling for a history of adolescent binge drinking, adult alcohol misuse predicted significantly lower GABA/Glx in the DLPFC (β=-.482, p=.048). Alcohol misuse did not predict individual variance in GABA or Glx levels. Additionally, DLPFC GABA/Glx ratio predicted habitual behavior (β=-.440, p=.038). Conclusions: Our results indicate that alcohol misuse in adulthood is associated with reduced GABA/Glx ratios in human DLPFC. Furthermore, reduced DLPFC GABA/Glx ratio is associated with habitual behavior. These results suggest that alcohol misuse disrupts E/I in the DLPFC, which further impairs executive function.

Disclosures: E. Vidrascu: None. **S. Dove:** None. **X. Zong:** None. **R. Corr:** None. **K. Meyer:** None. **M. Robertson:** None. **M. Sheridan:** None. **D. Robinson:** None. **C. Boettiger:** None.

Digital Abstract Session

P343. Prefrontal Circuit Mechanisms

Program #/Poster #: P343.09

Topic: H.04. Executive Functions

Title: Acute moderate running boosts positive mood and executive function coinciding with prefrontal activation: An fNIRS study

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Abstract: Running influences the genus Homo (Bramble and Lieberman, Nature 2004), and is one of the most popular exercises worldwide. Much evidence supports that running has a beneficial effect not only on the body, but also on the brain. In recent studies including our own, physical exercise that enhances positive mood has been reported to stimulate the left dorsolateral

prefrontal cortex (l-DLPFC), which improves executive function (Byun et al., NeuroImage 2014, Suwabe et al., Neuroscience 2020). Many of these studies have used cycling as a popular and easy exercise, but no study has investigated the neuronal substrate with which running enhances executive function, even though running may be a beneficial mode of exercise for the brain. The present study aimed to determine the acute effect of a single bout of moderate-intensity running on mood and executive function. We hypothesized that running would have a beneficial effect on executive function by enhancing mood-related prefrontal activation. Twenty-six healthy participants (8 females; age 23.12 $\pm$ 2.12 years) completed both a 10-minute running session on a treadmill at 50%VO_{2peak} and a resting control session in random order. Stroop interference time from the Color-word matching Stroop task (CWST) and mood based on the Two-Dimensional Mood Scale were measured before and after both sessions; additionally, functional near-infrared spectroscopy (fNIRS) was used to investigate prefrontal hemodynamic changes while performing the CWST. The results corresponded to our hypothesis in that running significantly enhances positive mood compared to control, and in particular revealed pleasure levels not observed in our previous cycling studies ($p < 0.001$, paired *t*-test; Byun et al., NeuroImage 2014, Suwabe et al., Neuroscience 2020). Running also led to a significantly greater reduction of Stroop interference time ($p = 0.002$, paired *t*-test) coinciding with a significant increase of Oxy-Hb signal in the l-DLPFC ($p = 0.001$, paired *t*-test, FDR correction). A new finding revealed activation in the right ventrolateral prefrontal cortex (r-VLPFC), an important brain locus for emotional regulation (Wager et al., Neuron 2008), which was significantly greater than that of control ($p = 0.007$, paired *t*-test, FDR correction). The coincidence frequency between r-VLPFC activation and positive mood was subsequently checked and the results showed significant coincidence ($p = 0.001$, McNemar test). This is the first study to report that 10 minutes of moderate-intensity running has a strong beneficial effect on inducing positive mood and enhancing executive function coincidentally with specific sub-regional prefrontal activation.

Disclosures: C. Damrongthai: None. **R. Kuwamizu:** None. **K. Suwabe:** None. **G. Ochi:** None. **Y. Yamazaki:** None. **K. Adachi:** None. **M.A. Yassa:** None. **H. Soya:** None. **W. Churdchomjan:** None.

Digital Abstract Session

P343. Prefrontal Circuit Mechanisms

Program #/Poster #: P343.10

Topic: H.04. Executive Functions

Support: University of Illinois Campus Research Board Award RB17155

Title: Effect of clinical sensory organization test on prefrontal cortical activation among older women with and without osteoarthritis

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Abstract: Osteoarthritis (OA) is a musculoskeletal condition that has been associated with a higher risk of fall. However, the underlying mechanisms that result in balance and gait changes and potential contributions from cognitive processes remain unclear. An individual's posture relies on visual, vestibular, and proprioceptive senses to maintain a stable upright posture, but if this is altered as per aging or due to disease, it increases the fall risk of an individual. This is in part due to a decline in neuromuscular functioning and reduced cognitive and visuospatial processing abilities. Although osteoarthritis is known to lead to functional changes in daily activities, however, little is known about the effect of OA on brain activation. This is the first study examining the effect of sensory organization test (SOT) on prefrontal cortical (PFC) activation patterns evaluated by functional near infrared spectroscopy (fNIRS) among older women with and without osteoarthritis (OA, HOA, respectively). We hypothesize that there will be increase in activation going from the easiest to most difficult SOT condition among HOA and OA. In a cross-sectional study design, we examined 7 OA (66.33±3.64yrs; WS:3.74; BMI:27.84) and 12 HOA (67.25±6.51yrs; WS:0.35; BMI:21.83). There were significant differences between OA and HOA in BMI and Womac pain score (WS) ($p<0.001$). Participants completed baseline cognitive and motor testing before starting the SOT on Neurocom Clinical Research System. In SOT, participants completed 3 trials each of 6 conditions: Eyes open (EO), Eyes closed (EYC), Eyes open sway reference moving (EYO_SR), Eyes open plate reference moving (EYO_PR), Eyes closed plate reference moving (EYC_PR), Eyes open sway and plate reference moving (EYO_SPR). They were instructed to stand upright on the force plate while experiencing different perturbations throughout the test. The results of the linear mixed model analysis revealed condition (CND) ($p<0.001$) and trial (TR) ($p<0.0001$) effects, and 2-way interactions among CND*TR ($p<0.01$) and Cohort*CND ($p<0.01$) and a 3-way interaction between Cohort, CND & TR ($p<0.0001$). In addition, post hoc analysis confirmed these interactions between CND*TR, Cohort*CND and Cohort*CND*TR. We found significantly higher PFC activation i.e. higher mean oxyhemoglobin & lower mean deoxyhemoglobin levels among both cohorts while going from easier to difficult task. This study confirms the use of increased attentional resources in the PFC as sensory disturbances increase and suggests an inability of older women with OA to recruit additional attentional resources, which merits further exploration. Keywords: fNIRS, PFC, OA, SOT.

Disclosures: **A. Bishnoi:** None. **Y. Hu:** None. **M. Hernandez:** None.

Digital Abstract Session

P344. Pharmacology and Behavior: Antidepressants, Stress and Memory

Program #/Poster #: P344.01

Topic: H.08. Learning and Memory

Title: Fluoxetine administration during adolescence impacts rodent cognition and behavior

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Abstract: Recent epidemiological data suggest that approximately 5-10% of adolescent children are diagnosed with major depressive disorder. In addition, problems related to adolescent depression are well documented to continue into adulthood. Existing treatment regimens for adolescent major depressive disorder include both talk therapy and drug therapy using fluoxetine (Prozac), with fluoxetine being the only drug currently approved by the US Food and Drug Administration for the treatment of depression in adolescents. Yet long term effects of adolescent fluoxetine treatment have only recently begun to be studied. Given that central nervous system growth and maturation continues throughout adolescence and into early adulthood, the effects of fluoxetine treatment on developing neurocircuitry and behaviors is unclear. The goals of these experiments were to examine the long term effects of adolescent fluoxetine treatment and compare those data to adult animals treated with fluoxetine in a rodent model of behavior and cognition. Male and female rats that were obtained from commercial sources (Envigio) were used as subjects in these experiments. These experiments used a 2 (age) x 2 (drug treatment) factorial design, with about 10-12 animals in each of the 4 experimental groups (adolescent+fluoxetine; adolescent+vehicle; adult+fluoxetine; adult+vehicle). Animals were administered 10 mg/kg of fluoxetine hydrochloride (Toronto Research Chemicals), or vehicle, for 14 days either during adolescence or adulthood. Afterwards during adulthood, all animals experienced a series of behavioral and cognitive tasks that assessed locomotor behavior in an open field, memory for novel objects after a short delay period, and behavior in an elevated zero-maze, which is a well validated task to assess anxiety-like behavior. Our hypotheses were that fluoxetine treatment during adolescence, but not adulthood, would be more likely to impact those behaviors more closely related to symptoms observed during major depressive disorder (anxiety, cognition). Data from these experiments suggested that adolescent fluoxetine dosing produced sex-selective effects on measures of cognition, exploratory, and anxiety-like behaviors. Taken together, these results may suggest that drugs like fluoxetine that alter serotonergic activity may produce functional changes in aspects of behavior and cognition during adolescence.

Disclosures: J.S. Rodefer: None. **A. Griggs:** None. **D. Agudelo:** None.

Digital Abstract Session

P344. Pharmacology and Behavior: Antidepressants, Stress and Memory

Program #/Poster #: P344.02

Topic: H.08. Learning and Memory

Support: FONCICYT-DADC OBTEEN 273553

Title: High fat diet intake during adolescence induces deregulation of norepinephrine neurotransmission and impairs spatial memory in male Wistar rats

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Abstract: According to the World Health Organization obesity worldwide has tripled since 1975. Not only adults have been affected with this obesity epidemic; in 2019, 38 million children and adolescents were overweight or obese. In addition to its association with chronic diseases like hypertension and diabetes, obesity affects neurocognitive features including learning and memory. To assess the effects of juvenile obesity on hippocampal function, we used a model of diet induced obesity in male Wistar rats. Rats were exposed after weaning to a regular diet (control group, 2.9Kcal/g) or a high-fat diet (HFD group, 4.7Kcal/g) for 3 months thus covering adolescence. HFD induced increased basal glucose blood levels, diminished glucose tolerance and insulin insensitivity. Once completed the diet exposure time, rats were implanted with microdialysis guide cannulae in the ventral hippocampus. While rats from both groups were submitted to an exploration task, we collected 4 minutes samples to assess catecholamines, glutamate and GABA. We found that in control rats norephinephrine release increased during exploration, and this increment was absent on the HFD group despite having similar exploration times. No significant differences between groups were observed regarding the other neurotransmitters. To further evaluate the effect of the HFD on the hippocampus, rats were trained in the Morris water maze (WM) and during the long-term test we found that HFD group showed an impaired performance taking significantly more time to reach the platform area. This impairment in hippocampal dependent long-term memory could be due to the dysregulation of norepinephrine transmission.

Disclosures: P. Salcedo-Tello: None. **D. Osorio-Gómez:** None. **A. Hernandez-Matias:** None. **C. Pérez:** None. **R. Gutierrez:** None. **G. Ferreira:** None. **G. Pacheco-López:** None. **K.R. Guzman-Ramos:** None. **F. Bermudez-Rattoni:** None. **S. Hernandez-Ramirez:** None.

Digital Abstract Session

P344. Pharmacology and Behavior: Antidepressants, Stress and Memory

Program #/Poster #: P344.03

Topic: H.08. Learning and Memory

Support: DGAPA-PAPIIT IA201420

Title: Establishment of conditioned place avoidance induced by gastric malaise requires the functional integrity of dorsal and ventral hipocampus

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Abstract: Literature concerning learning and memory is based on several studies of the hippocampus, a brain structure necessary for the formation and expression of episodic memories. The hippocampus has well delimited areas and its level of organization has promoted specific regional analyses. Thus, in the hippocampus, a dorsal and a ventral portion can be distinguished. Traditionally, the dorsal area has been thought to mainly contribute to spatial or contextual information processing, while the ventral hippocampus is considered a critical region related to the codification of the emotional content, specifically stressful or anxiety-related situations. However, there are scarce results about the functional role of the hippocampal regions on conditioned place avoidance induced by gastric malaise. Therefore, we aimed to determine the role of dorsal and ventral subregions of the hippocampus in the association of a context with aversive stimulation provided by gastric malaise, first by means of general inactivation and subsequently through blockade of specific receptors. For this purpose, male Wistar rats were trained in a protocol of Conditioned Place Avoidance (CPA) in which the animals received intraperitoneal injections of lithium chloride and then were confined in a compartment. In another set of experiments, dorsal or ventral portions of the hippocampus were inactivated by the administration of a mixture of muscimol and baclofen applied before the conditioning. Finally, we assessed the dependence of CPA on the NMDA receptors infusing APV within the ventral or dorsal regions before and after the conditioning. Our results indicate that LiCl promotes conditioned place avoidance to the compartment associated with the injection. Furthermore and interestingly, the inactivation of either dorsal or ventral hippocampus led to a memory impairment for the task. Moreover, we provide evidence that NMDA receptors are necessary for consolidation, but not acquisition, of CPA. Considering these results, it is proposed that contextual memories associated to gastric malaise are driven by both portions of the hippocampus, suggesting a joint activity of dorsal and ventral subregions through NMDA receptors activity.

Disclosures: **A. Hernandez-Matias:** None. **D. Osorio-Gómez:** None. **F. Bermudez-Rattoni:** None. **L.A. Cruz-García:** None.

Digital Abstract Session

P344. Pharmacology and Behavior: Antidepressants, Stress and Memory

Program #/Poster #: P344.04

Topic: H.08. Learning and Memory

Support: UWM Research Foundation Catalyst Grant Program
MCW Therapeutic Accelerator Program
NIH Grant R01MH107886

Title: A novel non-toxic histone deacetylase inhibitor enhances spatial memory consolidation in male mice

Authors: *S. B. BEAMISH[1], J. B. BELAYET[2], T. RAHMAN[2], M. RAHAMAN[2], S. ALANANI[3], M. M. HOSSAIN[2], D. A. STEEBER[3], K. M. FRICK[1];
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Abstract: Memory dysfunction is a hallmark of Alzheimer's disease (AD) and other dementias, yet truly effective treatments for memory loss do not exist. Memory dysfunction stems in part from reduced gene expression that leads to decreased levels of proteins essential for neural plasticity. Gene expression is facilitated by histone acetylation, an epigenetic mechanism that regulates chromatin accessibility. Compounds that maintain histone acetylation, called histone deacetylase inhibitors (HDACi), enhance memory by preventing deacetylation of core histone proteins, which allows for transcriptional machinery to bind open chromatin and increase gene expression. Although HDACi are promising therapeutics that could be used to prevent or delay memory loss associated with AD and other dementias, existing HDACi have poor solubility and undesirable toxicity. To address these shortcomings, our group has developed a novel HDACi compound, MJM-1, that is brain-penetrant and shows no evidence of toxicity. Here, we determine the extent to which MJM-1 can enhance spatial and object recognition memory consolidation. Male C57BL/6 mice were tested in object placement (OP) and object recognition (OR) tasks, which were used to assess spatial and object recognition memory consolidation, respectively. Mice received a post-training intraperitoneal (i.p.) injection of either negative control (100% DMSO), positive HDACi control (sodium butyrate; 0.6 g/kg NaBu), or one of three doses of MJM-1 (20, 30, or 40 μg/g). During testing, one training object was moved to a new location in the open field (OP task) or was replaced with a novel object (OR task). Mice who remember the training objects should spend more time with the moved and/or novel objects. Mice receiving NaBu, 20 μg/g MJM-1, or 40 μg/g MJM-1 spent significantly more time with the moved object, whereas DMSO-treated controls mice did not, suggesting that the established HDACi NaBu and two doses of the novel HDACi MJM-1 enhanced spatial memory consolidation. Mice receiving the 30 μg/g dose of MJM-1 also tended to prefer the moved object. Conversely, none of the doses of MJM-1 affected object recognition memory. The novel non-toxic brain penetrant HDACi MJM-1 can enhance spatial memory consolidation, suggesting encouraging proof of principle for future testing in models of aging and AD.

Disclosures: J.B. Belayet: None. **S. Alanani:** None. **T. Rahman:** None. **M. Rahaman:** None. **D.A. Steeber:** None. **M.M. Hossain:** None. **S.B. Beamish:** None. **K.M. Frick:** None.

Digital Abstract Session

P344. Pharmacology and Behavior: Antidepressants, Stress and Memory

Program #/Poster #: P344.05

Topic: H.08. Learning and Memory

Support: merit award I01BX003512 from Veterans Adminstration
William and Ella Owens Medical Research Foundation
Jess Hay Chancellor's Fellowship

Title: An adjunct treatment of sub-effective ketamine and fear extinction reverses cognitive deficits induced by chronic stress

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Abstract: Treatments for stress-related psychiatric disorders are inadequate. Behavioral therapies such as exposure therapy can be effective in ameliorating cognitive dysfunction associated with post-traumatic stress disorder and depression. We have recently established extinction learning in rats as a behavioral intervention that models the beneficial effects of exposure on cognition. In our recent studies, we have shown that extinction used as an intervention in rats, reverses chronic stress-induced deficits in cognitive flexibility on the attentional set-shifting test (AST), a medial prefrontal cortically-mediated executive process. Extinction requires the activity of pyramidal neurons in the infralimbic cortex, and Brain Derived Neurotrophic Factor (BDNF) signaling mediates these effects. The combined use of psychotherapy and pharmacotherapy may be more effective than either alone. Since extinction shares BDNF-mediated molecular mechanisms exerted by ketamine, we reasoned that extinction and ketamine used in combination will have enhanced efficacy. Methods: In these studies, we developed a model of sub-effective extinction therapy in rats that showed impairment in two readouts of prefrontal cortex function, AST and evoked local field potentials in the infralimbic cortex, following chronic unpredictable stress. Results: We found that reducing the duration of extinction attenuated its therapeutic effects on set shifting performance and activity of the infralimbic cortex in Sprague-Dawley rats after stress ($n=6$-9/group, $p<0.01$, $d=1.87$). We then established sub-effective doses of ketamine on the same measures of cognition and electrophysiology. Combining sub-effective extinction with a sub-effective dose of ketamine (1mg/kg) reversed the effects of stress on set shifting ($n=5$-11/group, $p<0.01$, $d=2.27$). Conclusions: We have developed a model to study adjunct treatment combining extinction and candidate drug therapies such as ketamine. Ongoing experiments will be conducted to determine the effects of the combined extinction plus ketamine treatment in female rats. This work was supported by merit award I01BX003512 from the US Department of Veterans Affairs Biomedical Laboratory Research and Development Program, and by a grant from the William and Ella Owens Medical Research Foundation.

Disclosures: D. Paredes: None. **D.A. Morilak:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Veterans Administration. **A.R. Knippenberg:** None.

Digital Abstract Session

P345. Mechanisms of Synaptic Plasticity: Invertebrates

Program #/Poster #: P345.01

Topic: H.08. Learning and Memory

Support: NIH Grant SC3GM111188
Texas Comprehensive Research Fund
Texas A&M University-Corpus Christi Division for Research, Commercialization and Outreach

Title: Role of serotonin in the memory impairment caused by food deprivation in Aplysia

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Abstract: Brain dysfunctions including memory deficits are caused by malnutrition. The mollusk *Aplysia* can be a suitable model for study of memory deficits raised from starvation because of its quantifiable behaviors, simple neural circuits and its ability to sustain long-term food deprivation without health deterioration (e.g., Kandel, 2001; Mac Leod et al., 2018). The mechanisms of an elementary form of learned fear known as sensitization have been extensively studied in *Aplysia* (e.g., Kandel, 2001). In normally-fed *Aplysia* (i.e., animals fed every two days, 2DFD), four strong electrical shocks to the body wall, mimicking attacks of a predator, induce long-term sensitization (LTS), which is exhibited as increased duration of defensive responses, including the tail-induced siphon withdrawal reflex (TSWR), 24 h after delivery of the shocks (Kandel, 2001). Serotonin (5-HT) is known to contribute to LTS in *Aplysia* (e.g., Kandel, 2001). Following shocks, 5-HT is released both in the neuropil and in the hemolymph (Glanzman et al., 1989; Levenson et al., 1999). LTS can be induced by 5-HT bath application without electrical shocks (Levenson et al., 2000). Fourteen days of food deprivation (14DFD) prevent LTS (Mac Leod et al., 2018). This study aimed to investigate the role of 5-HT in the absence of LTS in food deprived *Aplysia*. We hypothesize that 1) 5-HT levels may be reduced by 14DFD and 2) 5-HT bath application may rescue the prevented LTS in food deprived *Aplysia*. To investigate the first hypothesis, high-performance liquid chromatography with triple quadrupole mass spectrometer (HPLC-MS/MS) was used to detect 5-HT level in *Aplysia*. 5-HT concentrations in ganglia and hemolymph were measured and compared between the 2DFD and 14DFD *Aplysia*. 5-HT was significantly reduced in the hemolymph of 14DFD animals compared to 2DFD animals, whereas there was no significant difference in 5-HT levels in the ganglia between the two groups. To test the second hypothesis, four groups of animals were investigated: 2DFD *Aplysia* treated with 5-HT, 2DFD *Aplysia* treated with artificial seawater (ASW, vehicle solution to dissolve 5-HT), 14DFD *Aplysia* bathed with 5-HT and 14DFD *Aplysia* bathed with ASW. TSWR duration was measured prior to and 24 h after the treatment with either ASW or 500 µM 5-HT for 1.5 h. 5-HT induced LTS in both 14DFD and 2DFD animals. However, the amount of LTS was significantly smaller in 14DFD *Aplysia* compared to the 2DFD *Aplysia*, indicating that 5-HT alone was not sufficient to fully recover the memory deficits caused by 14DFD. Altogether, the above data suggest that shortage of 5-HT may be one of the causes that lead to the memory impairment caused by prolonged food deprivation in *Aplysia*.

Disclosures: **X. Deng:** None. **I. Huang:** None. **K. Williams:** None. **M. Wainwright:** None. **P.V. Zimba:** None. **R. Mozzachiodi:** None.

Digital Abstract Session

P345. Mechanisms of Synaptic Plasticity: Invertebrates

Program #/Poster #: P345.02

Topic: H.08. Learning and Memory

Title: Involvement of protein phosphatase 1 in conditioned inhibition-related reductions in excitability of Hermissenda type B photoreceptors

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Abstract: Explicitly unpaired (EU) presentations of light and rotation produce behavioral changes (e.g., increased phototaxis) in the sea snail Hermissenda crassicornis that satisfy the behavioral criteria for conditioned inhibition (CI) learning. This is accompanied by decreased excitability of type B photoreceptors (B cells) due to corresponding increases in potassium (K^+) currents. We've previously shown that protein phosphatase 1 (PP1) inhibitors (e.g., Calyculin A): 1) depolarize Untrained B cells and reduce somatic K^+ currents, partially mimicking the effects of associative conditioning (light - rotation pairings), 2) contribute to the excitability (spike frequency) decreases produced by extinction training (a form of learning that overlaps mechanistically with EU conditioning). Conversely, injection of catalytically-active PP1 (caPP1) into Untrained B cells partially mimicked the spike frequency declines produced by extinction training, and occluded further reductions in spiking by additional extinction training. Thus, we hypothesized that PP1 activity may also contribute to EU-training effects on B cells. To test this, we measured B cell photoresponses from Untrained and EU-trained animals following treatment with caPP1. B cells showed marked reductions in steady state generator potentials (SSGPs) and spike frequencies over the course of five 30-sec test lights, mimicking EU training. Prior EU training occludes the effects of caPP1 injection. Additional measurements showed that caPP1 increased both the transient (I_A) and delayed ($I_{K\text{-}Ca}$) components of K^+ current, previously shown to mediate the EU-related decrease in B cell excitability. We next injected B cells from Untrained and EU-trained animals with the core peptide fragment of the PP1 regulatory G subunit (G_M), which acts as a competitive inhibitor of PP1. Iontophoresis of 25uM G_M increased B cell SSGPs from untrained (19.5%; n.s.) and EU-trained (52.7%; p=0.0167) animals. Whereas EU training resulted in a 40.0% reduction in B cell SSGP ($P<0.001$), SSGPs of G_M treated B cells from EU-trained animals were indistinguishable from untreated B cells from Untrained animals. Thus, G_M treatment abolished the EU-related decrease in B cell excitability. Our results suggest that: 1) upregulation of PP1 activity is an important component of the EU-mediated decrease in B cell SSGP, 2) PP1 is constitutively active in B cells of Untrained animals. These data indicate that EU training increases PP1 activity in B cells, decreasing excitability through effects on downstream K^+ currents.

Disclosures: **J.B. Anderson:** None. **J. Cavallo:** None. **J. Farley:** None.

Digital Abstract Session

P345. Mechanisms of Synaptic Plasticity: Invertebrates

Program #/Poster #: P345.03

Topic: H.08. Learning and Memory

Support: NIH grants NS019895
NIH grants NS102490

Title: Quantitative description of the dynamic interactions among kinase cascades underlying long-term neuronal plasticity of Aplysia sensory neurons

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Abstract: Protein kinase A (PKA) and mitogen-activated protein kinase (MAPK) pathways are critical components of a molecular network underlying serotonin (5-HT) - induced long-term synaptic facilitation (LTF) of the *Aplysia* sensorimotor synapse and long-term enhancement of sensory neuron intrinsic excitability (LTEE). Nevertheless, the details of how these pathways interact and contribute to the temporal patterns of activity of these and other kinase pathways remain unclear. In this study, we used immunofluorescence analysis to measure the activities of PKA and three key components of MAPK cascades after a single brief treatment with 5-HT in the presence and absence of kinase inhibitors. The MAPK cascade components were extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (p38 MAPK), and p90 ribosomal S6 kinase (RSK). Dishes of SNs cultured from the same animals were paired for all the 5-HT treatments. One dish received a solution consisting of 50% isotonic L15 and 50% artificial seawater (L15-ASW) as vehicle control (Veh). The other received the same solution with the addition of 5 HT. At least five animals were used in each experiment. All experiments were performed in a blind manner so that the investigator analyzing the images was unaware of the treatment the SNs received. The results suggest that one pulse of 5-HT induced substantially different dynamics of PKA, ERK, p38 MAPK and RSK activation. The PKA inhibitors KT5720 and Rp-cAMP blocked activation of ERK and RSK. The blocks occurred at distinct time points after 5-HT, indicating temporally restricted roles for PKA in regulating the dynamics of activation of ERK and RSK. BI-D1870, a specific
inhibitor of RSK, blocked the delayed (~45 min) activation of p38 MAPK, suggesting a novel activation of p38 MAPK by the ERK/RSK pathway in *Aplysia*. Empirical data from this and previous studies, including engagement of MAPK by the growth factors *Aplysia* neurotrophin (NT) and transforming growth factor-β (TGF-β) that are critical for LTF, were incorporated into a computational model. The model simulated the temporal patterns of ERK activity produced by training procedures that induce long-term memory and made predictions. The model represents the most detailed quantitative description of the complex interactions in *Aplysia* sensory neurons among PKA pathways, MAPK pathways and the neurotrophin and TGF-β extracellular loops

necessary for LTF and LTEE.
This research was supported by NIH grants NS019895 and NS102490.

Disclosures: Y. Zhang: None. **P. Smolen:** None. **L. Cleary:** None. **J. Byrne:** None.

Digital Abstract Session

P346. Temporal Processing

Program #/Poster #: P346.01

Topic: H.08. Learning and Memory

Support: JSPS KAKENHI 19H01087

Title: The multiple look effect enhances the cutaneous rabbit illusion

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Abstract: The cutaneous rabbit illusion is a phenomenon that reveals "postdiction." For example, when two successive tactile stimuli, S1 and S2, are delivered to two locations on the forearm (e.g., the sides of the wrist and elbow) with a short inter-stimulus interval (ISI) such as < 100 ms, S1 and S2 are perceived at the locations as attracted to each other. Here, it should be noted that the relatively future event S2 affects the perception of the past event, S1. Goldreich (2007) proposed a Bayesian model of cutaneous rabbit illusion. According to this model, if decreasing uncertainty of the ISI between S1 and S2, the amplitude of the cutaneous rabbit illusion should increase. To verify the theoretical predictions, we focused on the following psychophysical phenomenon. After exposure to repeated stimuli with a constant ISI, participants can more accurately detect the deviation of stimulus timing from the constant ISI (multiple-look effect, Schulze 1989). Thus, the multiple look effect is a phenomenon that increases accuracy (i.e., decreases uncertainty) in the discrimination of stimulus intervals. The multiple-look effect increases as the number of repetitions of prior stimuli increases. Based on the Bayesian model and the multiple look effect, we hypothesized that a repetition of S1 prior to S2 with a coherent ISI would increase the amplitude of the cutaneous rabbit illusion. Eight volunteers participated in our experiments. We set four conditions of repetitions of S1 prior to S2: (1) S1 only, (2) S1-S2, (3) S1-S1-S2, and (4) S1-S1-S1-S1-S2. We used 60 ms for the ISI between S1 and S2 as well as among S1s. Results show that, as the number of repetitions of S1 increased, the perceived location of the last S1 was closer to the S2 location, which is consistent with our hypothesis. That is, the multiple look effect enhanced the cutaneous rabbit illusion. The multiple look effect is considered to occur due to the "prediction" generated by a prior stimulus sequence with constant rhythm (Jones et al. 2002). A recent psychophysical study on individuals with autism spectrum disorder proposed that postdiction involves different neural processes than does prediction (Wada et al. 2020). However, our results suggest that there is an interaction between

prediction and postdiction, which may mean that prediction and postdiction partially share neural processes.

Disclosures: D. Yoshioka: None. **M. Miyazaki:** None.

Digital Abstract Session

P346. Temporal Processing

Program #/Poster #: P346.02

Topic: H.08. Learning and Memory

Title: Encoding Stimulus Duration in Sensory Guided Behavior

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Abstract: Timing and making predictions are integral to most behaviors. Predicting a change in the traffic light, foraging, or speech perception all necessitate accurate timing of an event. Thus, understanding the circuit mechanisms of how temporal features of sensory input are represented and stored in cortical circuits is crucial to understanding complex behavior. One hypothesis of timing is that stimulus intervals are encoded in the population responses of locally connected neurons ("Intrinsic model'). This is supported by several psychophysical studies and stands in contrast to a "dedicated" clock model (Ivry and Schlerf, 2008; Goel and Buonomano, 2014). In support of the intrinsic model, we demonstrated that isolated cortical circuits can "learn" temporal intervals, a process mediated by local changes in network dynamics via temporally defined changes in excitation-inhibition (E-I) balance (Goel and Buonomano, 2016). To test this behaviorally, we used a novel **temporal interval sensory discrimination (TISD) paradigm**, in which water-deprived adult wild-type mice were presented with two identical visual sinusoidal gratings separated by gray screens of two different durations (0.2s vs 0.9s). One duration was associated with a water reward (preferred interval) and the other was not (non-preferred interval). Our preliminary results show that mice learned to discriminate between the two intervals, indicated by a discriminability index (d') of greater than 1.75 and a decrease in bias(c) (naïve session: c=1.714; learned session, c=-0.2356) and the normalized entropy of lick times across sessions. **These results suggest that stimulus duration features contribute to learning a goal directed task**. We hypothesize that TISD learning is accompanied by alterations in E-I balance mediated by parvalbumin (PV) and vasoactive intestinal peptide (VIP) interneurons. Specifically, ***enhancement of VIP function reduces PV output, via the disinhibitory circuit during the duration of gray screen (0.2s vs 0.9), which allows interval specific pyramidal cell activity patterns to emerge.*** To test this, we are performing two-photon calcium imaging in V1 during the TISD task. Similar to our *in vitro* studies (Goel and Buonomano, 2016), we expect that trained mice will exhibit a difference in the slope of the pyramidal cell population activity such that distinct patterns of activity emerge that encode information of about a 0.2s or 0.9s duration. Simultaneous imaging of pyramidal and PV or VIP cells will be performed using PVCreAi9 and

VIPCreAi9 transgenic mice. This study will ***provide novel insights into how shifts in E-I balance via stimulus durations influence sensory guided behavior.***

Disclosures: S. Post: None. **S. Ali:** None. **A. Goel:** None.

Digital Abstract Session

P346. Temporal Processing

Program #/Poster #: P346.03

Topic: H.08. Learning and Memory

Title: Effect of hippocampus lesions on temporal discrimination learning and maintenance

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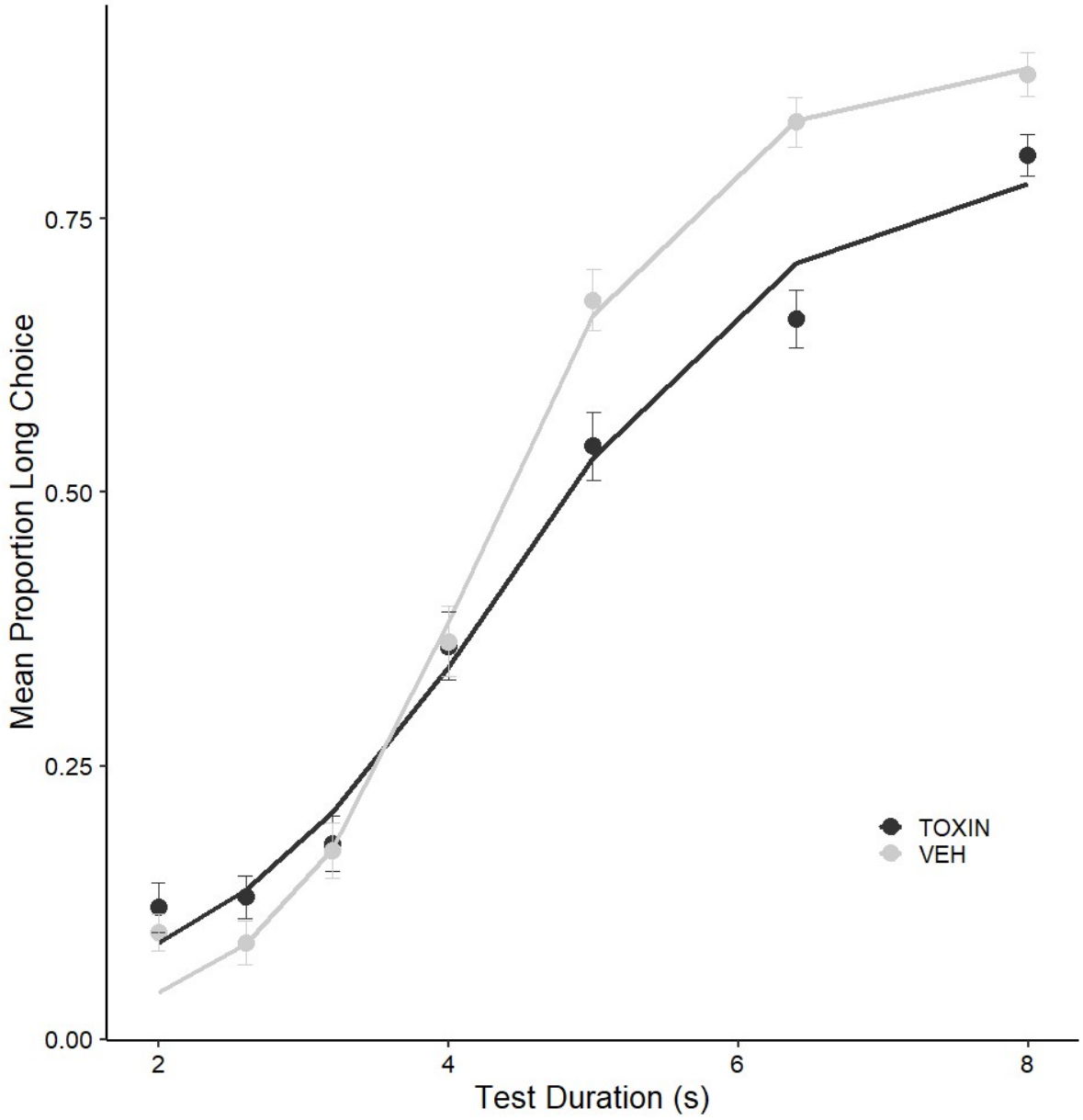
Abstract: This study assessed the ability of rats with hippocampus lesions to acquire an operant chamber temporal discrimination task. Lesions were made by infusing 0.5 µl (0.1 µl/min) of NMDA (20mg/ml) dissolved in sterile saline at stereotaxic coordinates targeting the dorsal hippocampus (AP - 2.8; ML ±1.6; DV - 3.6 and AP - 3.6; ML ±2.6; DV - 3.6 mm from bregma). Control animals were injected in the same manner but with sterile saline. Following recovery, animals were trained to discriminate two light cues of different duration by pressing one of two levers. Cue-lever pairings were counterbalanced across subjects. In the first phase of training, the house light in the chamber was illuminated for either a short (2 s) or long (8 s) duration, followed by the insertion of one of the corresponding, paired levers. A lever press was rewarded with food delivery. In the next phase, each house light cue was followed by the insertion of both levers. Incorrect trials were repeated until a correct response occurred. In the third phase, the correction procedure was removed and rats were trained until 80% of trials per session were correct for at least five consecutive sessions with no visual trends. For the fourth and final phase, intermediate house light cue durations (2.6, 3.2, 4, 5, and 6.4 s) were intermixed with the previously trained "anchor cues" (2 and 8 s). Only correct responses to the anchor cues were reinforced. Data from this final testing phase provided measures of timing accuracy and precision at the individual and group level. We found no differences between treatment groups in the number of trials to acquire the discrimination (phase 3). Multi-level, non-linear (sigmoid) regression analyses during phase 4 of training revealed that treatment group was not a significant predictor of the center/bisection point or slope parameters, but was a significant predictor of the asymptote parameter, $t = 2.27$, $p = 0.023$ (see figure). These results suggest the hippocampus is involved in maintaining the acuity of temporal reference memories, particularly for events of longer duration.



Disclosures: **W.E. DeCoteau:** None. **J. Ladison:** None. **L. Donohue:** None. **E. Golden:** None. **L. Gulbicki:** None. **A.E. Fox:** None.

Digital Abstract Session

P346. Temporal Processing

Program #/Poster #: P346.04

Topic: H.08. Learning and Memory

Support: JSPS KAKENHI 19H01087
JSPS KAKENHI 17KK0004

Title: Acquisition of multiple prior distributions in human coincidence timing: testing effects of motor-effector specificity

Authors: *Y. MATSUMURA[1], R. SATO[1], J. HERON[3], N. ROACH[4], M. MIYAZAKI[2,1]; [1]Grad. Sch. of Integrated Sci. and Technol., [2]Dept. of Computer Science, Fac. of Informatics, Shizuoka Univ., Hamamatsu, Japan; [3]Sch. of Optometry and Vision Sci., Univ. of Bradford, Bradford, United Kingdom; [4]Sch. of Psychology, Univ. of Nottingham, Nottingham, United Kingdom

Abstract: The brain can acquire prior distributions of sensory input and use them to optimize performance on sensorimotor tasks. Roach et al (2017) demonstrated that when exposed to two different prior distributions during a duration-reproduction task, participants first acquired a mixed distribution of the two priors ("generalization"). Moreover, they showed that if the two priors were assigned to two different motor outputs (keypress/vocalization), participants could acquire the two priors respectively ("motor specificity"). Here we hypothesized that if two prior distributions are assigned to two different motor effectors (right/left hand), participants can learn two prior distributions respectively even though the motor outputs are identical (keypress). To test the hypothesis, we conducted psychophysical experiments using a coincidence-timing task. Target timings were sampled from short (424-988ms) or long (1129-1694ms) time distributions. Participants in the effector-specific group responded to targets sampled from the two distributions with a specific index finger (right/left hand), whereas participants in the effector-fixed group used their dominant index finger throughout. In line with our hypothesis, we found that only participants in the effector-specific group were able to acquire both short-time and long-time distributions. Interestingly however, initial results for both groups were consistent with selective learning of the long-time distribution, rather than generalization across both distributions. This asymmetry was not a consequence of the short-time prior being inherently difficult to acquire; we found that an additional group of participants were able to quickly acquire the short-time distribution when it was presented in isolation. Instead, our results suggest that the brain initially prioritizes acquisition of the long-time prior over the short-time prior for coincidence timing. This difference in prior acquisition strategy compared to previous findings obtained with duration-reproduction tasks may reflect the ready availability of feedback in this paradigm and a desire to mitigate against large magnitude errors caused by duration-dependent internal noise (i.e. scalar variability).

Disclosures: Y. Matsumura: None. **R. Sato:** None. **J. Heron:** None. **N. Roach:** None. **M. Miyazaki:** None.

Digital Abstract Session

P346. Temporal Processing

Program #/Poster #: P346.05

Topic: H.08. Learning and Memory

Support: Simons Foundation Autism Research Initiative

Hock E. Tan and K. Lisa Yang Center for Autism Research at MIT
James and Patricia Poitras Center for Psychiatric Disorders Research at MIT
Stanley Center for Psychiatric Research at the Broad Institute of MIT and Harvard.

Title: Temporal Expectation in Marmosets: Global influences of task structure and local modulation by trial history

Authors: *T. DRAGOI[1], H. SUGIHARA[1], N. LE MINH[1], E. ADAM[1], J. SHARMA[1], G. FENG[2], R. DESIMONE[2], M. SUR[1];
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Abstract: It is generally believed that in absence of overt cues, humans and macaques implicitly use prior information to predict upcoming events and reduce uncertainty. Temporal expectation has been modeled using the hazard rate, which posits the likelihood of an event to occur in the future provided it has not occurred already. However, previous studies have not addressed how internal models of temporal expectation are acquired as a consequence of learning. To answer this question, we implemented a simple timing task in which freely behaving marmosets were required to make a timed response prompted by a visual stimulus change with a uniform distribution of stimulus durations. Our results demonstrate that, similar to previous findings in humans and macaques, marmoset reaction times follow the hazard rate model of expectation, consistent with the global task structure. Further, we examined how this model emerges from learning and found that with repeated task exposure, trial history and hence local task structure begins to influence reaction time. The combined effects of global and local task structure are well described by a multiple regression model, and computationally by Bayesian updating of the hazard function. Parallel experiments in human subjects similarly demonstrate global and local influences on reaction times and temporal expectation, which are also well captured by multiple regression and Bayesian models. These results demonstrate the evolution of dynamic internal models of temporal expectation based on multiple cues, along with the richness of temporal cognition in marmosets comparable to that in humans. An open question is whether the effects of local and global influences are distinct parallel processes or rather if they synergistically interact to influence temporal expectation. To reveal the neural underpinnings of temporal expectation, future studies in marmosets will measure large scale population activity across visual, parietal and dorsomedial prefrontal cortex.

Disclosures: **T. Dragoi:** None. **H. Sugihara:** None. **N. Le Minh:** None. **E. Adam:** None. **J. Sharma:** None. **G. Feng:** None. **R. Desimone:** None. **M. Sur:** None.

Digital Abstract Session

P348. Place Cell Dynamics in Spatial Memory Tasks

Program #/Poster #: P348.01

Topic: H.08. Learning and Memory

Support: NIH Grant R01 MH119179
NIH Grant R01 NS086947

Title: Flexible spatial memory encoding and hours-long retention reduce the stability of the hippocampal place code but promote goal selective representations

Authors: *S. AHMADI[1], A. RAMEZANI[1], C. K. LUONG[1], S. LEUTGEB[1,2], J. K. LEUTGEB[1];
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Abstract: The hippocampus is important for flexible memory-guided navigation, a process thought to be supported by the stability of hippocampal spatial representations. However, over the timescale of hours to days, hippocampal place fields gradually reorganize in familiar environments. This raises the question how spatial memory can be supported after many hours when hippocampal representations no longer correspond to the spatial locations they initially encoded. One possibility is that hippocampal representations remain stable even across hours-long periods when animals perform a memory task that requires them to retain recently encoded spatial information over extended intervals. To test this, we trained two groups of rats to perform either a spatial memory or a non-memory task on an 8-arm radial maze. In the memory task, rats learned a new goal arm location daily (SAMPLE) and were tested 6 hours later for memory retention (TEST). The goal arm was selected randomly each day and remained consistent for five SAMPLE trials and at TEST. In the behaviorally matched non-memory task a randomly chosen arm was rewarded in each of the five SAMPLE trials as well as at TEST. Memory task performance led to lower single-cell place map stability in comparison to the non-memory task (29.3% vs. 20.9%, $P = 0.011$, prop. SAMPLE-TEST map correlations < 0.1), and a greater decrease in the correlation between pairs of highly overlapping place representations across SAMPLE and TEST (Cohen's $d = -0.535$). These changes were deleterious for Bayesian position decoding accuracy, suggesting that a purely spatial hippocampal code cannot support memory performance. However, memory task performance also gave rise to a distinct goal-arm-selective population. SAMPLE correlations between goal-selective cells and the rest of the population were better preserved over the retention period until TEST in the memory task (54.6% vs. 48.4%, $P < 10^{-5}$). During the retention period between SAMPLE and TEST, hippocampal spikes were also preferentially biased toward long-duration sharp-wave ripples in the memory task (SWRs, $P < 10^{-57}$). Moreover, pairwise co-activations measured in SAMPLE were more strongly re-expressed in SWRs during the entire six hour long retention period between SAMPLE and TEST compared to the non-memory task. Taken together, our results describe neural dynamics that support memory of a new goal location after hours-long retention intervals despite the rapid reorganization of place selective representations. Furthermore, these findings segregate task-related network changes in service of memory from those related to environmental features, such as space and context.

Disclosures: **S. Ahmadi:** None. **A. Ramezani:** None. **C.K. Luong:** None. **S. Leutgeb:** None. **J.K. Leutgeb:** None.

Digital Abstract Session

P348. Place Cell Dynamics in Spatial Memory Tasks

Program #/Poster #: P348.02

Topic: H.08. Learning and Memory

Support: NIH Grant R01MH115304

Title: Stability of memory representations in the Hippocampus

Authors: *E. R. J. LEVY, E. PARK, A. A. FENTON;
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Abstract: The Hippocampus is crucial for the formation and persistence of memories. Two hypotheses describe how to decode information from neuronal discharge and how these signals store memory. One hypothesis states that information is encoded in the discharge rate of single neurons. A second hypothesis asserts that information is encoded in the co-firing relationships between the neurons' discharge.

To test these two hypotheses, we evaluated the activity of CA1 neurons across the entire multi-week experience of two pairs of environments: the first were physically distinct (cylinder and box), and the second were behaviorally distinct (conditioned and neutral). Each time, we recorded neuronal activity from mouse CA1 principal neurons expressing virally-induced GCaMP6f with a bright-field miniature microscope placed above a surgically implanted GRIN lens.

In the two environments, firing field stability measured by correlated firing rate maps is unstable across days, yet within-environment stability and across-environment discriminability increase with experience. Temporal reliability measured by correlating population activity vectors was similarly stable and discriminable but did not develop across experience. The activity vectors contain information that is both conserved and distinct between the cylinder and box, changing with environments and across experience.

We also evaluated how environment-specific information is organized across the CA1 population activity by projecting it onto two different sets of dimensions: we measured environment-specific discriminability using an SVM decoder and evaluated the natural non-linear temporal structure using an unsupervised Isomap algorithm. Both methods confirmed the preceding results. The SVM analysis indicates that both place and non-place cells are important for discriminating the environments and that the discriminative information distributes across more of the population with experience. Indeed, Isomap analysis reveals that the population activity readily collapses onto a low dimensional subspace that discriminates the two environments.

We conclude that the results from the first pair of geometrically-distinct environments are insufficient to decide between the two hypotheses. Results from the comparisons between distinct memories in the second pair of environments may be more appropriate for evaluating the differential validity of the two coding hypotheses.

Disclosures: **E.R.J. Levy:** None. **A.A. Fenton:** None. **E. Park:** None.

Digital Abstract Session

P349. Grid Cells

Program #/Poster #: P349.01

Topic: H.09. Spatial Navigation

Support: NIH NINDS grant K99NS116129
NIH NIMH grant R01MH60013
NIH NIMH grant R01MH120073
NIH NIMH grant R01MH052090
ONR MURI N00014-16-1-2832
ONR MURI N00014-19-1-2571
ONR DURIP N00014-17-1-2304

Title: The role of visual inputs in theta rhythmicity and coding of location and running speed in the medial entorhinal cortex

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Abstract: Neuronal representations of spatial location and movement speed in the medial entorhinal cortex (MEC) during the "active" theta state of the brain are important for memory-guided navigation. A key question is how external sensory cues such as visual inputs are integrated with internal self-motion cues in path integration to update self-location on a cognitive map. In this study, we use the spatially periodic firing pattern of grid cells in the MEC as a neuronal readout of such an integration mechanism and test the role of visual inputs on the spatial stability of grid cell firing and two proposed speed signals in the MEC, a speed signal represented directly by firing rate and an oscillatory speed signal represented by the slope of the local field potential (LFP) theta frequency to running speed relationship. By manipulating visual inputs in mice, we demonstrate that spatial stability of grid cell firing increases and decreases fast (< 10 s) and slowly (10-180s) during dark-to-light and light-to-dark transitions, respectively. The fast changes in grid cell stability were matched by fast changes in the mean firing rates of many MEC neurons, including speed cells, as well as fast changes in theta rhythmic firing and spike-to-theta phase coupling. Intriguingly, both fast and slow changes in grid cell stability over the course of three minutes were strongly correlated to changes in the slope of the LFP theta frequency to running speed relationship. In contrast, we did not observe slow changes in the potential speed signal represented by firing rate indicating that the slow change in grid cell stability is not driven by changes in speed cell firing. We further demonstrate that changes in the slope of speed cells' speed tuning curves during darkness can be fully explained by changes in mean firing rates as opposed to changes in the slope of the speed cells' running speed-dependent

gains in firing rates. Finally, we characterize changes in theta rhythmic firing in MEC neurons as a function of visual inputs and running speed.
In summary, data in this study demonstrate that spatial accuracy of grid cell firing is intrinsically linked to the slope but not y-intercept of the LFP theta frequency to running speed relationship. Moreover, changes on a slow time scale of tens of seconds suggest that grid cell firing is a function of velocity signals integrated over past time and thus malleable by internal memory processes.

Disclosures: H. Dannenberg: None. **H. Lazaro:** None. **P. Nambiar:** None. **A. Hoyland:** None. **M.E. Hasselmo:** None.

Digital Abstract Session

P349. Grid Cells

Program #/Poster #: P349.02

Topic: H.09. Spatial Navigation

Support: NIH Predoctoral Training in Quantitative Neuroscience 1T32NS105595-01A1

Title: A dynamical systems approach to understanding spatial localization in recurrent neural networks

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Abstract: Grid and place cells produce a spatial map of the environment suggesting the hippocampal system also maps abstract concepts using a similar map. A recurrent neural network trained to path-integrate, or calculate its position from past velocities, can show a similar grid-like firing pattern as grid cells. This suggests that there may be a universal computation for spatial mapping. Here we study the recurrent neural network as a dynamical system to understand this computation. Attractor states are stable fixed points, or a basin of attraction, in a dynamical system. A working model for grid cell computation is that localized grid-like bump activity corresponds to attractor states. The network's inputs move the network to different attractor states, which updates the network's internal prediction of position. We investigate whether the network uses attractor states for computing a spatial map. To understand how the network's spatial map may generalize to a cognitive map, we explore how the network's dynamics depend on different geometric properties. Training the network on different curved manifolds, we show how the environment's curvature, dimensionality, and symmetry affects the network's computation.

Disclosures: J. Gornet: None. **G. Raghavan:** None. **C. Lin:** None. **M. Thomson:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.01

Topic: H.09. Spatial Navigation

Support: Howard Hughes Medical Institute

Title: Circuit motifs for vector navigation and goal-directed action selection: insights from the Drosophila central complex connectome

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Abstract: The neural basis of goal-directed navigation has been the subject of decades of study, with a growing catalog of neuron types that form internal representations of an animal's spatial relationship to the external world. Yet, it has proven challenging to understand how these neurons acquire these representations and how navigation circuits ultimately guide an animal's behavior. The navigation center of insects resides in a highly recurrent, evolutionarily conserved region known as the central complex. The region houses a ring attractor network that computes the fly's head direction (HD) using both self-motion and allothetic sensory cues (Seelig & Jayaraman, 2015; Kim et al., 2017). Within this network, whose topography matches its function (Green et al., 2017; Turner-Evans et al., 2017, 2020), HD cells are arranged into columns according to their preferred firing direction, and population activity is organized as a traveling activity 'bump' whose columnar position encodes the fly's HD. Yet, how this 'compass network' is used to guide the fly's behavior remains largely unknown. Here we analyze a recently-released, complete connectome of the *Drosophila* central complex and identify circuit motifs downstream of the fly's compass network whose structure strongly suggests the implementation of vector-based computations for navigation. We used these motifs to build a conceptual model that describes how cosine-formatted activity bumps could form a four-vector basis set for navigational computations. The motifs suggest that the amplitudes of the basis vectors could be independently gain-modulated by self-motion signals to perform coordinate transformations, such as converting head direction into body direction. We highlight the utility of this motif in the context of path integration, a canonical vector-based navigation strategy used by a diverse array of both flying and walking insects. Specifically, we describe how the four-vector basis set could be used to compute an insect's translational velocity vector, which need not be the same as its head direction. We end by describing central complex output neurons that appear anatomically-configured to compare the fly's current HD to that of an allocentric goal direction to generate egocentric motor commands, ensuring a return to the goal. These results establish general circuit motifs for vector computation in the central complex, with specific implementations that likely depend on cell type, species, and behavioral need. More broadly, they highlight how the fly

connectome can be used to generate functional hypotheses about broadly relevant navigational circuit computations.

Disclosures: **B.K. Hulse:** None. **D.B. Turner-Evans:** None. **H. Haberkern:** None. **R. Franconville:** None. **S. Takemura:** None. **T. Wolff:** None. **M. Noorman:** None. **M. Dreher:** None. **C. Dan:** None. **A.M. Hermundstad:** None. **G.M. Rubin:** None. **V. Jayaraman:** None. **R. Parekh:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.02

Topic: H.09. Spatial Navigation

Support: EMBO Long-Term Postdoctoral Fellowship ALTF 382-2017
Sir Henry Wellcome Postdoctoral Fellowship 206491/Z/17/Z
CIHR Project Grant 155957
Canadian Research Chair
Canadian Foundation for Innovation

Title: Broad and sharp tuning to head-direction in the brainstem

Authors: *L. Y. WANG, A. J. DUSZKIEWICZ, G. VIEJO, H. BEAUDOIN, A. PEYRACHE; Montreal Neurolog. Inst. and Hosp., McGill Univ., Montréal, QC, Canada

Abstract: Head direction (HD) cells serve as the brain's internal 'compass' and each of them is tuned to the specific direction the animal is facing, independently of its location and ongoing behaviour. Brain state-invariant dynamics and computational models suggest that HD network is endowed with particular connectivity constraining the neuronal population to represent a single HD, made possible by mutual interaction between excitatory and inhibitory circuits and forming a so-called 'ring attractor'. While anatomical and lesion studies indicate that attractor dynamics in the HD system are likely supported by the reciprocal interactions between the dorsal tegmental nucleus (DTN) and the lateral mammillary nucleus (LMN), the underlying neuronal circuits and dynamics transforming vestibular angular head velocity (AHV) inputs into a coherent HD signal remain largely unknown.In order to elucidate the neural basis of the mammalian HD signal generator, we conducted bilateral population recordings in mouse DTN using high-density multi-shank silicon probes. We first confirmed previous observations of lateralized AHV coding in DTN: many recorded cells showed either unidirectional (tuned to clockwise or clockwise head turns) or bidirectional AHV tuning, and clockwise and anticlockwise AHV cells were enriched in right and left DTN, respectively.We then focused our analysis on the HD tuning present in the DTN and characterized two discrete populations of DTN-HD cells based on their millisecond-timescale spike train autocorrelograms: 'broad' and 'sharp' HD cells with average refractory period half-widths of 9 ms and 2 ms, respectively. These two DTN-HD cell types differ in their HD and AHV tuning: HD tuning curves of broad HD cells and sharp HD cells have average half-

widths of 180 degrees and 120 degrees, respectively, and broad HD cells are more likely to be asymmetrically tuned to AHV than sharp HD cells. Importantly, these two cell populations differ in their anticipatory firing: broad HD cells anticipate animal's HD 60 ms and sharp HD cells do so 30 ms into the future. A direct comparison of the two cell types with LMN-HD cells highlighted similarities in HD tuning and anticipatory firing between LMN-HD cells and sharp but not broad DTN-HD cells.Together, these observations indicate that these two DTN-HD cell types may be parts of different sub-circuits of the HD signal generator, with sharp HD cells positioned downstream of broad DTN-HD cells and possibly influenced by HD signal fed back from LMN. Further experiments will elucidate whether the directional tuning of these two newly characterized HD cell types depends on intact DTN-LMN feedforward and feedback connectivity.

Disclosures: L.Y. Wang: None. **A.J. Duszkiewicz:** None. **G. Viejo:** None. **H. Beaudoin:** None. **A. Peyrache:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.03

Topic: H.09. Spatial Navigation

Support: EMBO Long-Term Postdoctoral Fellowship ALTF 382-2017
Sir Henry Wellcome Postdoctoral Fellowship 206491/Z/17/Z
CIHR Project Grant 155957
Canadian Research Chair
Canadian Foundation for Innovation

Title: Non-uniform tuning in the cortical head-direction system

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Abstract: Head-direction (HD) cells are each tuned to a specific direction the animal is facing, independently of its location. The HD signal is generated subcortically and is relayed via the anterior thalamus, the postsubiculum (PoSub) and the medial entorhinal cortex (MEC), where it serves as a critical component of the grid cell code. While MEC grid cells are anatomically organized into modules with different grid spacing, it is unclear whether the HD system also exhibits a kind of anatomical organization. To address this, we used 64-channel linear electrode arrays to conduct population recordings across the cortical layers in mouse PoSub. A major obstacle in investigating the anatomical distribution of HD tuning is that it requires high-density local sampling of HD cells. To overcome this, we instead focused on the broad HD tuning of locally connected fast-spiking (FS) cells (putative interneurons) as a proxy for local HD tuning distribution. First, we confirmed that PoSub-FS cells are postsynaptic to local HD cells within

200 μm radius and their tuning is positively correlated with the population tuning of locally recorded HD cells. Further analysis revealed unexpected symmetries in PoSub-FS cell HD tuning: approximately half of recorded FS cells showed either two-fold or three-fold radial symmetry in their HD tuning, indicating PoSub FS cells pay be part of local modules of HD cells with preferred directions biased towards specific equally spaced angular values. We then looked for patterns in anatomical distribution of FS cells with radially symmetrical tuning. While FS cells with two-fold symmetries were enriched in middle cortical layers, FS cells with three-fold symmetries were instead present in the deep as well as the most superficial layers. These differences in anatomical distribution were further corroborated by subsequent PoSub recordings conducted parallel to the cortical layers. Overall, these results constitute the first evidence of local anisotropy in the HD system. Future experiments will aim to increase the number of locally sampled HD cells in order to directly investigate the fine details of functional organization in PoSub.

Disclosures: A.J. Duszkiewicz: None. **A. Peyrache:** None. **E.R. Wood:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.04

Topic: H.09. Spatial Navigation

Support: EMBO Long-Term Postdoctoral Fellowship ALTF 382-2017
Sir Henry Wellcome Postdoctoral Fellowship 206491/Z/17/Z
CIHR Project Grant 155957
Canadian Research Chair
Canadian Foundation for Innovation

Title: Fast-spiking interneurons shape symmetrical tuning to head-direction

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Abstract: Neuronal representations of external variables are organized into strikingly symmetrical maps. These geometrical organizations form the backbone of neuronal computations, how can they emerge robustly? In this work we show that as soon as fast-spiking interneurons are randomly connected into a network, they promote the emergence of symmetrical maps. We focus on the postsubiculum (PoSub), the cortical relay of the head-direction (HD) signal. It projects to the superficial layers of the medial entorhinal cortex, transferring HD inputs from the thalamus. Recent evidence from our laboratory suggests a topographical arrangement of the HD signal within the PoSub, as evidenced by symmetrical tuning of fast-spiking (FS) inhibitory neurons. Specifically, FS cells show a 1-, 2- or 3-fold symmetries in their tuning to HD, the 2- and 3-fold symmetries corresponding to peaks in the

tuning curves separated by exactly 180 or 120 degrees, respectively. The origin of this arrangement could reflect a topographical arrangement of local (<200 um) excitatory HD cells or could result from a transformation of thalamic inputs directly by the inhibitory networks.
To address this question, we have investigated the problem from a computational perspective. Surprisingly, models built with random thalamocortical (AD-to-PoSub) connections capture the emergence of the FS interneuron tuning curves. This is valid under the assumptions of linear integration by PoSub FS cells and the existence of inter-FS cell connectivity. The fold distribution of FS cell tuning curves is well reproduced over a wide range of AD-to-Posub connectivity parameters. In addition, the pyramidal cells in the PoSub are more selectively tuned to head-direction than in AD, yet some PoSub pyramidal cells also present symmetrical tuning to HD. This selectivity also emerged in a model based on random thalamocortical connections. These findings suggest that FS-FS connections and linear integration of thalamic inputs by FS cells in a randomly connected thalamocortical network are sufficient prerequisites for the emergence of symmetric and selective sensory tuning.

Disclosures: P. Orhan: None. **A. Peyrache:** None. **A.J. Duszkiewicz:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.05

Topic: H.09. Spatial Navigation

Support: NIH Grant NS053907
NIH Grant DC009318

Title: Landmark-based representations of spatial context in postrhinal cortex

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Abstract: Animals use visual landmarks to establish a sense of orientation and spatial context. This process requires the brain to integrate visual inputs with information about the animal's head direction (HD). The rat postrhinal cortex (POR), which receives inputs from visual brain areas as well as areas carrying a vestibular-driven HD signal, may play a role in this integration. We recorded from single HD cells in POR and found that, while they had a single preferred firing direction (PFD) in the presence of a single visual landmark (a large white cue card placed along one wall of the square enclosure), they adopted a second PFD when a second identical landmark was placed into the environment, with the orientation of the second PFD depending on the placement of the second landmark. These cells showed higher firing rates for a more familiar landmark, and they generally did not respond to a novel landmark (a black cue card) that was visually distinct from the familiar landmark. When the familiar landmark was removed from the environment, the cells still fired relative to the previous location of the landmark but with reduced firing rates, suggesting that they retained a memory for the familiar landmark. In

contrast, HD cells recorded from the anterior thalamic nuclei (ATN) maintained a single PFD under all circumstances. In addition, we could use the firing of a few co-recorded POR HD cells to discriminate among different spatial contexts (landmark configurations). In contrast again, ATN HD cells did not provide adequate information for the same contextual decoding. This study provides insight into how visual landmarks are integrated into a spatial framework that enables the neural encoding of landmark-based orientation and spatial context.

Disclosures: P.A. Lachance: None. **J. Graham:** None. **B.L. Shapiro:** None. **J.S. Taube:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.06

Topic: H.09. Spatial Navigation

Support: NIGMS P20GM103430
NIMH R01MH108729
NSF IOS-1656488

Title: A matter of complexity? The role of the rodent posterior parietal cortex in processing spatial layouts

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Abstract: The posterior parietal cortex (PPC) has long been associated with spatial cognition in rodent models. This region is thought to integrate multiple streams of information; however, most rodent studies have focused on simple tasks, for example, recognition of spatial relocation of one or two objects. Whereas these methods provide a necessary foundation for understanding PPC contributions to spatial cognition, new approaches are needed to expand on these results. In this study, we used experimental lesion methods to examine PPC function across a series of object exploration tasks which varied in spatial complexity. In Experiment 1, both PPC-lesioned and sham-lesioned rats were run on separate, single-feature change tasks including object recognition (OR), object location (OL), and object in place (OiP). Two object were presented in the study phase. In the test phase, one object was replaced (OR), moved to a novel location (OL), or moved to a familiar location (OiP). In Experiment 2, subjects were run on a complex spatial change (CSC) task in which object manipulations were similar to those in Experiment 1 but were presented in a more complex environment including four objects and four or five locations (Save, Poucet, Foreman, & Buhot, 1992). Our results show that in Experiment 1, all subjects preferred the novel spatial feature in the OR and OL tasks; however, control rats, but not lesioned rats, preferred novelty in OiP. In Experiment 2, all subjects preferred novelty for the combined OL/OiP change; however, control rats, but not lesioned rats, preferred novelty during the OR change. Interestingly, lack of response to specific changes (OR. OL, OiP) did not align

across experiments for lesioned rats. This suggests that exploration of spatial novelty may be dependent on the complexity of an environment when the PPC is intact. Further discussion is presented regarding PPC function and the ability to shift attention between environmental stimuli.

Disclosures: T.B. Wise: None. **V.L. Templer:** None. **R.D. Burwell:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.07

Topic: H.09. Spatial Navigation

Support: NIH BRAIN Initiative grant U01 NS094368
Simons Collaboration on the Global Brain Grant 542949
NIH grant NIDCD DC014686

Title: Spatial representations in macaque hippocampal formation

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Abstract: The hippocampal formation is linked to spatial navigation but there is little direct evidence in primates during active, unconstrained behavior. We recorded neurons across hippocampal regions in rhesus macaques during free foraging in an open arena while tracking their head and eye. We found that neurons encode a broad spectrum of spatial variables beyond place fields, and generally show mixed selectivity. Spatial representations were dominated by the allocentric location the head is facing and head tilt relative to vertical, whereas only a small fraction of neurons showed place or grid fields. Spatial facing selectivity was better explained by head-, rather than gaze-related properties. These findings reveal that the macaque hippocampal formation represents space using a multiplexed code, with heading properties dominating over simple place coding during free behavior.

Disclosures: D. Mao: None. **E. Avila:** None. **B. Caziot:** None. **J. Laurens:** None. **D. Dickman:** None. **D. Angelaki:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.08

Topic: H.09. Spatial Navigation

Support: NUS Memory Networks Program
Singapore Ministry of Education Tier 2 Research Fund
Singapore Ministry of Education Tier 3 Research Fund

Title: Representing place and view in the hippocampus of the non-human primate

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Abstract: The hippocampus and surrounding regions of the hippocampal formation are critical for spatial memory in multiple mammalian species, with single-neuron representations of allocentric place, direction or boundary well-established in rodents. Conjunctive, or mixed-selective, representations of two or more spatial variables are also abundant, even after isolating the independent contributions of each spatial variable. In contrast, clear examples in non-human primates of neurons exclusively selective for place are scarce. Instead, neurons selective for allocentric view of the environment, as well as a mix of spatial variables, have been found. However, many primate studies of spatial memory to date have used environments where place was heavily confounded with view, making it difficult to assess accurately the prevalence of different types of representations. The present study addressed that by recording hippocampal activity from a male non-human primate (macaca fascicularis) during a goal-directed virtual navigation task, where the direction of motion and view direction were not constrained by location. Neural activity was recorded from the right hippocampus with a 124-channel chronically-implanted microelectrode array, while eye gaze was tracked concurrently with location in the maze. Spatial information content was used to determine place and view selectivity, and the independence of the contribution of either variable to cell activity was assessed. 364 putative hippocampal cells were recorded, 38 of which were jointly selective for place and view, 20 selective for place only, and 10 selective for view only. Our results suggest that cells exhibiting mixed selectivity may be more prevalent than cells exhibiting exclusive selectivity for place or view in the primate hippocampus.

Disclosures: H. Tan: None. **T.P.Y. Ng:** None. **C. Owens:** None. **C. Libedinsky:** None. **S. Yen:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.09

Topic: H.09. Spatial Navigation

Title: Navigation using spatial view cells

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Abstract: A new theory is proposed of the mechanisms of navigation in primates including humans in which spatial view cells found in the primate hippocampus (Rolls and Wirth 2018) are used to guide the individual from landmark to landmark. The sequence of landmarks for the route could be stored in a time-cell sequence memory in the hippocampus (Rolls and Mills 2019), or in a continuous attractor network for a well-learned route, or in humans in a working memory in the prefrontal cortex (Rolls 2021). The individual moves along each leg of the route towards the next landmark, and then looks for the next landmark, and moves towards that. This type of navigation does not require a Euclidean topological map. Two other cells types found in primates, whole body motion cells, and head direction cells, can be utilized in the spatial view cell navigational mechanism, but are not essential. It is shown how if the landmarks are temporarily obscured, the spatial view cells can be idiothetically updated using mechanisms in the dorsal visual system areas in the parietal cortex (Rolls 2020), which reach the hippocampus via the retrosplenial and posterior cingulate cortex. The theory has been implemented in a model, and simulations will be presented showing the operation of the navigational mechanism. It is proposed that this navigational mechanism using spatial view cells is frequently used by humans. The mechanism is relatively simple because primates have visual neurons that respond allocentrically to locations in spatial scenes (Rolls and Wirth 2018). An advantage of this approach to navigation is that spatial view neurons are also useful for episodic memory, and for imagery (Rolls 2021).
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Rolls, E. T. (2020) Spatial coordinate transforms linking the allocentric hippocampal and egocentric parietal primate brain systems for memory, action in space, and navigation. Hippocampus 30: 332-353.
Rolls, E. T. and Mills, P. (2019) The generation of time in the hippocampal memory system. Cell Reports 28: 1649-1658.
Rolls, E. T. and Wirth, S. (2018) Spatial representations in the primate hippocampus, and their functions in memory and navigation. Progress in Neurobiology 171: 90-113.

Disclosures:

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.10

Topic: H.09. Spatial Navigation

Support: NIH NINDS (NS103802)
McKnight Foundation (Technological Innovations Award)
Keck Junior Faculty Award

Title: Neural mechanisms of location-encoding for self and others

Authors: *M. STANGL[1], U. TOPALOVIC[1], C. S. INMAN[1], S. HILLER[1], D. VILLAROMAN[1], Z. M. AGHAJAN[1], L. CHRISTOV-MOORE[2], N. R. HASULAK[3], V. R.

RAO[4], C. H. HALPERN[5], D. ELIASHIV[1], I. FRIED[1,6], N. SUTHANA[1];
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Abstract: Everyday tasks in social settings require humans to encode not only their own spatial location, but also the location of other individuals within an environment. Currently, the vast majority of what is known about neural representations of space for self and others stems from research in rodents and other non-human animals. However, it is largely unknown how the human brain represents the location of others, and how aspects of human cognition may impact these location-encoding mechanisms. To address these questions, we examined participants with chronically implanted electrodes while they performed real-world spatial navigation and observation tasks. We report boundary-anchored neural representations in the medial temporal lobe that are modulated by one's own as well as another individual's spatial location. Moreover, these representations were modulated by one's momentary cognitive state and strengthened when encoding of location was of higher behavioral relevance. Altogether, these results provide the first evidence for a common encoding mechanism in the human brain to represent the location of oneself and others in shared environments, and shed new light on the neural mechanisms that underlie spatial navigation and awareness of others in real-world scenarios.

Disclosures: **M. Stangl:** None. **U. Topalovic:** None. **C.S. Inman:** None. **S. Hiller:** None. **D. Villaroman:** None. **Z.M. Aghajan:** None. **L. Christov-Moore:** None. **N.R. Hasulak:** None. **V.R. Rao:** None. **C.H. Halpern:** None. **D. Eliashiv:** None. **I. Fried:** None. **N. Suthana:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.11

Topic: H.09. Spatial Navigation

Support: National Science Foundation BCS-1829398
Institute for Collaborative Biotechnologies
Hellman Family Foundation

Title: The Emergence of Head Direction Signals in Human Navigation

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Abstract: Head direction is crucial in human wayfinding, but whether head direction signals can be classified in the brain in a complex environment, and how this signal relates to navigation performance remain open questions. In an fMRI study, we tested over 90 participants in a cardinal-direction-aligned virtual maze. The navigation task consisted of an exploration and a test phase. During the 16-minute exploration, participants freely explored to find 9 objects

located in the environment and were instructed to remember their locations. For each of the 48 test trials, participants started at one object and were directed to go to another object, without feedback and with a limited time. We conducted an intra-subject multivariate pattern classification for the four head directions in four a priori regions of interest (ROIs). Our preliminary results suggest that we can successfully discriminate between head directions in retrosplenial cortex, extrastriate cortex, precuneus, and thalamus, as well as the whole brain. For some regions, head direction signals could only be classified successfully during the exploration phase, whereas others could discriminate during both exploration and test phases. Furthermore, we observed a relationship between an individual's classification strength and their subsequent navigation performance. Although in some regions we observed that better performance in discriminating directions was related to better navigation performance, in other regions classifier strength was related to worse navigation performance. This study indicates that the basic navigational signal of head direction is important for successful navigation in a complex environment and could form the basis of individual abilities.

Disclosures: Y. Cheng: None. **E.R. Chrastil:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.12

Topic: H.09. Spatial Navigation

Support: ONR N00014-19-2571
ONR N00014-16-1-2832

Title: Medial temporal lobe subfields balance representational dimensionality during prospective planning

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Abstract: Prior research has identified a prominent role for medial temporal lobe (MTL) subfields in the disambiguation of overlapping episodes during navigational "look-ahead" periods. However, it remains unclear how these areas mediate disambiguation. One solution is to orthogonalize overlapping representations by expanding their dimensionality. We hypothesized that hippocampal and related MTL subregions perform a balancing act - compressing representational space by identifying and extracting the relevant dimensions of neural representations over the course of learning, while simultaneously leveraging a higher-dimensional space for initially overlapping representations. To test this hypothesis, we reanalyzed a high-resolution functional magnetic resonance imaging study of the MTL in healthy adults performing a navigational disambiguation task (Brown, Hasselmo, & Stern, 2013). During scanning, participants (N=15) navigated virtual mazes that were well-learned from prior training

while also learning new mazes. Each maze began with a unique contextual cue period where subjects prospectively planned their routes. Critically, some of the routes overlapped (OL) while others did not (NOL). We employed a data-driven approach as used in Mack, Preston, & Love, 2020 to examine the dimensionality of neural representations during prospective planning. Neural representations for each OL and NOL cue were extracted using a Least Squares - Separate GLM approach. We then derived a dimensionality score for these representations using principal components analysis. In accordance with our hypothesis, we predicted greater dimensionality for OL relative to NOL representations as well as a decrease in dimensionality over the course of learning. We discovered evidence in MTL subfields of a higher-dimensional space for OL relative to NOL route representations. Furthermore, we found evidence for dimensionality compression over the course of learning within entorhinal and parahippocampal cortices and hippocampal subfields CA1 and subiculum when we compared early and late trials, consistent with a changing role for these regions over the course of learning. Finally, we demonstrated that previously well-learned routes were represented in a low-dimensional space in entorhinal cortex. Taken together, these results suggest that MTL subfields play a critical role in disambiguating overlapping sequences during prospective planning by balancing between the orthogonalization and compression of neural representational space.

Disclosures: S.S.P. Liapis: None. **T.T.I. Brown:** None. **J.T. McGuire:** None. **C.E. Stern:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.13

Topic: H.09. Spatial Navigation

Support: NSF BCS-1630296

Title: Spatial learning through interaction: A hybrid route to a cognitive map?

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Abstract: A growing body of research has characterized how we learn information from maps vs. routes although how we differentially learn and interact with route and map information remains poorly understood. While the networks that underlie human spatial cognition are believed to be similar or homologous to other mammalian species, humans may be the only species that uses figural representations of spaces (i.e., maps) to learn how to navigate actual spaces. To better understand learning of "figural" spaces like cartographic maps compared to "environmental" spaces like routes, we developed a novel hybrid learning task in which a similar underlying mental process (e.g., navigation) can be observed through interaction with a 3D scale model of an environment. In the novel hybrid task, participants moved an avatar while viewing

the environment from above on a virtual table top. Participants learned the configuration of 8 buildings within a virtual city (102x142 meters) by either navigation, a map, or our hybrid task. After learning, participants then completed 56 memory trials of either the judgements of relative direction (JRD) pointing task or the scene- and orientation-dependent pointing (SOP) task. The pattern of results for navigation and map learning were qualitatively similar to previous findings from our lab, with overall lower error on the SOP task compared to the JRD task and a benefit from map learning on JRD performance relative to navigation learning. For hybrid learning, however, performance across both JRD and SOP tasks was worse than map or route learning. There are several possibilities for why hybrid learning may have shown the worst performance, despite its seemingly intuitive combination of figural and environmental spaces. One possibility is that the hybrid learning task involved additional cognitive load imposed by maintaining multiple frames of reference (observer and remote avatar), impairing tracking spatial location and orientation. Another possibility is that prior experience was relevant: because participants had experienced routes and cartographic maps, but not hybrid spaces, they were better able to learn based on strategies they had developed previously with these modalities. A final possibility relates to the difficulty of combining scales of space, suggesting a degree of inflexibility in combining information from figural and "vista" scales.

Disclosures: **M.J. Starrett:** None. **D.J. Huffman:** None. **A.D. Ekstrom:** None.

Digital Abstract Session

P350. Human and Animal Navigation

Program #/Poster #: P350.14

Topic: H.09. Spatial Navigation

Support: Hellman Family Foundation

Title: The relationship between navigation abilities and mental disorders

Authors: ***N. HATAMIAN**, R. F. WOODRY, B. TRANQUADA-TORRES, A. YEE, E. R. CHRASTIL;
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Abstract: The link between navigational abilities and Alzheimer's disease has provided an exciting early marker for detection of dementia in at-risk individuals. However, it is unclear whether navigational abilities can be used to detect other neurological and mental disorders. The present study examines the relationship between two aspects of navigational ability and behavioral traits associated with various mental disorders. We propose that navigational ability may utilize the same brain circuitry as certain mental disorders, and may be a beneficial early marker for these disorders. This study is inspired by the Research Domain Criteria (RDoC), a framework outlined by NIMH to study the basic dimensions of functioning that span the range of behavior from normal to abnormal. For example, we hypothesize that spatial perspective taking that is important for navigation could be associated with social perspective taking in disorders

such as autism spectrum disorder or eating disorders. To test this question, participants recruited online through Amazon Mechanical Turk completed two web-based spatial cognition tasks and a battery of standardized questionnaires to capture non-pathological ranges of mental disorders. The Open Field Task (OFT) is similar to a virtual Morris water maze. It tests an individual's ability to recall, locate, and navigate to four hidden objects scattered in an open field environment from a first-person perspective. The OFT tests allocentric spatial learning and path integration. In the Spatial Orientation Task (SOT), participants see a top-down view of a layout of objects, then are asked to imaging standing at one object, facing a second object, and then to point to a third object. The SOT tests spatial perspective taking ability. The behavioral questionnaires assess pathological and non-pathological levels of mental disorders such as depression, anxiety, autism, and schizotypy to examine individual variability within the healthy population. Preliminary correlations indicate relationships between navigational ability and several mental disorders, particularly between spatial task performance error and autistic traits.

Disclosures: N. Hatamian: None. **R.F. Woodry:** None. **B. Tranquada-Torres:** None. **A. Yee:** None. **E.R. Chrastil:** None.

Digital Abstract Session

P351. Learning and Cognition

Program #/Poster #: P351.01

Topic: H.10. Human Learning and Cognition

Title: Strategy development and feedback processing during complex category learning in young adults

Authors: *V. TILTON-BOLOWSKY, S. VALLILA-ROHTER, Y. ARBEL;
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Abstract: For individuals who have sustained a neurologic injury, the rehabilitation process involves learning and relearning everyday processes. Therefore, rehabilitation researchers are interested in characterizing individuals' learning ability. Category learning tasks are often used to examine learning ability. In the current study, we analyzed performance on a probabilistic A/B prototype category learning task under observational and feedback training conditions. We counterbalanced two stimulus sets across participants and training conditions. We examined differences in learning success (percent accuracy) and strategy use (optimal versus suboptimal) between training conditions. We also evaluated the feedback-related negativity (FRN) event related potential as a measure of feedback processing during feedback training. We used the Geodesic EEG System with a 32-channel HydroCel Sensor Net following the international 10-20 system to collect and analyze EEG data. Thirty-eight healthy young adults (M = 25 years, SD = 3.33; 25 females) participated. A dependent samples t-test revealed that the difference in testing accuracy between training conditions was not significant, $p = 0.13$. A chi-square test indicated that strategy proportions under the two training conditions were significantly different, $p = 0.03$. One-way ANOVAs revealed that optimal strategy users achieved significantly greater testing

accuracy than suboptimal strategy users across training conditions, $p = 0.001$. A mixed-design ANOVA revealed a significant three-way interaction between feedback valence, training phase, and strategy trajectory on FRN amplitude, $p = 0.02$. Specifically, participants who switched from a suboptimal to optimal strategy during training exhibited a significantly larger FRN to negative feedback in late training than in early training. We found significant relationships between testing accuracy and FRN amplitudes to negative feedback in late training, $p = 0.02$, as well as between testing accuracy and FRN difference scores in late training, $p = 0.02$. In conclusion, those who developed an optimal strategy outperformed suboptimal strategy users, and more participants developed an optimal strategy under feedback training than observational training. Few studies have combined strategy analyses with measures of feedback processing. Our findings are consistent with prior research that suggests that method of instruction influences learning, and also suggests that measures of feedback processing in late-stage training of probabilistic category learning may reflect strategy optimization and may further illuminate differences among individual learners.

Disclosures: V. Tilton-Bolowsky: None. **S. Vallila-Rohter:** None. **Y. Arbel:** None.

Digital Abstract Session

P351. Learning and Cognition

Program #/Poster #: P351.02

Topic: H.10. Human Learning and Cognition

Support: CAREER Award BCS1943767

Title: Emergence of distributed value representations and learning in naturalistic reward environments

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Abstract: Learning an appropriate representation of the reward environment is extremely challenging in the real world where there are many options to learn about and these options have many attributes or features. Although recent studies have provided important insights into behavioral adjustments during such representation learning, neural mechanisms underlying these processes are still unknown. To address this, we measured learning and choice during a novel multi-dimensional probabilistic learning task in humans and trained recurrent neural networks (RNNs) to capture our experimental observations. Experimentally, we found that participants learned about a single informative feature before learning about the conjunctions of the uninformative features that carried partial information about stimulus-reward associations. The trained RNN exhibited a similar transition in learning strategy. Further analysis of neural response in RNNs revealed that the representations of reward value emerge over time as a distributed code that differentially relied on distinct types of excitatory and inhibitory units. Specifically, we found a higher degree of similarity between representation in inhibitory pools

and the informative feature value, a higher degree of similarity between representation in excitatory pools and object value, and a higher degree of similarity between representation in pools with reward-dependent learning in their sensory inputs and conjunction value. In addition to these results, our model predicts that recurrent connections from inhibitory pools that are endowed with reward-dependent learning in their sensory inputs to homologous excitatory pools play a crucial role in emergence of the observed distributed representations.

Disclosures: **S. Farashahi:** None. **J. Xu:** None. **A. Soltani:** None.

Digital Abstract Session

P351. Learning and Cognition

Program #/Poster #: P351.03

Topic: H.10. Human Learning and Cognition

Support: R01 DA034685
F32 MH119796
F32 DA038927

Title: Optimal dopaminergic tone for reward learning and executive control in humans

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Abstract: The dopaminergic neurotransmitter system is known to play important roles in both learning and executive control. It has been proposed that these two roles are subserved by interacting, but dissociable systems, with executive control processes such as working memory dependent on prefrontal dopamine and learning processes dependent on ventral striatal dopamine. To test this hypothesis, healthy subjects performed a probabilistic learning task that involves both trial-by-trial learning and executive control. Subjects (n = 77) performed the same task on placebo, bromocriptine, an agonist of the dopamine D2/3 receptors that predominate in the striatum, and tolcapone, a COMT inhibitor that predominantly affects prefrontal dopamine. Moreover, our subjects were stratified according to their DRD2 Taq1A and COMT genotypes, which influence dopaminergic function predominantly in the striatum and prefrontal cortex, respectively. In addition, PET measurements of striatal dopamine synthesis capacity and D2 receptor density were collected in the same subjects. This dataset provides a unique opportunity to query the genetic, regional, and cellular aspects of dopamine function in a large cohort of subjects.
We found that both higher dopamine synthesis capacity in the ventral striatum and DRD2 Taq1A- status correlate with reinforcement learning performance, suggesting that baseline dopamine tone (perhaps in tandem with D2 receptors) influences the degree to which reward associations are learned. However, these higher-performing subjects are most impaired by

dopamine drugs, consistent with a model in which a moderate level of dopamine tone is most beneficial for performance. In contrast to the ventral striatum, D2 receptor density in the dorsal striatum was related to the ability to form explicit knowledge of probabilistic associations, consistent with this region's association with executive function. Finally, we found that tolcapone, which increases prefrontal dopamine, impairs executive control in our task (i.e., response variability), especially for COMT Met homozygotes who have higher prefrontal dopamine at baseline. Our results show that dopamine influences behavior differentially depending on the neural system, and that optimal behavior arises at a level of moderate dopamine tone.

Disclosures: I.C. Ballard: None. **D. Furman:** None. **A.S. Berry:** None. **R. White:** None. **W. Jagust:** None. **A. Kayser:** None. **M. D'Esposito:** None.

Digital Abstract Session

P351. Learning and Cognition

Program #/Poster #: P351.04

Topic: H.10. Human Learning and Cognition

Support: Intel Corporation
John Templeton Foundation
NIH Award R01 MH069456

Title: Sculpting new visual concepts into the human brain

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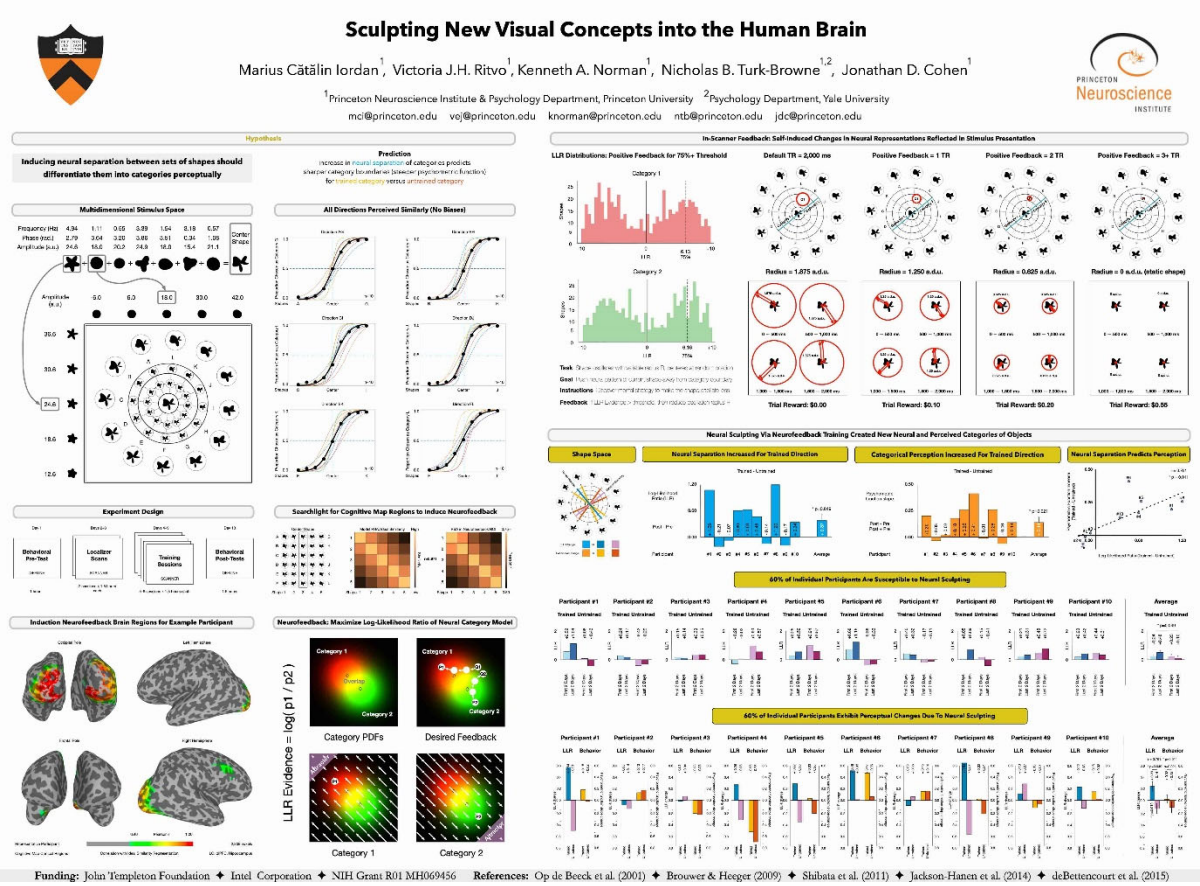
Abstract: Humans continuously learn through experience, both implicitly (e.g., statistical learning) and explicitly (e.g., instruction). As humans learn to group distinct items into a novel category, neural patterns of activity for those items become more similar to one another and, simultaneously, more distinct from patterns of other categories. We hypothesized that we could leverage this process using neurofeedback to devise a fundamentally new way for humans to acquire conceptual knowledge. Specifically, sculpting patterns of activity in the human brain that mirror those expected to arise through learning of new visual categories may lead to enhanced perception of the sculpted categories, relative to similar, control categories that were not sculpted. To test this hypothesis, we implemented a closed-loop system for neurofeedback manipulation using fMRI measurements recorded from the human brain in real time (every 2s) and used this method to sculpt/create new neural categories for complex visual objects. After training, participants exhibited behavioral and neural biases for the sculpted, but not for the control categories, and we observed a significant positive correlation between the increase in behavioral discrimination and the increase in neural separation of the categories. Neural

sculpting provides causal evidence (through direct experimental intervention) that distributed patterns of activity evoked by complex visual stimuli can be formed de novo in high-level cortex to create categories that didn't previously exist in the brain or behavior. The ability to sculpt new conceptual distinctions in the human brain has broad relevance to many domains of cognition such as perception, decision-making, memory, and motor control. It also broadens the possibility for non-invasive clinical intervention in humans with fMRI (e.g., brain-machine interfaces, neuroprosthetics, neurorehabilitation). Finally, this hints at the distant possibility of sculpting more extensive knowledge or complex concepts directly into the human brain, bypassing experience and instruction.



Disclosures: M. Iordan: None. **V.J. Ritvo:** None. **K. Norman:** None. **N.B. Turk-Browne:** None. **J.D. Cohen:** None.

Digital Abstract Session

P351. Learning and Cognition

Program #/Poster #: P351.05

Topic: H.10. Human Learning and Cognition

Support: JPMJER1801

102612/A/13/Z
JP18dm0307008

Title: Neurobehavioral value mediators of abstraction in human learning

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Abstract: Humans excel at creating concepts, organizing information in schemas and hierarchies, which in turn enable efficient learning and behavior. Here, we hypothesized that value assigned to sensory features is key in driving abstraction that subserves learning. While their brain activity was recorded with functional magnetic resonance imaging (fMRI), human participants repeatedly learned new associations between imaginary characters (made of a combination of visual features), and symbols (fruits). Leveraging reinforcement learning algorithms, we designed models differing in their state-space dimensionality to characterize participants' behavioral strategies in solving the task. We show that abstraction was associated with higher expected value, revealing superior performance. In turn, participants with higher abstraction ability solved the task problems faster, and were more confident in their performance. Participants' learning speed was predicted by the functional connectivity between visual cortex and the area encoding value signals (the ventromedial prefrontal cortex - vmPFC). Moreover, we found that feature-level information was represented in the hippocampus and vmPFC when behavior was 'abstract', suggesting that these areas may actively transform incoming sensory information, under a specific goal. In a second experiment, we used multivoxel neurofeedback to test for the causality of feature valuation as a mechanism of abstraction. Pairing the neural sensory representation of a task's feature with rewards subsequently led to a shift towards abstraction in behavioral learning strategies. Thus, these findings help carve a new understanding of valuation as a goal-dependent process, key to constructing the abstract representations at the basis of reasoning and intelligent behavior.

Disclosures: **A. Cortese:** None. **M. Kawato:** None. **B. De Martino:** None.

Digital Abstract Session

P352. Human Learning and Cognition

Program #/Poster #: P352.01

Topic: H.07. Long-Term Memory

Support: CIHR

Title: Episodic memory impairments in Parkinson's disease: a meta-analysis

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Abstract: While there is growing consensus that episodic memory is impaired in Parkinson's disease (PD), variability across studies has obscured our understanding of the severity and nature of these impairments. To address this, we are conducting a meta-analytic review of episodic memory performance on clinical tools in nondemented PD patients compared to controls. In addition to quantifying the severity of episodic memory deficits in PD, we are coding for moderators to inform a current debate — whether these impairments stem solely from fronto-striatal dysfunction or whether hippocampal degeneration also contributes. If memory decline is a secondary effect of frontal-striatal deficits in PD, tasks that provide less support at retrieval (e.g. free recall versus cued recall or recognition) would show larger group differences. On the other hand, if memory impairments in PD are also hippocampal-mediated, PD groups would show greater forgetting after delays and worse performance in associative compared to item memory. From a sample of 25 studies, we found preliminary evidence for a large effect in which patients perform worse than controls across all memory measures, $p<0.0001$, $g= -0.7$, 95% CI [-0.85, -0.50]. These impairments seem to diminish when patients are tested over delays ($p=0.08$), and there is no significant difference between item and associative memory ($p=0.18$), inconsistent with a strong hippocampal contribution. However, we also found that greater degree of retrieval support did not minimize memory deficits ($p=0.82$), inconsistent with fronto-striatal predictions. Although preliminary, our findings do not provide unequivocal support for either proposal; analyses of other features of memory performance is warranted. Ultimately, such analyses will help elucidate the neural underpinnings of memory dysfunction in PD and inform the development of compensatory learning strategies.

Disclosures: **A.Y. Youm:** None. **E. Williams:** None. **J. Koh:** None. **M. Vaziri:** None. **H. Lee:** None. **R. Simpson:** None. **K. Duncan:** None. **M. Cohn:** None.

Digital Abstract Session

P352. Human Learning and Cognition

Program #/Poster #: P352.02

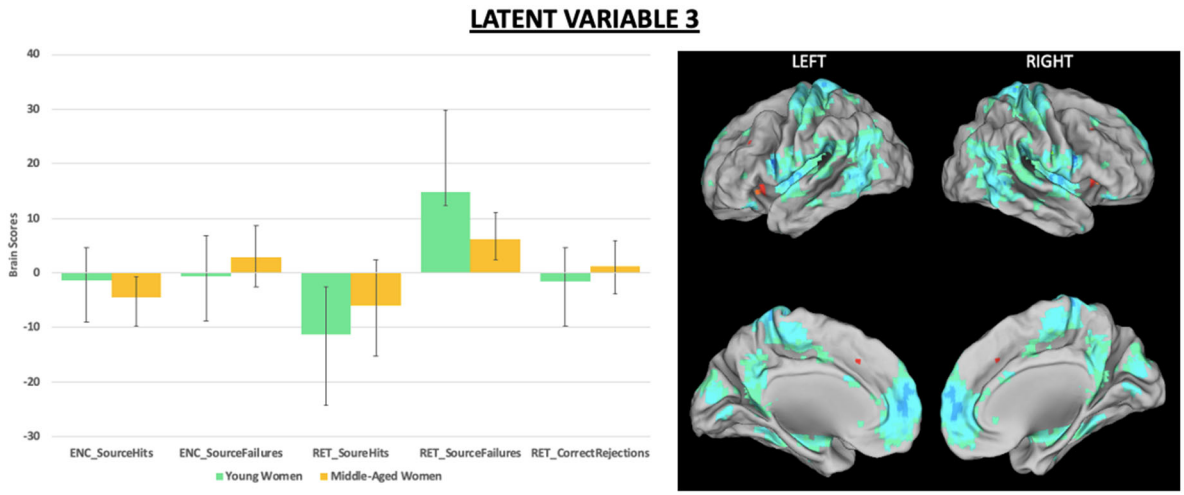
Topic: H.07. Long-Term Memory

Support: CIHR PJT201610 - 153321
CIHR GS9 - 171369
NSERC DISCOVERY GRANT # RGPIN-2018-05761
Healthy Brains, Healthy Lives Master's Fellowship

Title: Effects of aging on women's brain health: a multivariate analysis of task fMRI data

Authors: *A. CRESTOL[1], S. RAJAGOPAL[4], A. ELSHIEKH[1], L. KHAYYAT[4], R. YOUNG[4], J. SNYTTE[2], S. LOPARCO[1], A. DUVAL[2], S. SUBRAMANIAPILLAI[2], S. RABIPOUR[3], S. PASVANIS[4], M. N. RAJAH[3];
[1]Integrated Program in Neurosci., [2]Psychology, [3]Psychiatry, McGill Univ., Montreal, QC, Canada; [4]Brain Imaging Centre, Douglas Inst. Res. Ctr., Montreal, QC, Canada

Abstract: Hormonal fluctuations resulting from menopause in middle-aged (MA) women have a robust impact on episodic memory - our ability to encode, store and retrieve past experiences in rich contextual detail. In a previous study, we demonstrated that sex differences were present in the effect of aging on brain function associated with episodic memory. The objective of the current task fMRI study was to contribute to this field of knowledge by assessing the impact of aging on brain function when targeting women at midlife. We compared fMRI task-related brain activity patterns in 20 young (18-39) and 26 MA (40-65) women as they encoded and retrieved face-location associations while being scanned. Multivariate partial least squares (PLS) analyses were performed to assess age differences in brain activity associated with different memory phases (encoding vs. retrieval), response types (source hits [SH] vs. source failures [SF] vs. correct rejections [CR]), as well as performance in the behaviour PLS (proportion of SH vs. SF). A mean-centered PLS identified three significant latent variables (LV; $p<.02$). The first two LVs demonstrated main effects of encoding vs. retrieval and encoding vs. CR in both groups. LV3 identified age-related differences in encoding and retrieval activations such that young women activated negative salience regions, including regions of the recollection network during SH retrieval to a greater degree than MA women, while MA women activated these regions to a greater degree during SH encoding (see figure). Both groups activated positive salience regions during SF retrieval, which comprised of the ventrolateral prefrontal cortex. Parallel sets of regions were identified in the behaviour PLS, which predicted encoding success in young women and retrieval success in MA women. These results reveal that an aging effect in both encoding and retrieval activations arise at midlife in women. A post hoc analysis was performed to compare pre/peri and post-menopausal MA women. While no significant results were found ($p>.05$), these preliminary results are currently underpowered.



Disclosures: **A. Crestol:** None. **S. Rajagopal:** None. **A. Elshiekh:** None. **L. Khayyat:** None. **R. Young:** None. **J. Snytte:** None. **S. LoParco:** None. **A. Duval:** None. **S. Subramaniapillai:** None. **S. Rabipour:** None. **S. Pasvanis:** None. **M.N. Rajah:** None.

Digital Abstract Session

P352. Human Learning and Cognition

Program #/Poster #: P352.03

Topic: H.07. Long-Term Memory

Support: CIHR PJT201610 - 153321
CIHR GS9 - 171369
NSERC DISCOVERY GRANT # RGPIN-2018-05761

Title: Bilingual language experience and education moderate the relationship between menopause and cognition.

Authors: *A. DUVAL[1], A. BEATTY-MARTINEZ[1], A. CRESTOL[1], A. ELSHEIKH[1], S. PASVANIS[2], L. KHAYYATT[2], R. YOUNG[2], J. SNYTTE[1], S. SUBRAMANIAPILLAI[1], D. TITONE[1], M. N. RAJAH[1,2];
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Abstract: Cognitive reserve (CR) refers to how individual differences in lifestyle impact one's ability to maintain normal cognitive processes in the presence of brain pathology. CR has been shown to mitigate age-related cognitive decline, such that people with higher levels of CR can withstand greater levels of neuropathology associated with neurodegenerative diseases i.e., Alzheimer's Disease. Education and bilingual language experience are common proxy measures of one's level of CR. Recently, our group found that women at midlife were more likely to show cognitive improvements with increased bilingual experience, yet midlife is also the time when women transition into menopause (Subramaniapillai et al., 2019). Menopause causes a decrease in 17b-estradiol levels, which have been linked to cognitive decline, however, little is known about how CR impacts the relationship between menopausal status and cognition. Thus, in this study, we investigated this issue. Specifically, we hypothesized that postmenopausal women would exhibit worse task performance compared to other groups, however, this effect would be smaller for women with high CR. We recruited 138 healthy adult women (pre and postmenopausal) with an age range of 19-66 (mean 38.75, SD 13.12). A Language and Social Background Questionnaire assessed proxy measures of CR (number of languages known, percentage of non-native language use and age of second language acquisition), as well as years of education. Cognitive performance was measured using the California Verbal Learning Task (CVLT; episodic memory) and the Wisconsin Card Sorting Task (WCST; cognitive control). Preliminary robust regression analyses revealed an interaction of age of second language acquisition and menopause status on CVLT long-free recall ($p < .01$, $B = -.33$) and WCST non-perseverative error ($p < .001$, $B = .22$) scores, such that postmenopausal women performed worse than premenopausal women with a later onset of second language. Additionally, a significant interaction effect of menopause status and the number of languages known on WCST non-preservative error scores was observed, such that postmenopausal women performed worse than premenopausal women ($p < .05$ $B = .14$), and premenopausal women with a higher number of languages known showed improved scores. These data provide preliminary evidence that CR,

operationalized as bilingual language experience and education, impacts the relationship between menopause and cognition. We continue to evaluate whether more social aspects of bilingual language experience play an especially critical role.

Disclosures: **A. Duval:** None. **A. Beatty-Martinez:** None. **A. Crestol:** None. **A. Elsheikh:** None. **S. Pasvanis:** None. **L. Khayyatt:** None. **R. Young:** None. **J. Snytte:** None. **S. Subramaniapillai:** None. **D. Titone:** None. **M.N. Rajah:** None.

Digital Abstract Session

P352. Human Learning and Cognition

Program #/Poster #: P352.04

Topic: H.07. Long-Term Memory

Title: Extrapolating the unobserved past and future in other people's autobiographical timelines

Authors: ***X. XU**[1], Z. ZHU[2], J. R. MANNING[1];
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Abstract: In our social interactions, we readily make inferences about what happened to other people in their past, and what will happen in their future. This can happen on short timescales, e.g. when we use contextual cues to join an in-progress conversation, or on longer timescales, e.g. when we make inferences about someone's past or future when we first meet them. To study these phenomena, we had participants view sequences of segments excerpted from the TV series *Why Women Kill*. Participants used free-form text responses to either predict what would happen next, or retrodict what had happened just prior, to the just-watched segment. We systematically varied whether participants watched the segments in forward or reverse temporal order, and how many segments preceding or proceeding the target segment they had seen prior to making a response. We also varied whether participants were told the main characters in the target segment. Participants were better at retrodicting past events than predicting future events. One reason is that, when characters referenced specific events from their past or future, participants better retrodicted and predicted those events. These references also indirectly affected predictions and retrodictions about their temporally proximal (non-referenced) events. When controlling for these references, participants were equally good at retrodicting and predicting. This suggests that asymmetries in how often characters referenced their past versus their future might account for the asymmetry in how well participants retrodict and predict past versus future events. Our work elucidates how we retrodict others' pasts and predict others' futures by showing how we draw on our observations in the present (including specific references to past or future events), and from our own relevant prior experiences and knowledge. This also explains the apparent contradiction between temporal symmetry in the information that the present gives people about the past versus the future, and the empirical observation that people tend to be better at retrodicting what happened in other people's pasts than predicting their futures.

Disclosures: **X. Xu:** None. **Z. Zhu:** None. **J.R. Manning:** None.

Digital Abstract Session

P352. Human Learning and Cognition

Program #/Poster #: P352.05

Topic: H.06. Social Cognition

Support: NIH grant 229468

Title: High resolution imaging of a parieto-occipital cortical network involved in monitoring peri-personal space

Authors: ***Z. NASIRIAVANAKI**[1], R. TOOTELL[1], B. BABADI[1], S. NASR[1], D. GREVE[2], J. POLEMINI[2], D. HOLT[1];
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Abstract: **Background**: The regulation of preferred physical distance from another individual ('personal space') is altered in several neuropsychiatric conditions, including autism and schizophrenia. Many prior studies have described regions in inferior parietal cortex that responds selectively to visual stimuli (including faces) that intrude into near (personal) space. To better understand that functional organization, we conducted high-resolution functional imaging (7T, 1.1 mm isotropic), extensively signal-averaged in within-subject analyses. **Methods:** Seven healthy subjects were scanned using three different tasks. In the main task, subjects viewed human face images that appeared to approach or withdraw from the subjects. In the second (control) task, we presented random dot stereograms, which formed a stereoscopic (3D) percept of a cuboid at different distances from a subject. In a third (control) task, we tested responses to stationary (rather than moving) face stimuli. **Results:** The first task (approaching vs. withdrawing faces) activated discrete patches in inferior parietal cortex. Approach-biased patches dominated the withdrawal-biased patches, in size and number. All patches were radially elongated, similar to cortical columns in lower visual areas. The near-vs. far columns based on personal space were systematically interdigitated with the columns based on visually-defined (stereoscopic) distance. The third task showed that the approach-biased patches showed a BOLD amplitude x distance response that was similar to that found earlier in behavioral tests: i.e. highest to the nearest faces, decreasing to increasingly further faces as a power function, reaching baseline near and well beyond the behaviorally-defined personal space boundary in each subject. **Conclusions:** Specific radially-elongated patches ('columns') located in inferior parietal cortex are activated by faces that intrude into personal space. The topography suggests that 'near-vs. far' columns in eye-based coordinates (based on stereopsis) are systematically paired with another set of columns that are selective for 'near vs. far' distances in visually-defined space.

Disclosures: **Z. Nasiriavanaki:** None. **R. Tootell:** None. **B. Babadi:** None. **S. Nasr:** None. **D. Greve:** None. **J. Polemini:** None. **D. Holt:** None.

Digital Abstract Session

P353. Associative Learning

Program #/Poster #: P353.01

Topic: H.10. Human Learning and Cognition

Support: NIH Grant 5R01MH116005-03

Title: Midline Thalamus Modulating Mnemonic Discrimination

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Abstract: Anxiety is a common pediatric psychopathology that affects 1 in 3 youth and increases the risk of comorbid pathologies, such as depression. This pernicious disorder begins during peripuberty – a transitioning period between childhood and adolescence. One of its common features is negative overgeneralization, or responding similarly to innocuous situations that share features with past aversive events. Prior research has investigated bottom-up mechanisms, such as amygdala-salience detection. Yet, evidence in rodents shows the nucleus reuniens (RE), a medial thalamic nucleus, exercises control over fear memory specificity by modulating medial prefrontal cortex inputs to the hippocampus. To our knowledge, no studies have examined the role of the RE in negative overgeneralization. We analyzed data from 34 children, between 9-14 years of age with varying degrees of anxiety. Clinic and community samples were recruited to maximize our effect of interest: negative overgeneralization. To approximate the location of the RE, we used probabilistic tractography and a k-means clustering approach based on ipsilateral connectivity to cortical and subcortical targets. We collected brain activation to positive, neutral, and negative valenced pictures during Study and Test sessions separated by 12 hours. During Test, participants performed a memory recognition test with target, foil, and lure (i.e., similar but not identical) pictures. False alarming (FA) to negative, relative to neutral images was our operational definition of negative overgeneralization. We predicted decreased RE activation at encoding for negative stimuli that were subsequently replaced by a lure and FA. Analyses were made with either a paired samples t-test or a Wilcoxon signed rank test, if assumptions of normality were violated. We found a significant increased RE activation for negative compared to neutral images that were replaced by lures and FA ($t(33) = 1.99$, $p = 0.05$). To examine an extension of our original definition of negative overgeneralization, we assessed activation differences for negative images subsequently replaced by a lure and FA, relative to those correctly rejected (CR). We expected RE activity at encoding would decrease for negative lures FA, compared to those CR. Our results showed no differences in RE activation ($t(33) = 0.08$, $p = 0.93$). The present findings shed light onto a novel limbic thalamus mechanism of negative overgeneralization that could further our understanding of anxiety.

Disclosures: M.V. Rivera Nunez: None. **A.T. Mattfeld:** None. **D. McMakin:** None.

Digital Abstract Session

P353. Associative Learning

Program #/Poster #: P353.02

Topic: H.10. Human Learning and Cognition

Support: EOS Grant G0F3818N
FWO Grant 1102519N

Title: The neural signatures of extensive training

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Abstract: Objectives: Frontal theta-frequency rhythms are important for dealing with cognitive tasks, but their exact role remains obscure. Theta oscillations might resolve conflict or error arising during task execution. Alternatively, they may support task processing at a slower time scale. For example, theta may support learning task-relevant stimulus-response (S-R) mappings. Furthermore, alpha-frequency rhythms have been implicated in optimizing task performance via inhibition of task-irrelevant processing pathways. The differential roles of theta and alpha have not been empirically clarified, however. For example, it is unknown whether they operate at similar (fast or slow) time scales, and whether they support different learning processes.
Research question: We present an EEG study to address this issue. Within each task block (fast time scale), 8 S-R mappings of a procedural learning task were presented repeatedly. Across blocks, the same S-R mappings were repeated (slow time scale; interleaved with novel S-R mappings). At behavioral level, we expect that reaction times (RT) decrease, and accuracy increases with training. Neurally, we expect that theta power decreases and alpha power increases with training. Moreover, we investigate whether theta and alpha evolved (resp., decrease and increase) according to fast or slow time scales.
Materials and methods: We recorded EEG in 28 participants while they performed a procedural learning task. Participants learned which stimulus is associated to which response. Some S-R pairs were repeated throughout the task. The experiment consisted of 16 blocks, where eight blocks showed new stimuli, and eight blocks (interleaved) showed recurring stimuli. This allowed us to disambiguate the fast time scale (i.e. number of times one saw a stimulus in a block) from the slow time scale (i.e. the number of blocks completed so far).
Results: Behaviorally, both RT and accuracy improve (resp. decrease and increase) over both fast and slow timescales. Neurally, theta significantly decreases following the slow timescale, whereas alpha instead significantly increases along the fast timescale.
Conclusion: We demonstrate that theta and alpha both play a role during procedural learning, but operate at different time scales. Specifically, our results suggest that theta and alpha may operate on slow and fast time scales, respectively.

Disclosures: P. Huycke: None. **P. Verbeke:** None. **C. Boehler:** None. **T. Verguts:** None.

Digital Abstract Session

P353. Associative Learning

Program #/Poster #: P353.03

Topic: H.10. Human Learning and Cognition

Support: Simons Center for the Social Brain at Massachusetts Institute of Technology, Cambridge, MA
Simons Foundation for Autism Research Initiative in New York City, NY

Title: Prediction in Autism Spectrum Disorder: What Does the Empirical Evidence Say?

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Abstract: According to a recent influential proposal, several phenotypic features of autism spectrum disorder (ASD) may be accounted for by differences in predictive skills between autistic and neurotypical individuals. In the past five years, over 700 studies have explored this possibility. In this presentation, we will describe the results from a systematic review of the subset (n=47) that have used empirical methods to directly test this hypothesis. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to identify these articles, and assessed the results based on two observable aspects of prediction: learning a pairing between an antecedent and a consequence and responding to an antecedent in a predictive manner. The aggregated results highlight distinct differences in both predictive learning and predictive response. Studies showing differences in habituation and perceptual adaptation suggest low-level predictive processing differences in ASD. Studies documenting differences in learning predictive pairings indicate challenges in detecting such relationships, especially when predictive features of an antecedent have low salience or consistency. These challenges may account for the observed differences in the influence of predictive priors, in spontaneous predictive movement or gaze, and in social prediction. These results point to several promising avenues for future research. These include studying prediction within naturalistic contexts, assessing the effect of prediction-based interventions, and testing multiple types of prediction within the same individuals, which may help establish a link between low-level processing differences and predictive learning impairment.

Disclosures: J. Cannon: None. **A.M. O'Brien:** None. **L. Bungert:** None. **P. Sinha:** None.

Digital Abstract Session

P353. Associative Learning

Program #/Poster #: P353.04

Topic: H.10. Human Learning and Cognition

Support: NSERC Discovery Grant (RGPIN-2017-06753) to MLM

Canada Foundation for Innovation and Ontario Research Fund (36601) to MLM
Brain Canada Future Leaders in Canadian Brain Research Grant to MLM
NSERC CGS-M Grant to EMH

Title: Investigating hippocampus sensitivity to learning sequence in a categorization task using a neural network model

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Abstract: Introduction. Category learning is key to navigating an information-rich world. However, many categories contain exceptions that violate a general rule. Previous research has implicated hippocampus (HC) in rule-plus-exception (RPE) category learning, but limited work has explored hippocampal sensitivity to changes in learning sequence. Specifically, does delaying the introduction of exception stimuli (ES) in an RPE category learning task impact learning outcomes? We predicted that participants who were able to learn the rule before learning ES would be better able to detect and properly categorize ES. The delayed introduction of ES would elicit surprise, prompting the formation of distinct, pattern-separated memories for ES that would improve subsequent ES categorization. We explored HC's role in this manipulation using an anatomically inspired neural network model. **Methods**. Ninety-three participants completed the experiment. Participants sorted eight flower stimuli that varied across three binary-valued dimensions. Two stimuli were defined as category prototypes and were maximally dissimilar. Rule-followers differed from their category's prototype along one dimension, and ES differed from their category's prototype across two dimensions (i.e., they were more similar to the prototype of the opposite category). Participants completed three learning blocks with 48 trials. Full feedback was provided after each trial. Participants were randomly assigned into one of two conditions. In the "early exceptions" (EE) condition, participants viewed ES in all three learning blocks. In the "late exceptions" (LE) condition, participants were not exposed to ES until the second learning block. Following the learning blocks, participants completed a "no-feedback" (NF) block with 48 trials. This task was then simulated with a previously developed neural network model of HC to assess HC's sensitivity to trial order. **Results**. In the behavioural experiment, ES categorization improved significantly more with repetition in the late condition than in the early condition throughout the learning blocks ($P<.001$). ES categorization accuracy was also higher in the NF block for the LE condition ($P=.001$). These results were replicated by the model: ES categorization accuracy in the LE condition was significantly higher than in the EE condition ($P=.005$). **Conclusion**. This work offers an empirical demonstration of how learning sequence effects performance in a category learning task. Moreover, we provide novel computational evidence of HC's sensitivity to learning sequence in an RPE task that lends further support to HC's broad role in cognition.

Disclosures: E.M. Heffernan: None. **M.L. Mack:** None.

Digital Abstract Session

P354. Human Cognition

Program #/Poster #: P354.01

Topic: H.10. Human Learning and Cognition

Title: Flexible frameworks of thought advance creativity and innovation

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Abstract: From children building a pillow fort to technology giants in Silicon Valley, creativity is overwhelmingly apparent in learning and innovation. To uncover factors that modulate the generation of new ideas, we investigated creativity as a flexible ability rather than a fixed trait. To do this, 133 human subjects were randomly assigned to hypothetically adopt an eccentric mindset, a simple mindset, or to the control condition in which no instructions regarding mindset were given. An Alternate Uses Task (AUT), in which subjects generated as many uses as possible for a series of ten everyday objects, was used to measure divergent thinking ability under the assigned mindsets. Hypothetically adopting an eccentric mindset enhanced subjects' divergent thinking ability, while no difference was found between adopting a simple mindset or receiving no instructions regarding mindset. These findings shed light on the bounds of divergent thinking. We conclude that the framework in which humans generate new ideas is flexible and can be modulated by mindset. Moreover, these findings lay a foundation for the development of interventions that may hold power in advancing humans' ability to generate new ideas. Further, we are using functional near-infrared spectroscopy (fNIRS) to investigate the neural networks and time course of activation of the brain regions underlying creative thought. By contrasting activation patterns observed during completion of the AUT to those evoked by a control task in which subjects name the characteristics of a set of objects, we are able to determine the neural correlates of divergent thinking. However, our collection of neural data is currently paused due to the COVID-19 environment. This study is innovative in its use of stimuli that reflect the way humans encounter information in the real world, as well as its ability to collect detailed behavioral responses.

Disclosures: N.C. Catanzarite: None. **K.N. Dunbar:** None.

Digital Abstract Session

P354. Human Cognition

Program #/Poster #: P354.02

Topic: H.10. Human Learning and Cognition

Title: Test-retest, internal consistency, and split-half reliabilities of the Gibson Assessment of Cognitive Skills, a brief tool for screening cognitive function across the lifespan

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Abstract: Human cognition researchers need a rapid tool for assessing cognitive skills. Robust tools to quickly screen performance across the lifespan on primary cognitive skills or that can be administered and scored by non-clinical research personnel are in high demand. The aim of the current study was to examine reliability of the Gibson Assessment of Cognitive Skills (GACS), a paper-based, brief cognitive screening tool for children and adults measuring working memory, processing speed, visual processing, fluid reasoning, and three auditory processing constructs: sound blending, sound segmenting, sound deletion along with work attack skills. The sample (n = 103) for the current study consisted of children (n = 73) and adults (n = 30) between the ages of 6 and 80 (M = 20.2, SD = 17.7), 47.6% female, and 52.4% male. Analyses of test data included calculation of internal consistency reliability, split-half reliability, and test-retest reliability. Internal consistency reliability was conducted using JMetrik's Item Analysis procedure which produced a coefficient alpha for each subtest. Split-half reliability was determined by dividing the test items into odd and even halves and running Pearson's correlations in JMetrik for each subtest and applying a Spearman-Brown formula to each one. To determine test-retest reliability, we used SPSS to run Pearson's correlations between the two test administrations spaced one week apart. A regression analysis in SPSS examined differences in subtest scores based on sex. Overall coefficient alphas range from .80 to .94, producing a strong source of internal consistency reliability evidence. The split-half reliability coefficients ranged from .83 to .96 overall, producing a strong second source of reliability evidence. Across all ages, the test-retest reliability coefficients ranged from .83 to .98. For adults ages 18 to 80, test-retest reliability coefficients ranged from .73 to .99. For children ages 6 through 17, test-retest reliability coefficients ranged from .89 to .97. All correlations were statistically significant at $p < .001$, indicating strong test-retest reliability and stability across administrations. Post hoc analysis of sex differences found no statistically significant differences in mean scores on any subtest. The evidence collected for the current study suggests that the GACS is an accessible and reliable brief screening tool for assessing cognitive skill performance across the lifespan. Each subtest was stable over time, indicating it is reliable method for assessing performance in pre-post intervention studies as well as longitudinal studies with cognition outcomes.

Disclosures: **C. Ledbetter:** Other; Gibson Institute. **A.L. Moore:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Gibson Institute.

Digital Abstract Session

P355. Timing and Temporal Processing

Program #/Poster #: P355.01

Topic: H.10. Human Learning and Cognition

Support: BBSRC BB/R01583X/1
NIH-NIDA (R01-DA048096)
NIH-NIMH (R01-MH121099)
NIH-NINDS (R01-NS092701)

Title: Sub-second dopamine fluctuations in human striatum and their role in trial-to-trial variations in interval timing.

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Abstract: As a factor critical to most forms of learning, behaviour, and sensory-motor processing, time is a fundamental dimension of human experience. However, even though the ability to perceive time belongs to the most important and basic functions and the timing of intervals is a ubiquitous daily task, our timing estimates are notoriously variable and prone to biases. Multiple strands of evidence implicate dopamine systems in interval timing, including the pharmacologically induced altered temporal performance or poorer temporal precision in clinical populations with aberrant striatal dopamine profiles such as the Parkinson's disease or schizophrenia. Here, we investigated how striatal dopamine tracks trial-by-trial variations in interval timing in a sample of patients with Essential tremor undergoing deep brain stimulation treatment. Using fast scan cyclic voltammetry (FSCV), we recorded the in vivo electrochemical signal (at 10 Hz) during completion of a visual temporal bisection task (stimulus range: 500 - 1100 ms). On each trial, patients classified the duration of a stimulus based on its similarity to short or long reference intervals which they learnt prior to the testing phase. The behavioural responses yielded standard psychophysical measures of temporal bias and precision. The FSCV approach additionally involved in vitro experiments which generated recordings with known concentrations of dopamine and other analytes for the purpose of cross-validated training of an elastic net regression model for optimal dopamine signal prediction. This model was subsequently applied to infer human dopamine concentrations. Our primary analyses and predictions are based on a similar study reporting on midbrain dopamine tracking of temporal performance in rodents. Accordingly, we predicted that the human data would show analogous trends with high and low dopamine concentration levels associated with a tendency to under- and over-estimate temporal intervals, respectively. To evaluate this prediction, we will divide behavioural responses into high, medium, and low terciles of the distribution of dopamine response following the end of the stimulus interval. We expect that the psychometric function

fitted to low-tercile trials will show a leftward shift reflecting temporal overestimation whereas that fitted to the high-tercile trials will show a rightward shift reflecting temporal underestimation. If these predictions hold, these results will help to advance current understanding of the neurochemical bases of fluctuations in time perception.

Disclosures: **R. Sadibolova:** None. **E. DiMarco:** None. **A. Jiang:** None. **D. Terhune:** None. **K.T. Kishida:** None.

Digital Abstract Session

P356. Language and Speech

Program #/Poster #: P356.01

Topic: H.11. Language

Support: Dr. Stanley Ho Medical Development Foundation
Innovation and Technology Fund (Hong Kong) #ITS/067/18

Title: Neural responses to speech predict future language and communication development in healthy infants

Authors: *P. C. M. WONG[1], C. M. LAI[1], P. H. Y. CHAN[1,2], T. F. LEUNG[2], H. S. LAM[2], G. FENG[1], A. R. MAGGU[1,3], **N. NOVITSKIY**[1];
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Abstract: Spoken language development requires support of the structures along the auditory neural pathway from the auditory brainstem to the cortex. Children's early language development is highly variable despite growing up in the same language environment. We hypothesize that one source of this behavioral variability is physiological variability in the auditory neural system across individuals. To test this hypothesis, EEG responses were collected from 118 Cantonese-learning infants (1-12 months of age) while they listened to three speech stimuli, that differed principally in pitch, during natural sleep. Between 3 to 16 months after the initial EEG recording, their language and communication skills were assessed using the Words and Gestures form of the Chinese Communicative Development Inventories – Cantonese version (CCDI-C). Predictive models were constructed via support vector machine classification with cross-validation to classify infants into either two or three groups. In the two-way classification, children were classified into those with future CCDI-C scores below 25th percentile and those above 25th percentile for their age and sex group, while the three-way classification classified them into <25th, 25th – 75th, and >75th percentile groups. Age-corrected EEG data that included both early- and late-latency neural responses to speech such as FFR pitch strength and P1 latency as well as gestational age and birth weight were used as model inputs. The statistical significance of the predictive model was calculated as the proportion of the randomly permuted data above the mean of the model performances across 10,000 bootstrapping iterations. Across the CCDI-C

measures, the mean area under the receiver operating characteristic curve of the predictive models of early gestures, later gestures, vocabulary comprehension and vocabulary production were respectively at 0.92 ± 0.030, 0.91 ± 0.028, 0.90 ± 0.034, and 0.89 ± 0.04 for the two-way classification, and 0.88 ± 0.041, 0.88 ± 0.33, 0.85 ± 0.046 and 0.85 ± 0.049 for the three-way classification (ps<0.001). These results demonstrate a strong association between functions of the auditory neural pathway and children's future language outcome, to the extent that individual-level outcome prediction is possible and within the range of clinical utility. Early prediction enables caregivers and clinicians to plan for early intervention should it be needed. Measuring neural responses to speech coupled with language outcome prediction models is a potential avenue for early prediction and personalization of preventive early intervention for those infants who are susceptible to communication disorders.

Disclosures: **P.C.M. Wong:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); the founder of a startup company supported by a Hong Kong SAR Government startup scheme for universities. **C.M. Lai:** None. **P.H.Y. Chan:** None. **T.F. Leung:** None. **H.S. Lam:** None. **G. Feng:** None. **A.R. Maggu:** None. **N. Novitskiy:** None.

Digital Abstract Session

P356. Language and Speech

Program #/Poster #: P356.02

Topic: H.11. Language

Support: the Leading Initiative for Excellent Young Researchers, MEXT, Japan

Title: Revealing factors of subjective impressions affecting learnability of novel concepts using the semantic differential method

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Abstract: In knowledge acquisition or learning of the human, we actively select an item to be acquired. However, especially for the learning of abstract items such as semantic concepts, so far, attributes characterizing the items that are selectively learned better has not been studied in depth. To reveal such attributes, we conducted an experiment in which human subjects were asked to learn novel compositional words that should mean new semantic concepts. The novel compositional words used in the present study were constructed by randomly combining two two-character Japanese kanji nouns. We investigated relationships between the learnability and the features of each compositional word that were quantified based on the semantic differential (SD) ratings of it. The experiment was composed of the following five tasks: 1) flash presentation task, 2) N-back working memory task, 3) recognition memory test, 4) SD rating task, and 5) questionnaire survey of the Schwartz's core values. In the flash presentation task, 20 target and 20 dummy novel compositional words were presented to the subjects. This was

followed by the N-back task to wash the short-term memory of the compositional words. Next, we tested the recognition memory of the target compositional words. Then, we conducted the SD rating task in which the subjects were required to rate each target compositional word with 40 bipolar adjective scales. Finally, we investigated the individual attributes of values using the Schwartz's core value survey. We applied the factor analysis into the data of the SD ratings and found the following SD factors: "badness", "farcicality", "activity", "simplicity", "innovativeness", "importance", "beauty", and "bigness". One factor was empty. We also applied the same analysis to the data of the Schwartz's core value survey and identified three factors that roughly correspond to the "Social Focus", "Openness to change", and "Self-Enhancement", respectively. Using the linear model analysis, we investigated relationships between memory confidence of each compositional word and the SD and value factors. The values of coefficients corresponding to "badness", "farcicality", and "importance" factors are significantly positive. Importantly, the coefficient of the interaction between the "badness" SD factor and the "Openness to change" value factor is significantly negative. Those results suggest that human selectively learn important, bad, and/or farcical information to construct knowledge.

Disclosures: H. Kurashige: None. **J. Kaneko:** None.

Digital Abstract Session

P356. Language and Speech

Program #/Poster #: P356.03

Topic: H.11. Language

Title: Exploring Structural Brain Changes Induced by Vocabulary Learning of a Second Language: A Short Longitudinal MRI Study.

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Abstract: A number of neuroimaging studies have reported structural neuroplasticity as a result of life-long and short period language use and exposure. To this date, results have been inconsistent across studies, with variability in terms of the location and directionality of changes. We examined the structural brain changes regarding volume of the structures and cortical thickness, as a result of learning 132 selected words in a second language (L2) during four weeks. In this study, 24 healthy monolingual participants, matched for gender, age, motivation and previous language learning experience participated in a four week computerized audiovisual L2 vocabulary training for 15 minutes a day. Participants were scanned after the first week (Phase I; Accuracy rate: 35%; low proficiency level) and four week (Phase II; Accuracy rate 98%; high proficiency level). Behavioral assessment at phase I and II included confrontation naming of the vocabulary learnt during the training. Accuracy rates and response times were compared after the first week and the fourth week. Freesurfer was used to measure cortical thickness and volumetrics of the cortical and subcortical areas, using the default setting. The

output of cortical thickness and volumes of cortical and subcortical structures recorded at phase I and II were compared using SPSS. Behavioral measurements including accuracy rates and response times (RT) confirmed consolidated learning (RT_{T1}= (M = 2.1, S = 0.32); RT_{T2}=(*M* = 1.7, SD = 0.23), $t(12) = 4.52$, $p = .001$. Cortical thickness and volumetric measures reflected non-significant changes across phases. Our findings suggest that three weeks of vocabulary learning is not enough to make any significant structural brain changes. Compared with the functional changes reported previously, these findings reflect functional changes come before structural changes, which require longer or more intensive practice. Better understanding of how structural changes occur can have implications in teaching a second language, as well as in developing successful intervention plans for patients with aphasia in which the duration, frequency and intensity of language therapy is optimal.

Disclosures: A.E. Carrillo: None. **Z. Yang:** None. **L. Ghazi-Saidi:** A. Employment/Salary (full or part-time):; UNK, CB3. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NU-System. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UNK.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.01

Topic: H.11. Language

Support: NSF Grant 1607441
NSF Grant 1923144

Title: Modeling pronoun resolution in the brain

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Abstract: One unique machinery of human language is reference, that is, using a linguistic symbol such as pronouns to pick out certain entities in the discourse context. We typically have no difficulty linking a pronoun to its antecedent during language comprehension, yet the neural computations underlying this linking process remain elusive. To achieve a detailed, process-based understanding of pronoun resolution, we utilize computational models that lay out specified and carefully thought-out steps to achieve pronoun resolution. By evaluating the cognitive validity of these models against human brain activity, we provide insights on the constituting elements and their interactions during the cognitive process of pronoun resolution. We selected three symbolic models that formalize three influential theories on pronoun

resolution. The syntax-based Hobbs model implements the classic Binding Theory in formal linguistics, which states that pronouns cannot be coindexed with antecedents in the same clause. The discourse-based Centering model implements the Centering Theory that views pronominalization as a means to achieve discourse coherence. The memory-based ACT-R model conforms to the salience account for pronoun resolution, and selects the most highly-activated entity in the working memory as the antecedent of the pronoun. In addition to the knowledge-based models, we also included one data-oriented deep neural network model that learns a statistical pattern to cluster coreferential mentions from a labeled dataset. We correlated the four model predictions with brain activities during pronoun resolution. We recorded the BOLD signals while participants listened to a 100-minute audiobook of "The Little Prince" in the fMRI scanner. We collected data from English and Chinese speakers using the same paradigm to test whether linguistic typology would influence the strategies for pronoun resolution. To further explore the temporal dynamics of the model fit, we also collected an MEG dataset while English speakers listened to a 12-minute audio excerpt from the YouTube channel "SciShow Kids". We applied both multivariate RSA and univariate GLM analyses to compare the four models' relatedness to the BOLD responses and the source-localized MEG data time-locked at each third person pronoun in the narratives. Our combined results suggest that the memory-based ACT-R model best explains the neural signatures for third person pronoun processing, primarily localized at the left middle temporal gyrus (LMTG) at around 300-400 ms after the onset of the pronouns. We propose a domain-general mechanism for pronoun resolution that resembles memory retrieval.

Disclosures: J. Li: None. **W. Luh:** None. **L. Pylkkänen:** None. **Y. Yang:** None. **J. Hale:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.02

Topic: H.11. Language

Title: Convergence of Heteromodal Lexical Retrieval in the Lateral Prefrontal Cortex: A Brain Tumor Model

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Abstract: Lexical retrieval requires selecting and retrieving the most appropriate word from the lexicon to express a desired concept. Prior studies investigating the neuroanatomic underpinnings of lexical retrieval used lesion models that rely on stereotyped vascular distributions, functional neuroimaging methods that lack causal certainty, or direct electrical stimulation which is confounded by anesthesia and narrow cortical exposures. Furthermore, only a few studies have

probed lexical retrieval with tasks other than picture naming and have yielded conflicting results regarding the role of the temporal lobe as a convergence zone for both visual and auditory inputs. In the present study, we set out to identify areas of the brain required for heteromodal lexical retrieval by implementing a lesion-symptom model in patients with cytoarchitecturally disruptive brain tumors in perisylvian language areas. We asked fifty-three patients with lesions encompassing dominant frontal, temporal, and anterior parietal areas to perform four language tasks: picture naming, text reading, auditory naming, and describing line drawings with correct syntax. A subset of participants also underwent the Quick Aphasia Battery, which provides a validated measure of lexical retrieval via the word finding subtest. Generalized linear modeling and principal components analysis revealed multicollinearity between picture naming, auditory naming, and word finding, implying redundancies among these linguistic measures. Support vector regression lesion-symptom mapping across participants tested whether and where lesioned voxels were associated with lower accuracies on each of the four language tasks. We found that both picture naming and auditory naming survived cluster-level corrections. Specifically, lesions within overlapping clusters of 8,333 voxels and 21,512 voxels in the left lateral PFC were predictive of impaired picture naming and auditory naming, respectively. Notably, in contrast to prior studies that used correlational functional imaging techniques, we did not find a significant association between lesions in the temporal lobe and lexical retrieval task performance. Overall, these findings provide novel support for models that posit convergence of heteromodal lexical retrieval processes within the PFC. They also illustrate the feasibility of lesion-symptom mapping in patients with cytoarchitecturally disruptive brain tumors.

Disclosures: **A.A. Aabedi:** None. **S. Kakaizada:** None. **J.S. Young:** None. **O. Wiese:** None. **C. Valdivia:** None. **S. Krishna:** None. **M. Berger:** None. **D.H. Weissman:** None. **D. Brang:** None. **S.L. Hervey-Jumper:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.03

Topic: H.11. Language

Support: NIH Grant F32 DC018200
NIH Grant R01 NS109367
Leon Levy Fellowship in Neuroscience

Title: Neural mechanism underlying speech production during delayed auditory feedback

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Abstract: Monitoring the vocal output during speech production allows the detection and correction of vocalization errors in real-time, however the neural mechanism underlying this auditory feedback control of speech is poorly understood. We obtained electrocorticography

recordings from human subjects while they read aloud words and sentences during delayed auditory feedback. Subjects slowed down their speech rate to compensate for the delay and this behavioral effect was stronger for sentences. Neural responses in superior temporal, supramarginal, inferior frontal and dorsal prefrontal regions were both enhanced in amplitude and extended in duration for large delays reflecting the error signal caused by altered feedback and the subsequent longer articulation. Response enhancement was stronger across the speech network for sentences when delayed feedback had a stronger disruptive effect on speech. Our results highlighted dorsal precentral gyrus as a critical region for auditory feedback control of speech, which showed selective response enhancement during slowed down speech and was recruited much earlier compared to other vocal motor sites. Furthermore, our subjects performed an auditory repetition task, which we used to identify auditory sites that show suppressed responses during speaking compared to listening. Our results revealed that sites that show stronger auditory suppression also show stronger response enhancement to delayed feedback, providing evidence for a shared mechanism between speech-induced auditory suppression and sensitivity to altered feedback in humans.

Disclosures: **M. Ozker Sertel:** None. **W. Doyle:** None. **O. Devinsky:** None. **A. Flinker:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.04

Topic: H.11. Language

Support: NIH: R01EY025978
Emory University Research Council

Title: Neural basis of sound-symbolic pseudoword-shape correspondences

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Abstract: Sound symbolism is a form of crossmodal correspondence that refers to a non-arbitrary mapping between the sound of a word and its meaning. Research has mostly concentrated on the mapping of sound to shape, in which people assign auditory pseudowords, like “moh-loh” and “keh-teh”, to rounded and pointed visual shapes, respectively. However, the neural basis of this sound-symbolic correspondence is not well understood. Here, we used functional magnetic resonance imaging (fMRI) to investigate neural representations of the sound-to-shape correspondence. Participants (n = 24) were presented with audiovisual stimuli consisting of a visual shape (rounded or pointed) and an auditory pseudoword (“moh-loh” or “keh-teh”) that were either congruent (e.g., rounded/moh-loh) or incongruent (e.g., pointed/moh-loh) with respect to the sound-symbolic correspondence. Blood oxygen level-dependent (BOLD) responses were estimated using the general linear model with each trial modeled as a boxcar of

variable duration to control for differences in response time across conditions. All reported analyses were corrected for multiple comparisons at the group level. A univariate contrast showed greater activation in the left precentral gyrus for incongruent relative to congruent stimuli and greater activation in the right caudate nucleus for congruent relative to incongruent stimuli. Searchlight multivariate pattern analyses (MVPA) were then conducted after factoring out univariate effects. Higher above-chance classification of the audiovisual stimuli was found in the pars opercularis of the left inferior frontal gyrus (Broca's area) and the right middle occipital gyrus when the stimuli were congruent across modalities, relative to when the stimuli were incongruent. Cross-classification analyses showed that while visual and auditory cortices robustly differentiated visual or auditory stimuli, respectively, regardless of audiovisual congruency, the left precentral gyrus, the right thalamus, and the right caudate distinguished congruent from incongruent stimulus pairs, independent of the visual or auditory features. Taken together, these findings indicate distinct roles for different brain regions during the processing of sound-symbolic correspondences. For instance, visual and auditory cortices encode visual and auditory stimuli, respectively; the left precentral gyrus and right caudate nucleus are associated with audiovisual congruency; and Broca's area distinguishes audiovisual stimuli better when they are congruent than incongruent. Thus, sound symbolism engages neural processes in sensory, motor and language domains.

Disclosures: D.A. Barany: None. **S.A. Lacey:** None. **L.C. Nygaard:** None. **K. Sathian:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.05

Topic: H.11. Language

Support: National Institute of Neurological Disorders and Stroke (NS098981)

Title: Referential semantics implicates medial parietal cortex and hippocampus in electrocorticography

Authors: *E. MURPHY, K. FORSETH, *P. ROLLO, Z. ROCCAFORTE, N. TANDON; Univ. of Texas Hlth. Sci. Ctr., Houston, TX

Abstract: The ability to establish referential relations is a hallmark of language. While the formal processes of semantic computation have received detailed theoretical treatment, a compositional neural architecture for language remains at a distance. Here, we isolate the mapping of sentential representations onto successful versus unsuccessful referents. Currently, there is no consensus in the field about the neural localization of referential composition. We used intracranial electrocorticography with penetrating depth and surface grid electrodes with high spatiotemporal resolution to address this issue. Patients (n=71) were implanted with depth and surface grid electrodes (n=13298) for the evaluation of medically refractory epilepsy, with full coverage over language-dominant cortex. They were asked to quickly and accurately

articulate the name of common objects in response to descriptions (range: 3-12 words; average: 6.5; average response time: 1200ms), presented in rapid serial visual presentation (500ms per word). The final word in each sentence was either congruent or incongruent ("*A person at the circus who makes you* [*laugh/commute*]") (80 trials per condition). We analyzed broadband gamma (70-150Hz) activity to index cognitive engagement of cortical substrates. We identified greater gamma activity at the onset of the final word in congruent trials across left inferior frontal regions (~150ms post-onset), medial parietal cortex (~250ms) and hippocampus (-400ms post-onset). Medial parietal regions mediate action encoding, which possibly explains these effects since incongruent items often involved impossible/failed actions (e.g. "*Something that grows on your* [*face/name*]"). These regions are also part of the default network, which shows activity increases during endogenous attentional tasks, with representational search likely being greater for referential relations. Research into this fronto-parietal referential network will contribute to rehabilitative solutions and neuro-prosthetic designs for individuals with reading deficits, and in particular patients with anomic aphasia and alexia. In addition, we provide the first intracranial analysis in a large patient cohort of the central component of natural language semantics - reference.

Disclosures: **E. Murphy:** None. **K. Forseth:** None. **P. Rollo:** None. **Z. Roccaforte:** None. **N. Tandon:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.06

Topic: H.11. Language

Title: Dynamics of superior temporal sulcus in self-generated speech in large-scale electrocorticography

Authors: *L. BULLOCK, K. J. FORSETH, N. TANDON;
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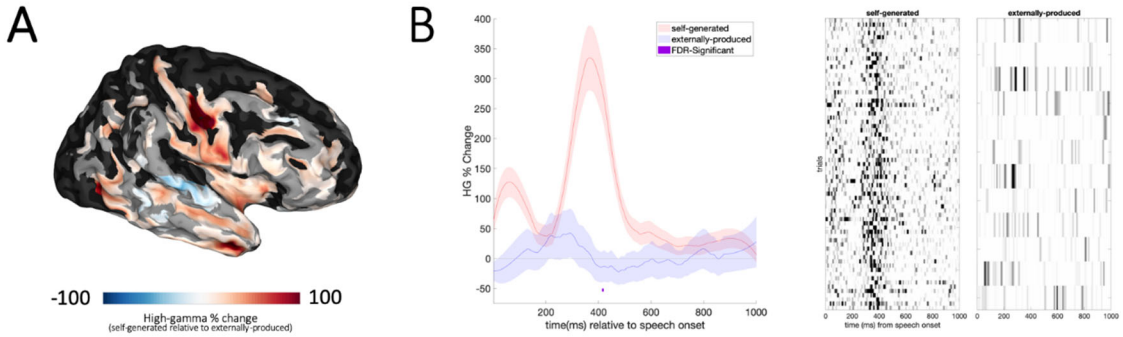
Abstract: To communicate effectively, humans readily recognize different voices in their environment. The means by which we perceive our own speech and that generated by another person are distinct in nearly all models of speech perception, but the mechanisms subserving this process remain unclear. While fMRI studies have implicated the right superior temporal sulcus (STS) in speaker recognition, we compile high-SNR intracranial EEG (iEEG) to paint a more detailed picture of the cortical dynamics of this region during the processing of self-generated speech. Epileptic patients (n=81) were implanted with electrodes (n=13218) for resective surgery evaluation. This included both stereotactic depth electrodes (n=8096) and cortical-surface grid electrodes (n=5122). Patients engaged in a picture-naming task in which they responded with the name of the object if the picture was intact, and "scrambled" if the image was incoherent. The latter condition served as the self-generated speech condition. In another cued-naming task, patients listened to pre-recorded definitions, many of which begin with the word "something".

The passive perception of this somewhat similar (fricative-initial, disyllabic) word served as the control condition of externally-generated speech. We first used surface-based mixed-effects multilevel analysis (SB-MEMA), with high-gamma activity (60-120 Hz) as a measure of cortical activity, to identify regions implicated in self-generated speech. We then narrowed our focus to the right STS, inspecting time-series high-gamma at individual electrodes and at the population level within the ROI.In addition to the expected increased activity in speech motor regions and suppressed activity in primary auditory cortex, we find right anterior STS (raSTS) is more active at articulation onset in the self-generated speech condition. Further, raSTS is re-engaged just after the offset of the first syllable (~300ms) through the end of the second syllable, remaining at least 30-40% higher for self-generated speech. See accompanying figure for tentative results.



Disclosures: L. Bullock: None. **K.J. Forseth:** None. **N. Tandon:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.07

Topic: H.11. Language

Title: Exploring the limitations of field potential recordings in electrocortocography; how referencing scheme, electrode size and inter-electrode distance confound level interpretability

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Abstract: Intrinsic to the analyses of electrocorticography (ECoG) are assumptions regarding the source and extent of neural activity a given electrode can detect. This problem of source localization is not new, and there are a diversity of methods in both human and animal models by which best estimations are implemented. Difficulties emerge when incorporating data across various electrode types and recording scales. These assumptions ultimately limit our understanding of what the informative resolution is when we record task-related human neural activity.
We explored these ambiguities across three scales of recording electrodes implanted in patients

monitored for intractable epilepsy (n=21). The largest are subdural electrodes (SDE; 3mm diameter and 3-4mm pitch (inter-electrode distance)), macro stereo-EEG electrodes (sEEG; 2mm length and 2mm pitch), and micro sEEG electrodes (0.5mm length and 1mm pitch). We compared activity bandpass filtered into theta (4-8Hz), alpha (8-15Hz), beta (15-30 Hz), narrowband gamma (30-60 Hz), and broadband high gamma (70-150 Hz) in the second following auditory stimulus onset. By fitting an exponential decay curve we estimate the full-width half maximum (FWHM) of the decay of signal cross-correlations over distance between electrodes.
The mean FWHM between SDE pairs (n=5152) was 18.7 ± 1.6 (Mean ± SE) and between macro-sEEG pairs (n=76646) was 23.2 ± 0.8 and between micro-sEEG pairs (n=471) was 24.1 ± 1.9. There was a significant effect of frequency band on the FWHM with gamma band activity showing significantly smaller spread across SDE and micro-sEEG electrode pairs. A pivotal confound of ECoG recordings is referencing scheme, and we compare correlation across multiple referencing conditions including common average, selective power, white matter, and bipolar references. We found FWHM of lower frequencies of macro-sEEGs to be most influenced by the referencing scheme, which suggests the referencing condition is pivotal to analyses which incorporate lower frequency ECoG recordings.
Invasive neural recordings provide a unique, high spatiotemporal window into human cognition, but the limitations intrinsic to this method must be considered. With continued progress in neuroengineering pushing advances in neural recording interfaces, the key question remains how best to integrate findings across varying recording scales. Our analyses not only explore caveats of field potential recordings across scale, but can also inform probe design, to eliminate redundancy and over-sampling of neural space while maximizing for signal to noise ratio.

Disclosures: **M.J. McCarty:** None. **O. Woolnough:** None. **N. Tandon:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.08

Topic: H.11. Language

Title: The crucial role of the mid fusiform cortex in lexical access

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Abstract: Resections of the temporal lobe for epilepsy or neoplasms often result in a decline in naming, especially for low frequency words. The resulting deficit can be socially disabling and impair the return of these patients to independent functioning. The exact substrates responsible for this cognitive loss are not known, and such knowledge might influence the surgical strategies to minimize language declines. We used voxel-based lesion symptom mapping (VLSM), which

allows for precise relation of cognitive function, measured as performance on a specific test, to anatomical substrate. In this study, we implement a multivariate VLSM with multimodal integration of lesion data with imaging to assess language function in in the dominant anterior temporal lobe. Data were obtained from 77 patients in whom a surgical procedure was performed for medication-resistant epilepsy originating in the dominant left-sided temporal lobe. This included either an anterior temporal lobe resection (n=51) or a mesial temporal ablation (n=26) for focal epilepsy. All patients underwent neuropsychological testing and MRI prior to and 6 months following surgery. Lesion masks were traced on postoperative MRI and aligned to a normative imaging space. The effects of preoperative scores and seizure outcomes (ILAE scores) were removed from the postoperative behavior scores via a linear regression prior to analysis. VLSM was then performed using a multivariate SVR model to assess postoperative neuropsychological test scores. Resulting Beta maps were converted to p values and corrected using a permutation-based cluster level correction. Lastly, we integrated an ecologically relevant language-based task fMRI (picture naming) with the VLSM results.
Beta maps from multivariate VLSM analysis revealed that the loss of basal temporal regions (mid and anterior fusiform gyrus and temporal pole) was associated with a prominent decline in Boston Naming Test (BNT) scores ($p<0.005$). Many of these voxels were spatially correlated with the susceptibility artifact on echo-planar imaging, perhaps explaining why this region has been under-appreciated as the locus responsible for postoperative deficits. This crucial contribution of the ventral temporal cortex in lexical access as shown using this multivariate VLSM approach should inform surgical approaches to this region.

Disclosures: **K. Snyder:** None. **C. Donos:** None. **P. Rollo:** None. **J. Breier:** None. **S. Fischer-Baum:** None. **N. Tandon:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.09

Topic: H.11. Language

Support: NINDS BRAIN Initiative Grant U01NS098981

Title: The neural correlates of modality-independent and modality-dependent semantic integration

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Abstract: Introduction: Current advances in the understanding of language and applications to patients with language disorders are limited by a lack of understanding of higher-order semantic

processes such as semantic integration. Semantic integration is the process of forming sequential relationships between concepts, such as perceiving the continuation of events by integrating information from event 2 with event 1. The regions involved in semantic integration remain unclear. Aims: The current research aims to address this limitation by analyzing intracranial neural recordings with high spatiotemporal resolution while 20 patients with intracranial electrodes for seizure monitoring in drug-resistant focal epilepsy performed a semantic integration task. Specifically, the aim was to identify the neural correlates of semantic integration by presenting pairs of events that followed a sequence (coherent) or disrupted a sequence (incoherent). Methods: The events were presented for three seconds on screen as sentences or images, with 95 unique pairs for each modality. Initially, regions that were more active (over 10% above a baseline of -400 to -100 ms before trial onset in 70-150 Hz) for the second stimulus compared to the first stimulus were identified at an FDR-corrected alpha level of .0099, particularly in the second time window (1500-2500 ms) which was selected to ensure completion of initial visual processing and reading. Results: For sentences and images, a wide network of frontal, parietal, temporal, regions was active during stimulus 2 for the first time window (500-1500 ms), with overlapping activation predominantly in visual areas for the second time window (1500-2500 ms). From 500-1500 ms, there was significant activation in parietal regions for images but not sentences and in frontal areas for sentences but not images. However, frontal electrodes in both sentences and images showed greater activation for stimulus 2 compared to stimulus 1. Parietal electrodes showed greater activation for stimulus 2 compared to stimulus 1, from 500-1500 ms for images and from 1500 to 2500 ms for sentences. Conclusions: The findings suggest potential roles for parietal and frontal regions in processes related to semantic integration. The time windows of activation may differ depending on the amount of time taken to process the stimulus, which may differ for images compared to sentences due to longer time for reading than visual processing.

Disclosures: **A. Medvedeva:** None. **O. Woolnough:** None. **P. Rollo:** None. **P.F. Dominey:** None. **N. Tandon:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.10

Topic: H.11. Language

Support: Air Force Office of Scientific Research research grants FA95501710218 and FA95502010118
Google Faculty Research Award
start-up funds in the Machine Learning Department at Carnegie Mellon University

Title: Functional magnetic resonance imaging and magnetoencephalography reveal different aspects of meaning composition

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Abstract: Understanding language in the real-world requires us to compose the meaning of individual words in a way that makes the final composed product more meaningful than the string of isolated words. For example, we understand the statement that ``Mary finished the apple'' to mean that Mary finished eating the apple, even though ``eating'' is not explicitly specified. This supra-word meaning is at the core of language comprehension, and its neurobiological bases and processing mechanisms must be specified in the pursuit of a complete theory of language processing in the brain.
The current work provides an operational definition of supra-word meaning by defining it as the multi-word meaning that is beyond the meaning of individual words. We further provide a computational representation of supra-word meaning using powerful neural network algorithms and a new approach to disentangle individual- from supra-word meaning.
We found that the supra-word meaning representation significantly predicts fMRI recordings in the bilateral anterior and posterior temporal lobes (ATL and PTL, FWE-corrected pvalue<0.05). Our finding poses questions for the theory that posits left PTL as primarily a site of lexical (i.e. word-level) semantics [1] and suggests a common substrate for lexical and combinatorial semantics. Strikingly, we found that the supra-word meaning representation did not significantly predict any MEG sensor-timepoints. Instead, the MEG recordings were significantly predicted by information unique to the two most recently-read words (FWE-corrected pvalue<0.01).
Our results suggest that fMRI recordings are sensitive to supra-word meaning, while MEG recordings reflect instantaneous processes related to both the current and the previously-read word. A likely candidate for this instantaneous process is the integration of a word with the previous context. MEG may be sensitive to the previously-read word because a word might take longer to process than the duration it is on the screen, or a word may constrain the processing of the following word, highlighting its relevant properties and aiding with composition.
The surprising finding that supra-word meaning is difficult to capture using MEG has implications for future neuroimaging research and applications where natural language is decoded from the brain. While high temporal imaging resolution is key to reaching a mechanistic level of understanding of language processing, our findings suggest that a modality other than MEG may be necessary to detect long-range contextual information.
[1] Peter Hagoort. “The meaning-making mechanism (s) behind the eyes and between the ears”. PhilTransRoSoc 2020

Disclosures: **M. Toneva:** None. **T. Mitchell:** None. **L. Wehbe:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.11

Topic: H.11. Language

Support: NSF 1912286

R01 NS109367

Title: Task-specific Network Dynamics in ECoG during Language Perception and Production

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Abstract: It is well established that resting state network dynamics emerge during cognitive tasks as well as rest. However, it is unclear how these connectivity patterns emerge and change over time during language related tasks and speech production. Here we use Electocorticography (ECoG), with a combined high signal to noise ratio, temporal and spatial resolution to investigate the functional connectivity (FC) during rest compared to language tasks. Seven adult epilepsy patients undergoing intracranial, pre-surgical evaluation completed a prompted rest task and 5 speech production tasks involving auditory (repetition, sentence completion, naming to definition) and visual (picture naming, reading aloud) modalities with matched stimuli across tasks. We used Pearson's r of the high-gamma (70-150 Hz) signal to generate FC correlations between all electrode pairs within subjects, for each task (epoched into stimulus perception and item production) as well as rest. Permutation testing (1000 iteration, $p<0.01$ threshold) was used to test for significant electrode pair FC (r values) change from rest. We find a distinct temporal pattern of network states from perception to production characterized by a significant network change from baseline during perception (across language tasks) followed by a reduction (L2 distance from baseline) during pre-articulation and finally a return to a network state closely matching perception (non-significant difference in L2 network distance). Surprisingly the perception and production network states were similar regardless of task modality. This was most striking during visual perception vs. auditory feedback during production, likely representing a task-specific network dynamic which is distinct from local high gamma changes.

Disclosures: J. Jeschke: None. **W. Doyle:** None. **O. Devinsky:** None. **A. Flinker:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.12

Topic: H.11. Language

Support: NIH R01 NS109367
NSF 1912286

Title: Distributed frontal cortex activity encodes prearticulatory single word retrieval

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Abstract: Speech production critically depends on frontal cortex activity to retrieve, plan, and execute speech utterances, but the timing and extent of cortical activation across different task demands, modalities, and the nature of the neural representations remain unknown. To investigate this, we employed a battery of five functional language tasks which prompt the subject to produce the same word through varying word retrieval routes: word reading, picture naming, auditory naming, auditory word repetition, and auditory sentence completion. We obtained direct cortical ECoG recordings in a cohort of 27 neurosurgical patients undergoing treatment for refractory epilepsy. We focused on high gamma (70 ~ 150 Hz) spectral responses shown to correlate with the spiking rate of underlying neuronal populations. We employed analyses based on specific regions of interest, unsupervised clustering of the data as well as Representational Similarity Analysis in order to investigate which areas are recruited and when in time stimulus identity is encoded. Based on anatomy, robust activity was evident in STG locked to perception for the three auditory tasks as well as pre- and post-central gyri activation locked to production. We found a pre-articulatory response profile in the posterior IFG (pars opercularis) and dorsal MFG, with significant activity prior to speech onset for all five production tasks.
While IFG and MFG activity was significant prior to speech, it varied greatly across tasks. In order to test if a more distributed representation, rather than specific anatomical regions, encode retrieval we applied non-negative matrix factorization, an unsupervised clustering technique. This technique revealed a pre-articulatory cluster distributed across frontal cortex. Unlike IFG and MFG alone, the distributed activity of the frontal cluster showed significant activity prior to speech similarly across all five tasks. We employed an RSA analysis in order to probe the nature of this distributed neural activity over time. The dissimilarity matrix of the frontal cluster was most correlated with word identity models prior to speech onset and with semantic models (word embedding vectors) locked to and during speech. Our results suggest that frontal cortex represents stimulus identity during retrieval in a distributed manner across frontal regions prior to and throughout speech production.

Disclosures: **L. Yu:** None. **D. Werner:** None. **O. Devinsky:** None. **A. Flinker:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.13

Topic: H.11. Language

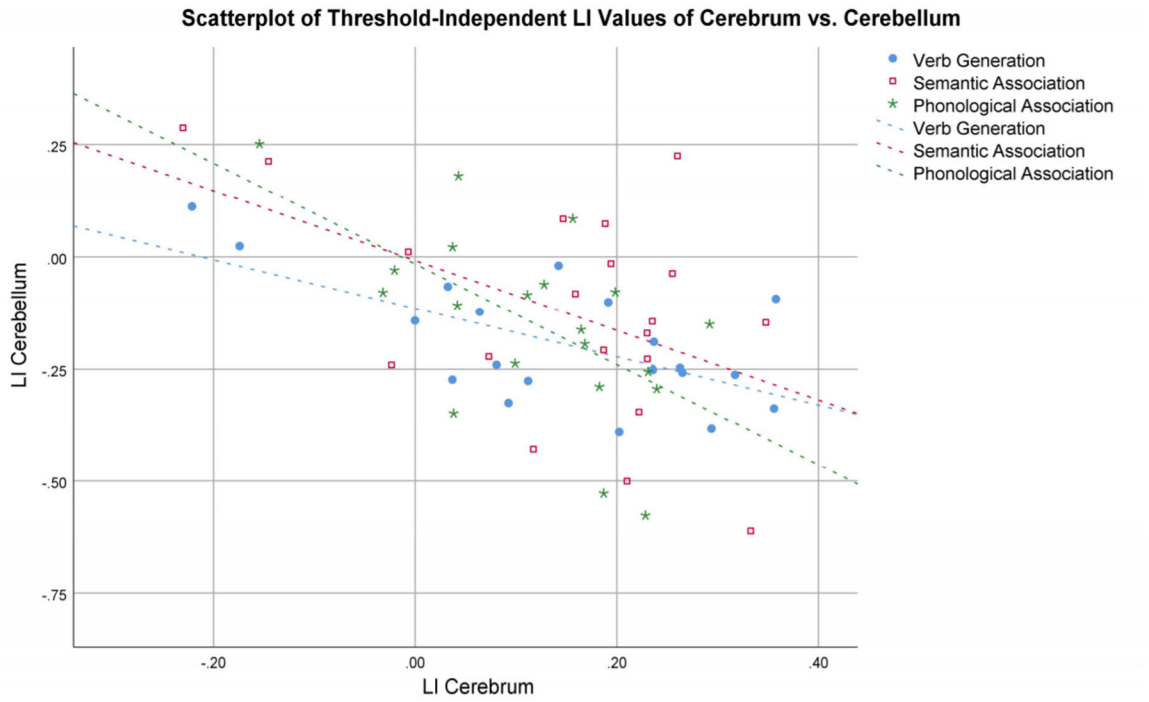
Support: FONDECYT Initiation into Research Study Nº 111150429
Beca ANID doctoral Nº 21201983

Title: BOLD activations in the cerebellum as an additional diagnostic feature for determination of language lateralization

Authors: ***I. R. THAKKAR**[1], L. ARRAÑO-CARRASCO[1], B. CORTÉS RIVERA[2], R. ZUNINO-PESCE[1], F. MERY-MUÑOZ[1], M. SMITS[3], C. MENDEZ-ORELLANA[1];

[1]Pontificia Univ. Catolica De Chile, Santiago, Chile; [2]Univ. Santo Tomás, Santiago, Chile; [3]Erasmus MC–University Med. Ctr., Rotterdam, Netherlands

Abstract: Postsurgical preservation of language is a focus in life-saving tumor removal surgeries. Thus, presurgical language localization is important in surgical resection planning. fMRI studies assessing language lateralization in patients mainly focus on the cerebrum. But recently, crossed cerebro-cerebellar BOLD activations during language paradigms have come to light as additional diagnostic features (Hubrich-Ungureanu et al, 2002; Mendez-Orellana et al, 2015). A major advantage of considering these contralateral cerebellar activations is that they remain undisturbed by cortical tumor presence. Currently, in the clinic, language lateralization determination based on BOLD is done through visual inspection by neuroradiologists. Recently, automatized calculation of lateralization index (LI) using the number of active voxels is gradually being adapted to the clinic. Yet, these techniques rely on the statistical threshold chosen during preprocessing. Here, we use a threshold-independent LI calculation technique (Branco, et al., 2016). Healthy subjects (n = 23) performed three language paradigms: a verbal fluency task, and button-press semantic and phonological association tasks. BOLD images were preprocessed using SPM and LI values were obtained separately for cortical and cerebellar activations. To test contralaterality between cerebral and cerebellar activations, Pearson's correlation was performed between their LI. Pearson's *r* for the three tasks was -0.63, -0.53, -0.63 respectively. Language activations were then assigned left-, right-, or bi-laterality depending on their LI. A McNemar test for all three tasks also showed significant contrast between cerebral and cerebellar laterality (p = .007, .019, .018 respectively). A Cohen's Kappa between laterality based on LI versus by neuroradiologists also showed fair agreement. Thus, substantial contralateral activations between the cortex and cerebellum were observed pointing to the possibility of cerebellar activations being used as additional language lateralization determination tools in the clinic.



Disclosures: I.R. Thakkar: None. **L. Arraño-Carrasco:** None. **B. Cortés Rivera:** None. **R. Zunino-Pesce:** None. **F. Mery-Muñoz:** None. **M. Smits:** None. **C. Mendez-Orellana:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.14

Topic: H.11. Language

Support: NIH RO1DC009659

Title: Network-level differences in the dual-stream language network in post-stroke aphasia: a resting-state fMRI study

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Abstract: The neurobiology of language has been studied for a century but remains highly debated. A dual-stream model has been proposed to represent the cortical networks involved in speech processes. However, it is not yet known if the dual-stream model represents an intrinsic brain network that exhibits functionally connectivity at rest, and the right hemisphere's ventral stream is still debated. It also is unclear how stroke affects the functional connectivity of the dual-stream model beyond the area of focal damage. To investigate these questions, 30

neurotypical participants (20-79 years, native English-speaking, right-handed) and 14 chronic left hemisphere stroke survivors (28-80 years, native English-speaking, right-handed) underwent resting-state functional MRI and T1-weighted structural MRI. SPM12 and in-house Matlab and R scripts were used to calculate: 1) the average functional connectivity within and between the bilateral streams within the dual-stream model in the control group, 2) compare this connectivity to other well-defined brain networks (i.e. default-mode and visual networks) and 3) group-level comparisons of functional connectivity between the control and stroke groups in each stream and hemisphere of the dual-stream network. Pearson correlations and pairwise t-tests indicate: The dual-stream model regions are significantly functionally correlated, including a bilateral ventral stream and left-lateralized dorsal stream. The functional connectivity within the dual-stream model is significantly higher than the functional connectivity within the default mode network, and the functional connectivity within the dorsal and ventral streams is significantly higher than between the dual-stream network and the comparison networks (default mode and visual). Two-sample t-tests indicate functional connectivity within the ventral stream and between ventral and dorsal streams is significantly lower in the stroke than the control group, including within the right ventral stream and the
right hemisphere homologue of the left dorsal stream. These preliminary results suggest regions depicted in the dual-stream model of speech processing represent an intrinsic, bilateral, cortical network. The left hemisphere stroke survivors exhibiting significantly lower resting-state functional connectivity not only in the left dorsal and ventral streams, but also in the right hemisphere's ventral stream and the right homologue of the left-lateralized dorsal stream, suggests that the neural disruptions affecting language abilities extend well-beyond the stroke's area of infarct, including contralateral (right hemisphere) disruptions.

Disclosures: H. Zhu: None. **A.N. LaCroix:** None. **M.C. Fitzhugh:** None. **E. DePinto:** None. **S. Mallapragada:** None. **C. Rogalsky:** None.

Digital Abstract Session

P357. Language: Neural Circuits and Mechanisms

Program #/Poster #: P357.15

Topic: H.11. Language

Support: Marie Sklodowska-Curie grant H2020-MSCA-IF-2017-795807 to G.L.-U.

Title: The gradient of population receptive field stimulus-dependence in ventral visual cortex

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Abstract: Regions of ventral occipito-temporal (VOT; Figure 1A) visual cortex are more responsive to written words than other categories of visual stimuli. We refer to these regions as

the VOT reading circuitry (VOTRC; Figure 1B, red clusters). The population receptive field (pRF) of a voxel is the portion of the visual field in which stimuli evoke a response. The pRFs of voxels in the VOTRC are stimulus-dependent (Le et al., 2017). Measured with word stimuli, the pRF centers are mainly confined to the central visual field; measured with checkerboard stimuli, the pRF centers span a wider range of locations, extending to the periphery. To understand this stimulus dependence, we analyzed pRFs in visual field-maps (V1-3, hV4, VO1) whose signals are the likely inputs to the VOTRC. We measured pRFs in 34 subjects (20 readers of English only; 14 readers of Hebrew and English) using the moving bar paradigm with several types of contrast patterns within the bar (checkerboards, English or Hebrew words of different sizes, false fonts). We found that stimulus dependence emerges gradually along the visual field map hierarchy. The stimulus dependence is small in visual areas V1 and V2, becoming more pronounced in V3, hV4, and VO-1 (see Figure 1C). The pRF center can be expressed in Cartesian [x,y] or Polar [eccentricity,angle] coordinates. The largest variation in stimulus dependence is seen in the eccentricity of the pRF center; the angle remains relatively constant. Given that the pRF position is stimulus dependent, it is important to use the relevant stimulus when using pRFs to model the visual components of different tasks. With an interest in reading development, it seems best to use word stimuli in a legible orthography. Establishing pRF properties using such stimuli in good readers may help understand the visual reading circuitry and provide a baseline for comparing responses in poor readers.

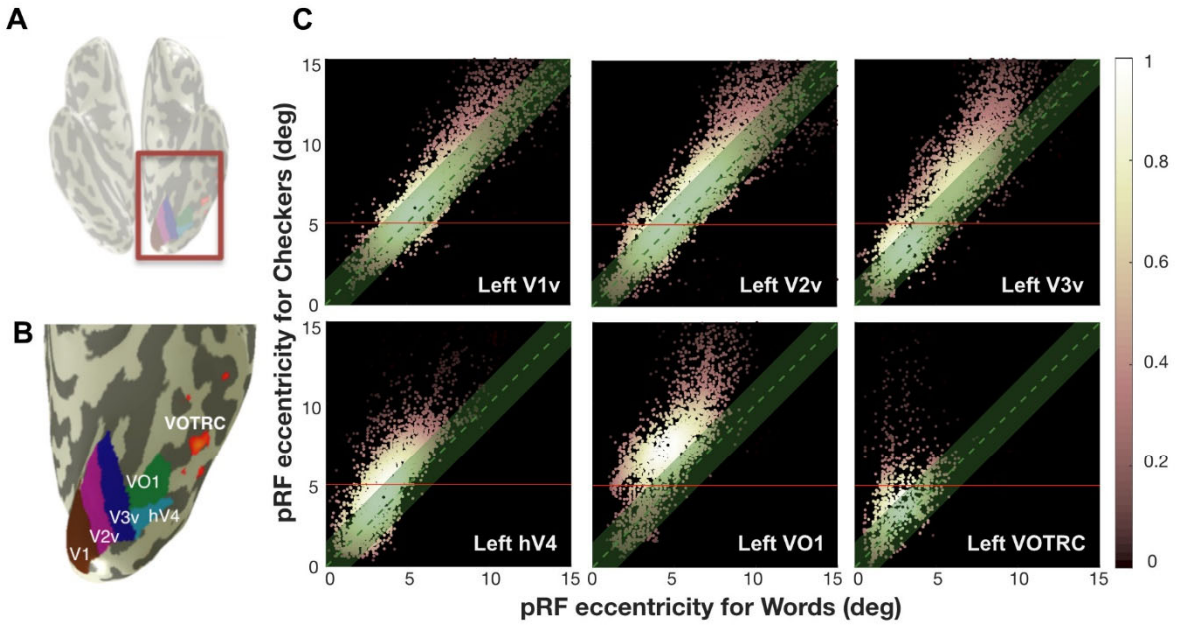

FIGURE 1. In the VOTRC, pRFs measured with word stimuli are more foveal with word stimuli compared to checkerboard stimuli.

A. Ventral view of an inflated brain surface, VOT inside red square. B. Detail of the left VOT, with the ventral maps V1v, V2v, V3v, hV4, VO1 and the VOTRC. C. The eccentricity of pRF centers for words versus the eccentricity for checkerboards for the left ventral V1, V2, V3, hV4, VO1 and VOTRC. The color bar indicates relative density of points; the green band express 5 deg around the identity line. The pRFs shown have > 20% variance explained.

Disclosures: **G. Lerma-Usabiaga:** None. **R. Le:** None. **C. Gafni:** None. **M. Ben-Shachar:** None. **B. Wandell:** None.

Digital Abstract Session

P358. Human Cognitive Development

Program #/Poster #: P358.01

Topic: H.12. Aging and Development

Support: Wu Tsai Neurosciences Institute Big Ideas Project
NIH 1 R21 EY030588-01

Title: Catch me if you can: Least myelinated white matter bundles develop fastest during early infancy

Authors: ***M. GROTHEER**, M. ROSENKE, H. WU, H. KULAR, F. R. QUERDASI, V. NATU, J. D. YEATMAN, K. GRILL-SPECTOR;
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Abstract: White matter myelination is one of the key hallmarks of infant brain development and abnormalities in myelination have been linked to a plethora of neurological and cognitive disorders. Early histological work has suggested heterogeneous myelin content of white matter bundles at birth and heterogenous myelination rates after birth. However, it is unclear if white matter that is less myelinated at birth continues to develop slower during infancy (starts-first/finishes-first hypothesis), or if it develops faster to catch up with the rest of the brain (catch-up hypothesis). To distinguish between these hypotheses, we collected diffusion (dMRI) and quantitative MRI (qMRI) longitudinally at birth (N=9), as well as, 3 month (N=10) and 6 months (N=10) after birth. Using the dMRI data, we developed a novel method for automated fiber quantification in individual infant brains (babyAFQ), which we validated by comparing to manually identified "gold-standard" bundles. From qMRI, we derived T_1, which is directly related to white matter myelination. In all bundles, we find a linear decrease of T_1, which suggests increasing myelination, from birth to 6 months of age (Fig 1a). Consistent with histology, we find differential T_1 at birth and differential rates of T_1 development during infancy across the white matter. Critically, we discovered a significant negative correlation between T_1 at birth and the rate of T_1 development both across bundles (Fig 1b) and within bundles along their length. These data suggest that less mature white matter at birth develops faster to catch up with the rest of the brain. This, in turn, leads to the equation of differences in myelination during early infancy, which may promote efficient communication across the entire brain.

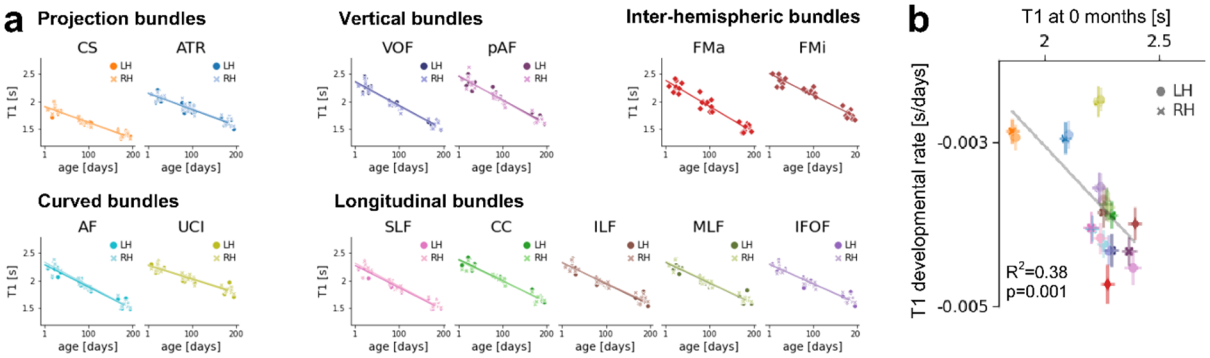

Figure 1. T_1 at birth and T_1 developmental rate are negatively correlated, supporting the catch-up hypothesis. a. Mean T_1 of each bundle decreases linearly from 0 to 6 months of age. Dots correspond to individual's left and right hemispheres. b. Across bundles, mean T_1 at birth and rate of T_1 development are negatively correlated.

Disclosures: **M. Grotheer:** None. **M. Rosenke:** None. **H. Wu:** None. **H. Kular:** None. **F.R. Querdasi:** None. **J.D. Yeatman:** None. **K. Grill-Spector:** None. **V. Natu:** None.

Digital Abstract Session

P358. Human Cognitive Development

Program #/Poster #: P358.02

Topic: H.12. Aging and Development

Support: New Faculty Startup Fund from Seoul National University
National IT Promotion Agency GPU award
Intel PRTI award

Title: Identifying Prepubertal Children with Risk for Suicides Using Deep Neural Network Trained on Multimodal Brain Imaging

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Abstract: Suicide is among the leading cause of death in youth worldwide. Early identification of children with high risk for suicides is key to effective screening and intervention strategies. Yet, little is known about the neural pathways to the clinical outcomes of suicide, such as suicidal ideation and attempts, and methods to screen youth with high risk for suicide. In this study, we tested what brain functional signals are associated with the risk for suicidality in young children. Based on the large, multi-site, multi-ethnic, representative, prospective developmental population study in the US, we trained the state-of-the-art interpretable deep neural network for tabular data, TabNet (https://github.com/dreamquark-ai/tabnet), on functional brain imaging. Our model shows the best model predicting suicidality contains several functional estimates of the

brain circuitry, such as intrinsic functional couplings among middle and inferior temporal gyrus, paracentral gyrus, and activation patterns during the emotional memory task among cingulate gyrus, inferior frontal gyrus. Of note, our interpretable neural network shows that these brain functional features appeared to interact with the known risk factors of childhood suicidality, such as depression and impulsivity. This study demonstrates the novel application of the interpretable deep neural network to childhood suicidal research, uncovering the complex interactions between psychological and neural factors underlying childhood suicidality.

Disclosures: G. ahn: None. **B. kim:** None. **J. Cha:** None.

Digital Abstract Session

P359. Aging: Behavior and Mechanisms

Program #/Poster #: P359.01

Topic: H.12. Aging and Development

Support: NIH R00 AG047282
AARGD-17-529552

Title: Differential Influences of Alcohol by Type and Frequency on Body Composition in Older Adults: A UK Biobank Study

Authors: *B. A. LARSEN[1], B. S. KLINEDINST[2], T. WOLF[3], C. PAPPAS[2], S. T. LE[2], N. F. MEIER[4], Y.-L. LIMF[5], B. E. FRIZELL[6], L. LANNINGHAM-FOSTER[2], A. A. WILLETTE[2]; [1]Dept. of Biomed. Sci., [2]Dept. of Food Sci. and Human Nutr., Iowa State Univ., Ames, IA; [3]Dept. of Hlth. Sci., Western Carolina Univ., Cullowhee, NC; [4]Dept. of Kinesiology, Concordia Univ., Irvine, CA; [5]Dept. of Psychology,, Virginia Polytechnic Inst., Blacksburg, VA; [6]Sch. of Med. and Publ. Hlth., Univ. of Wisconsin, Madison, WI

Abstract: INTRODUCTION: Aging is characterized by physiological alterations in body composition, such as increased visceral adiposity accumulation and bone loss. Alcohol consumption is thought to partially drive these associations, but findings have been mixed. To clarify inconsistent findings, different types of alcohol--beer, liquor, and wine--may show different association patterns with body composition. **METHODS**: Our longitudinal U.K. Biobank study leveraged 1,874 White British participants (aged 40-79 years; 58.9% male). Participants self-reported demographic, alcohol and dietary consumption patterns, and lifestyle factors using a touchscreen questionnaire. Anthropometrics and serum for proteomics were collected and body composition was obtained via dual-energy X-ray absorptiometry (DEXA). Structural equation modeling was used to probe direct and indirect associations between adiposity and bone, alcohol types, and cardiometabolic biomarkers. **RESULTS**: Over a mean duration of four years, greater consumption of beer and liquor were significantly associated with more visceral adiposity (β=.069, $p<.001$ and β=.014, $p<.001$, respectively); these associations were driven by dyslipidemia and insulin resistance. In contrast, greater red wine consumption predicted less adipose mass (β=-.023, $p<.001$), and this association was mediated by reduced

inflammation and higher high-density lipoproteins (HDL) cholesterol. White wine consumption did not influence visceral adiposity but did predict greater bone mineral density (β=.051, p=.003). **DISCUSSION**: Taken together, these data suggest that beer and liquor may drive the "empty calorie" hypothesis related to adipogenesis, while red wine may be protective due to anti-inflammatory and eulipidemic effects. Furthermore, white wine may benefit bone mineral density in older adults.

Disclosures: B.A. Larsen: None. **B.S. Klinedinst:** None. **T. Wolf:** None. **C. Pappas:** None. **S.T. Le:** None. **N.F. Meier:** None. **Y. Limf:** None. **A.A. Willette:** None. **B.E. Frizell:** None. **L. Lanningham-Foster:** None.

Digital Abstract Session

P359. Aging: Behavior and Mechanisms

Program #/Poster #: P359.02

Topic: H.12. Aging and Development

Support: Priority Program, SPP 1772 from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG), grants BO 649/22-1
Priority Program, SPP 1772 from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG), grants VO 1432/19-1
European Social Fund and the Free State of Saxony grant 100342331

Title: Persisting effects of multitasking on car drivers' braking responses

Authors: *O. BOCK[1], R. STOJAN[2], K. WECHSLER[1], M. MACK[2], C. VOELCKER-REHAGE[2];
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Abstract: It is well established that participants' driving performance on one task suffers when they concurrently engage in another task. Here we investigate whether driving performance also suffers when participants had engaged in another task a few seconds ago. For potential practical implications, this work is carried out in a driving simulator. A total of 59 young (age: 23.2 ± 2.9 years) and 42 older adults (age: 70.0 ± 2.9 years) followed a lead car in a driving simulator, and had to brake when the lead car braked. Trials of the braking task were interspersed with trials form a battery of different loading tasks (typing, reasoning, and memorizing, each presented visually and auditorily). Stimulus onset asynchrony between a braking trial and the last preceding loading trial was 11.49 ± 1.99 s. In a control condition, the braking task was administered in absence of any loading task. Participants also completed eight executive-function tests. A multivariate general linear model with the factors Group and Condition, was followed up by Bonferroni corrected post-hoc tests. We found that (1) older participants kept a larger distance to the lead car, steered their car occasionally closer to the curb, and kept their car less steady in the lane; (2) participants in the multitasking condition kept a larger distance to the lead car before but not during the braking maneuver and steered their car occasionally closer to the median or to

the curb; (3) the effects of loading tasks on driving parameters were not substantially different between age groups. Adding an overall executive function score as a predictor reduced the effects of Age by about 41%, and the effects of Condition and of Group * Condition by about 25%. We conclude that our loading tasks had persistent effects on braking responses, which lasted for at least about 10 s. Those effects were associated with executive functions. We interpret our findings as evidence that each loading task establishes a persistent "task set", which needs to be reconfigured when a subsequent task - e.g., a braking task - is presented.

Disclosures: O. Bock: None. **R. Stojan:** None. **M. Mack:** None. **C. Voelcker-Rehage:** None. **K. Wechsler:** None.

Digital Abstract Session

P359. Aging: Behavior and Mechanisms

Program #/Poster #: P359.03

Topic: H.12. Aging and Development

Support: Institutional Development Award (IDeA) Network for Biomedical Research Excellence from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103430
Evelyn F. McKnight Brain Research Foundation
University of Florida Summer Neuroscience Internship Program

Title: The impact of long-term social housing on biconditional association task performance and neuron ensembles in the anterior cingulate cortex and CA3

Authors: *A. M. DANKERT[1], K. I. DAYAW[1], J. N. DAYAW[1], L. TRUCKENBROD[2], T. WISE[3,1], A. HERNANDEZ[4,2], S. N. BURKE[2], V. L. TEMPLER[1];
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Abstract: Cognitive decline and changes in neuronal activity are hallmarks of advancing age across species. Among the first brain regions to show age-associated dysfunction are the prefrontal cortex (PFC) and medial temporal lobe (MTL). Likewise, behaviors that rely on these structures are vulnerable to decline with advancing age. For example, aged rats are consistently impaired on biconditional association tasks (BATs), which require rats to learn that the target object in a pairwise discrimination problem changes trial-by-trial based on its location within the maze. Previous work using Compartmental Analysis of Temporal activation with Fluorescent *in situ* Hybridization (catFISH) has revealed that poorer BAT performance in aged rats is associated with altered activity patterns in the PFC and MTL. It has been observed that successful human agers with positive social relationships present larger anterior cingulate cortices (ACC) in the PFC and a higher density of von Economo neurons, which are thought to be important for social cognition, as compared to their less-social counterparts who experience

more cognitive difficulties. However, the mechanisms by which sociality might protect against cognitive aging and if this can be modeled in a rodent model have not been determined. Here we investigate the differences in performance on a BAT and the corresponding neuronal activity between socially housed (SH) and nonsocially housed (NSH) aged rats in comparison to individually housed young controls. All male Long-Evans were trained to criterion on BAT and were then tested on both BAT and an alternation control task on the day of sacrifice. On the final day of testing, aged NSH rats were significantly less accurate on BAT than the young control rats; however, there was no significant difference between aged SH rats and young control rats. These data suggest that social housing may have protected against cognitive decline with advancing age. Immediately following behavior, animals were sacrificed and brain tissue was processed to label the immediate-early gene mRNA for *Arc* and *Homer1A* mRNA. CatFISH was used to identify the neuronal populations in the CA3 subregion of the hippocampus and the ACC that were active during the BAT and the control task. SH rats had less overall neuronal activity in distal CA3 than the NSH rats and young controls. Furthermore, the SH and NSH aged rats had less overall neuronal activity in proximal CA3 than the young controls. These findings indicate that sociality may protect against the cognitive decline that occurs with advancing age and that physical, social, and cognitive enrichment are essential for preserving neuronal activity.

Disclosures: **A.M. Dankert:** None. **K.I. Dayaw:** None. **J.N. Dayaw:** None. **L. Truckenbrod:** None. **T. Wise:** None. **A. Hernandez:** None. **S.N. Burke:** None. **V.L. Templer:** None.

Digital Abstract Session

P359. Aging: Behavior and Mechanisms

Program #/Poster #: P359.04

Topic: H.12. Aging and Development

Support: Deparmental funding

Title: Microglial and Lipid Droplets Accumulation Age-Related Changes in Sleep Brain Regions in Cx3cr1 Mouse Model.

Authors: *M. BADAWY[1], S. GE[2], Q. XIONG[3];
[1]Neurobio. and Behavior, [3]Neurobio. & Behavior, [2]Stony brook university, Stony Brook, NY

Abstract: Natural aging affects brain homeostasis. Sleep pattern is reported to be dramatically disturbed with age. Older adults tend to have higher sleep latency, sleep stage transition, sleep-wake fragmentation, and less sleep duration when compared to younger adults. This is thought to be because of the global effect of aging on brain cells. One of these cell types is Microglia that is reported to behave differently as animals age. Microglia inflammation profile is reported to increase with age. Additionally, lipid droplets accumulation is another factor that is considered to increase with age. Pro- inflammatory microglia is reported to be more prominent with age. Furthermore, lipid droplets accumulation in the brain is reported to be associated with age and contributes, in part, to microglia activation. We used well-established morphological approaches

to investigate the changes that happen to microglia, and lipid droplets accumulation with age across sleep related brain regions. We had 8 Cx3cr1 mice, with ages ranging from 3 to 21 months, males and females. The animals were perfused, and their brains were sliced. Microglia and neuronal cell bodies were immunofluorescence-labelled for microglia, neuronal cell bodies, and lipid droplets and imaged in the cortical, preoptic, thalamic, midbrain, and medulla brain regions. We analyzed microglia total process length, sholl analysis, and lipid droplets accumulation within microglia and neurons to determine the pattern of change across ages. Our results indicate a global pattern of significant microglia cell processes retraction starting from mice with 6 months of age. Furthermore, we observed a prominent increase lipid droplets accumulation within microglia and neuronal cell bodies as mice aged. However, our sholl analysis' AUC indicates a gradual decrease of microglia coverage area and cell complexity as the mice age. Our work presents evidence of age effect on brain sleep regions. Nonetheless, based on our analysis parafacial zone, and ventrolateral preoptic area were affected the least. This work, when complete, will give a perspective to answer the question about the contribution of microglia, and lipid droplets accumulation in age-related sleep disturbance.***Keywords—*** Aging, Lipid droplets accumulation, Microglia, sleep brain regions.
*The Authors report no conflicts of interest in this work.

Disclosures: **M. Badawy:** None. **S. Ge:** None. **Q. Xiong:** None.

Digital Abstract Session

P359. Aging: Behavior and Mechanisms

Program #/Poster #: P359.05

Topic: H.12. Aging and Development

Support: Large Program Development Award, OSU

Title: Effects of age and non-combat military service on memory and cognitive flexibility in a virtual Morris water maze task

Authors: A. MOLLUSKY[1], N. REYNOLDS[1], D. LEE[1], J. ZHONG[2], *K. R. MAGNUSSON[1]; [1]Oregon State Univ., Corvallis, OR; [2]Air Traffic Mgmt. Res. Inst., Nanyang Technological Univ., Singapore, Singapore

Abstract: This study applied a virtual version of the Morris water maze (vMWM) task, which is commonly used for assessing spatial memory performance in rodents, to an examination of age-related differences between younger (18-30 years; N=5 male, 11 female) and older (60-86 years; N=8 female, 21 male) participants, and the impact of non-combat military service in the Vietnam and Korean war eras in older men (60-86 years; N=10 military, 11 non-military) on spatial memory and cognitive flexibility. Participants performed the Logical Memory task (WMS IV) that included immediate and delayed recalls, the NIH Toolbox Cognitive test battery that included the Fluid Cognition Composite (FCC) and the Crystallized Cognition Composite (CCC), and cognitive tasks in vMWM that included long-term memory, cognitive flexibility, and

working memory trials followed by visible control trials, while seated at a computer desk. Older individuals performed significantly worse than young (immediate recall; p<.0001; delayed recall, p=.0012) in the Logical Memory task. All NIH Toolbox Cognitive measures showed significant effects of age (p range: <.0001 to .035). For the FCC, older individuals performed worse than younger adults (uncorrected; p< .0001). In the CCC, older individuals performed better than young (p< .0001), but older male veterans performed worse than older male civilians (p=.026). In the vMWM tasks, older individuals showed significantly worse proximities than young in probe trials (p=.0004) and hidden trials (p=.0003) for spatial memory, and in delayed (15 seconds) match-to-place trials (p <.0001) for working memory. Older individuals had worse proximity scores than young (p=.016), and older male veterans showed significantly poorer performance than older male civilians (p=.0035) in reversal trials for cognitive flexibility. Although there was a significant effect of age in visible platform trials (p=.0003), the differences between scores were 8.7 fold higher in the hidden platform trials. There were no significant effects of gender (p = .13-.54) on vMWM performances. This study provided novel findings showing that military service in the Vietnam and Korean war eras may have reduced cognitive flexibility later in life.

Disclosures: **A. Mollusky:** None. **N. Reynolds:** None. **D. Lee:** None. **J. Zhong:** None. **K.R. Magnusson:** None.

Digital Abstract Session

P359. Aging: Behavior and Mechanisms

Program #/Poster #: P359.06

Topic: H.12. Aging and Development

Title: Characterizing the therapeutic application of amphetamine in age-associated cognitive decline

Authors: *S. SCOGNAMIGLIO, G. DEZFULI, K. J. KELLAR;
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Abstract: Memory impairment often accompanies normal aging and is thought toinvolve hypofunctional glutamatergic systems in the cerebral cortex andhippocampus. Glutamate, the major excitatory neurotransmitter in the brain,plays a fundamental role in functions such as cognition, motor behavior andemotion (Meldrum, 2000). Along with glutamate, norepinephrine (NE) is animportant modulator of memory, especially emotional memory (Tully *et al.*,2010). Our previous study demonstrated a marked deficit in glutamate-stimulated NE release in slices from the cortex and hippocampus of aged (22-24 months old) male Fischer 344 rats, which is rescued by addition ofamphetamine (Dezfuli *et al.,* 2019). Thus, amphetamine potentiatesglutamate-stimulated NE release (and possibly release of othermonoamines). The purpose of our current study was to test the cognitiveeffects of amphetamine treatment in aged rats. To do this, we investigatedwhether memory performance as measured in the novel object recognitiontask (NOR) is enhanced by acute treatment with amphetamine in agedFischer 344 rats. An additional

goal of this study, is to test the effects ofchronic amphetamine treatment on the NOR task. Our initial results indicatethat in aged Fischer 344 rats, acute treatment with amphetamine (0.5 mg/kg)increases object recognition memory in the NOR task. These results set thestage for our chronic studies, which will be reported.

Disclosures: S. Scognamiglio: None. **G. Dezfuli:** None. **K.J. Kellar:** None.

Digital Abstract Session

P359. Aging: Behavior and Mechanisms

Program #/Poster #: P359.07

Topic: H.12. Aging and Development

Support: VO 1432/19-1
BO 649/22-1

Title: The association of cognitive, motor, and cardiovascular fitness with street crossing performance under multitasking conditions

Authors: M. MACK[1], R. STOJAN[1,2], O. BOCK[3,2], *C. VOELCKER-REHAGE[1,2];
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Abstract: Human gait often changes under multitasking conditions, probably because walking and the additional loading task compete for processing resources. This interference is particularly prominent in older age. We investigate whether multitask interference is influenced by participants' individual levels of cognitive, motor, and/or cardiovascular fitness. For ecological validity, we use a virtual street crossing scenario. Younger adults (n = 63, 20-30 years) and older adults (n = 61, 65-75 years) participated in the study. A non-motorized treadmill translated their forward movement into forward shifts towards and across a virtual street, where cars were passing with a constant speed and increasing gaps. The participants' task was to cross the street without getting hit by a car, both under single-task (ST) and under multitask (MT) conditions. Performance was assessed as stay time at the curb, crossing speed, and crossing failures. Loading tasks were presented visually or auditorily, and required participants to remember a shopping list or to type three-digit numbers. Participants also performed seven cognitive tests (executive functions), five motor tests (balance, movement speed, and bimanual coordination), and one cardiovascular test (graded maximal exercise testing on a cycle ergometer). A linear mixed effect approach yielded significant performance decrements from ST to MT, which were age-independent for crossing failures, increased with age for stay time and existed only in older age for crossing speed. Effects of cognitive, motor and cardiovascular fitness on MT performance decrements in young and older adults will be analyzed. An association between MT decrements and fitness would suggest that MT represents an overarching ability rather than a task-specific ability.

Disclosures: C. Voelcker-Rehage: None. **M. Mack:** None. **R. Stojan:** None. **O. Bock:** None.

Digital Abstract Session

P359. Aging: Behavior and Mechanisms

Program #/Poster #: P359.08

Topic: H.12. Aging and Development

Support: McKnight Brain Research Foundation and the McKnight Brain Institute

Title: Acute effects of cannabis on cognition in aging

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Abstract: Adults over age 65 are the fastest growing demographic of cannabis users. These individuals use cannabis for a variety of reasons, including pain management, appetite stimulation, and sleep, but cannabis can exert effects on cognition as well. Compared to young adults, aged individuals exhibit deficits in cognitive functions supported by the prefrontal cortex (PFC) and the hippocampus. These same cognitive functions are impaired by acute administration of cannabis or delta-9-tetrahydrocannabinol (THC) in young subjects; however, effects in aged subjects are less well understood. The current study used a rat model to determine whether the effects on cognition of acute exposure to cannabis smoke differ between young and aged subjects. Male, fully mature young adult (6 months) and aged (24 months) Fischer 344 x Brown Norway F1 hybrid rats were tested on both a PFC-dependent delayed response working memory task and a hippocampal-dependent trial-unique non-match to location (TUNL) task in touchscreen operant chambers. The delayed response task required rats to remember the location of a visual stimulus over variable delay periods ranging from 0-12 s. The TUNL task required rats to remember the location of a visual stimulus with varying degrees of discriminability from other, distractor stimuli. A semi-randomized, within-subjects experimental design was used such that each rat was exposed to smoke from burning 0, 3, 5, and 10 cannabis cigarettes immediately prior to test sessions in each task. In the delayed response task, acute exposure to cannabis smoke impaired accuracy in young rats but enhanced accuracy in aged rats. In contrast, in the TUNL task, cannabis smoke had no effects on performance in either age group. Considered together, this pattern of results shows that in aged rats, which exhibit impaired cognitive performance, cannabis smoke can enhance PFC-dependent cognition, but has no effect on hippocampus-dependent cognition. A follow-up study evaluated plasma THC levels over time in young and aged rats following exposure to smoke from burning 5 cannabis cigarettes. Compared to young adults, aged rats exhibited greater variability in their THC concentration-time profiles and showed a trend toward higher THC levels overall. Future studies will evaluate the mechanisms underlying these age differences in cognitive and pharmacokinetic properties of cannabis.

Disclosures: S. Zequeira: None. **A.A. Güvenli:** None. **M.M. Bruner:** None. **C. Hernandez:** None. **J.F. Deslauriers:** None. **M. Febo:** None. **J.L. Bizon:** None. **B. Setlow:** None.

Digital Abstract Session

P359. Aging: Behavior and Mechanisms

Program #/Poster #: P359.09

Topic: H.12. Aging and Development

Support: 5R01NS092388
5U54GM104942-03

Title: Influence of Dim Light at Night and Sex on Cognitive Aging and Vascular Contributions to Cognitive Dementia

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Abstract: Several components of brain vasculature are altered in response to acute dim light at night (dLAN) and cardiac arrest, including reduced vascular density in the hippocampus, decreased VEGF, reduced BDNF in males, and increased neuroinflammation (IL-1β) in females. We previously reported that dLAN impairs performance on the Barnes maze task in Nile grass rats; thus, we investigated the interacting effects of disrupted circadian rhythms by dLAN in conjunction with aging on cognitive function. We hypothesized that chronic exposure to white dLAN impairs cognition in aged mice, and proposed that in addition to being a risk for poor ischemic outcome, dLAN may be a risk factor for vascular contributions to cognitive dementia-like impairments. Eighteen month old (+/- 2 months) male and female C57BL/6 mice were individually housed and exposed to 10 weeks of standard light/dark conditions (LD; 14:10 light/dark) or dim light at night (5 lux), then cognitive function was assessed using the Barnes maze, spontaneous alternation, novel object recognition, and an auditory fear associative learning task. We observed an interaction effect between lighting condition and sex on body weight as a percent change from baseline; females display a significant decrease in body weight after 10 weeks of dLAN exposure compared to females in dark nights, whereas males inversely displayed a reduced percentage of body weight loss in LD. In addition, chronic exposure to dLAN increased mortality in female mice. We also observed a sex difference in the acquisition aspect of the Barnes maze task, where aged males were significantly impaired in their ability to locate escape location compared to females, although no effect of lighting condition was observed. Aged male mice froze significantly more during contextual fear retention tasks compared to females, but there was no effect of sex or lighting condition on acquisition or CS/US pairings. No differences were observed in spontaneous alternation or novel object recognition. Together, light at night alters body mass and survival in aged females, and the data suggest that there may be a sex difference in cognitive aging irrespective of lighting conditions.

Disclosures: J.A. Liu: None. **J.C. Walton:** None. **J.R. Bumgarner:** None. **W.H. Walker:** None. **O.H. Melendez-Fernandez:** None. **A.C. DeVries:** None. **R.J. Nelson:** None.

Digital Abstract Session

P359. Aging: Behavior and Mechanisms

Program #/Poster #: P359.10

Topic: H.08. Learning and Memory

Support: NIH Grants RO1 AG049464, RO1 AG049465, McKnight Brain Research Foundation

Title: Quantitative and volumetric and diffusion weighted MRI analysis of rodent brains as a function of age and cognition

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Abstract: Animal models play an important role in preclinical and translational studies of the human brain. MRI, being both non-invasive and inherently translational, can play an important role in comparing brain anatomy in animal models and humans. Furthermore, diffusion weighted MRI is an established tool for the examination of white matter connectivity and microstructural changes as a function of age. Volumetric and diffusion weighted MR imaging have the potential to detect differences in rat brains as a function of age and cognition. The volumes and structural integrity of important brain regions as a function of age and cognition are continuing to be explored and these results will give important information as to the connectivity of different brain regions during both healthy cognitive aging as well as characterizing structural differences in rat brains with various levels of cognitive aptitude. Young (6mo), middle-aged (15mo) and old (23mo) male F344 rats were used in this study. A T2-weighted template image was registered to each animal's anatomical image using rigid body, affine and non-linear techniques and the deformation fields produced were applied to an 85 region labeled atlas to calculate the volume of individual regions of interest (ROIs) in the brain. This allowed for volumetric measurements across age and cognitive groups regionally. Image processing for the diffusion weighted images involved eddy current corrected and motion corrected, denoising, brain extraction and diffusion tensor generation. Scalar parameters such as fractional anisotropy (FA) can then be used to microstructurally characterize the white matter tracts. Volumetric MR imaging detected differences in rodent total intracranial volume and the data suggests that the rat brains are continuing to grow past 6 months of age. Body weight measurements confirm the imaging findings to middle aged however there is a deviation at old age where total intracranial volume (TIV) plateaus and body weight decreases significantly. These results will inform future analysis comparing regional brain volumes with age and cognitive performance. Diffusion MRI analysis is in progress and will show the microstructural integrity of white matter tracts both globally and regionally and those results will be compared across age and cognitive groups by comparing scalar indices such as fractional anisotropy, mean diffusivity and radial diffusivity.

Disclosures: **L. Do:** None. **M.A. Zempare:** None. **A.S. Bernstein:** None. **C.A. Barnes:** None. **C. Ugonna:** None. **P. Bharadwaj:** None. **N. Chen:** None. **T.P. Trouard:** None. **G.E. Alexander:** None.

Digital Abstract Session

P360. Cognitive Natural Aging: Hippocampus and Network Mechanisms

Program #/Poster #: P360.01

Topic: H.12. Aging and Development

Support: University of South Carolina Office of the Associate Dean for Research and Graduate Education to PWB
University of South Carolina Office of Undergraduate Research to NMR
NIH Grant P30AG028740 to SNB and JAM
NIH Grant P20GM103641 to JAM
NIH Grant K01AG061263 to JAM

Title: Metabolic Contributions to Synaptic Aging in the Brain

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Abstract: Aging is accompanied by irregularities in circulating glucose, altered secretion of glucocorticoids, and synaptic changes within corticolimbic structures that support normal cognition and regulate homeostasis. These disturbances are especially pronounced in Alzheimer's disease (AD), supporting a theory wherein age-related deficits in neural glucose metabolism underlie bioenergetic disturbances that contribute to memory loss and dyshomeostasis in AD. Our study evaluated metabolic, neuroendocrine and neurobiological differences between young adult (6 months) and aged (24 months) male F344 × Brown Norway F1 rats. First, we observed that blood glucose levels were significantly elevated in aged rats, compared to young adults, at the start of the active phase of the day. When challenged with 60 minutes of physical restraint, a potent stressor, aged rats effected no change in blood glucose while young adults showed increases in blood glucose. Age-related changes in blood glucose profile could not be attributed to differences in hypothalamic-pituitary-adrenal (HPA) axis function, as both age groups exhibited significant and similar modulation of corticosterone secretion in response to circadian cues and stressful experience. Next, synaptosomal fractions were prepared from the hippocampus and prefrontal cortex from all animals to evaluate changes to expression/localization and function of enzymes responsible for producing ATP that fuels synaptic activity. Using an antibody cocktail that probes representative subunits from each of the five complexes that comprise the electron transport chain, we determined there was no difference in the abundance of these subunits between age groups in hippocampal or prefrontal synapses.

Our initial findings suggest that normally aging rats are a translationally useful model to examine the antecedents and consequences of glucose dysregulation. Blood glucose levels in aging become uncoupled from the typical modulation by the HPA axis, which suggests defective glucocorticoid signaling is not the basis for these changes. The abundance of synaptic mitochondria does not appear to change in response to differences in blood glucose, but ongoing experimentation will determine if intervening processes such as glucose transport or glycolysis influence metabolic efficiency of aging synapses.

Disclosures: J.A. McQuail: None. **P.W. Braunstein:** None. **N.M. Ruggiero:** None. **S.N. Burke:** None. **A.M. Hampton:** None. **L.A. Newman:** None.

Digital Abstract Session

P360. Cognitive Natural Aging: Hippocampus and Network Mechanisms

Program #/Poster #: P360.02

Topic: H.12. Aging and Development

Support: NIH Grant K01AG061263 to JAM

Title: Age-associated memory impairments correlate with decline of excitatory and inhibitory synapses in hippocampal area CA3

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Abstract: Aging is characterized by declining memory and escalating risk for Alzheimer's disease (AD). Anomalous patterns of hippocampal activity manifest in prodromal AD, wherein a shift in the excitatory-inhibitory (E/I) dynamic signals the transition from normal brain aging to neurological disease. E/I imbalance is most evident within the dentate gyrus (DG)-CA3 network and is proposed to underlie age-related deficits in spatial learning that are ascribed to the dorsal, but not the ventral, hippocampus. Collectively, these observations indicate that a neuroanatomically precise characterization of changes to excitatory and inhibitory hippocampal synapses could elucidate the circuit-basis of memory changes that confer age-associated risk for AD. Consequently, we quantified changes to VGluT1, a marker of glutamatergic synapses, and VGAT, a marker of GABAergic synapses, in hippocampal sections from young 6-months old F344×Brown-Norway F1 rats (6M; n=9) relative to strain-matched 24-months old rats that were rated as spatial-learning unimpaired (24M-U; n=12) or impaired (24M-I; n=12) based on performance in the Morris water maze. We identified significantly lower VGluT1 in both dorsal and ventral divisions of area CA3 between 6M and 24M-I rats. Subsequent analysis of synaptic layers localized these changes to the stratum lacunosum moleculare of the dorsal CA3 and the stratum radiatum of the ventral CA3. Using a complementary approach that leverages the full range of individual differences in spatial learning, correlational analyses applied across the entire spectrum of aged performance revealed significant relationships between spatial learning decrements and lower level of VGAT in all synaptic layers of the dorsal CA3. These new

findings are consistent with our hypothesis that E/I disruption centered on dorsal CA3 is of special relevance to age-related memory loss. More specifically, impaired memory is related to diminished input from glutamatergic terminals synapsing upon apical dendrites of pyramidal neurons (putative perforant path terminals) in dorsal CA3 as well as more distributed decline of GABAergic terminals innervating proximal dendrites, neuronal soma and initial axon segments.

Disclosures: D.J. Horovitz: None. **L.A. Askins:** None. **G.M. Regnier:** None. **J.A. McQuail:** None.

Digital Abstract Session

P360. Cognitive Natural Aging: Hippocampus and Network Mechanisms

Program #/Poster #: P360.03

Topic: H.12. Aging and Development

Support: NIH/NIA 1R01AG049722
NIH/NIA R01AG055544
NIH T32AG061892

Title: Age-related changes in hippocampal lfp dynamics during behavior

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Abstract: The number of aged individuals is increasing, bringing with it medical challenges which includes increased rates of age-related cognitive impairment. Many of these impairments have been linked to neurobiological dysfunctions within the medial temporal lobe, though the specific mechanisms are not fully understood. Local field potential dynamics are one means of assessing network integrity, as this signal primarily reflects the accretion of synaptic activity over a population of neurons. Several well described changes within the hippocampus are likely to disrupt LFP activity in aged animals. For example, degradation in the perforant path, which connects the entorhinal cortex and hippocampus, has been reported in rats (Barnes and McNaughton, 1980) and humans (Yassa et al., 2010a). Additionally, the CA3 subregion of the hippocampus in aged rats (Wilson et al., 2005), monkeys (Thomé et al., 2016) and humans (Yassa et al., 2010b) have been found to be hyper-excitable. During waking behaviors, the theta (6-12 Hz) and gamma (50-100 Hz) rhythms are prominent. While theta frequency and theta-gamma coupling have been reported to change in old rats within the CA1 subregion (Shen et al., 1997; Jacobson et al., 2013), these LFP dynamics have not been examined in the dentate gyrus or CA3 in the context of aging. To investigate this phenomenon, 5 young (6 months) and 5 aged (24 months) were implanted with a silicon probe spanning the CA1, CA3, and the Dentate Gyrus (DG) of the hippocampus. Rats were then run on an inter-region dependent Object-Place Paired Association task, on which aged rats perform worse than their young counterparts (Hernandez et

al., 2015). Because this task requires the use of multiple regions working simultaneously it is possible to use it, in tandem with the silicon probes, to examine how age-related changes in one region may translate to changes in another region. Synaptic changes are likely to manifest as changes in network measures such as oscillatory activity. We hypothesize that age-related degradation of the perforant path and age-related hyperexcitability of the CA3 will result in not only changing peak frequencies in CA3, but also to less coherence of theta and gamma both within CA3 as well as between CA3 and CA1.

Disclosures: N.M. DiCola: None. **A.L. Lacy:** None. **J.L. Whitney:** None. **S.N. Burke:** None. **A.P. Maurer:** None. **A. Sheremet:** None.

Digital Abstract Session

P360. Cognitive Natural Aging: Hippocampus and Network Mechanisms

Program #/Poster #: P360.04

Topic: H.12. Aging and Development

Support: Paul G. Allen Frontiers Group (SH)

Title: Pair bonding slows epigenetic aging and alters methylation in brains of prairie voles

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Abstract: The quality of romantic relationships can be predictive of health consequences related to aging. DNA methylation-based biomarkers of aging have been developed for humans and many other mammals and could be used to assess how pair bonding impacts aging. Prairie voles (*Microtus ochrogaster*) have emerged as a model to study social attachment among adult pairs. Here we describe DNA methylation-based estimators of age for prairie voles based on novel DNA methylation data generated on highly conserved mammalian CpGs measured with a custom array. The multi-tissue epigenetic clock for voles was trained on 3 tissue sources (ear, liver, and samples of brain tissue from within the pair bonding circuit). A novel dual species human-vole clock accurately measured relative age defined as the ratio of chronological age to maximum age. According to the human-vole clock of relative age, sexually inexperienced voles exhibit accelerated epigenetic aging in brain tissue ($p = 0.02$) when compared to pair bonded animals of the same chronological age. Epigenome wide association studies identified CpGs in four genes that were strongly associated with pair bonding across the three tissue types (brain, ear, and liver): *Hnrnph1*, *Fancl*, *Fam13b*, and *Fzd1*. Further, four CpGs (near the *Bmp4* exon, *Eif4g2* 3 prime UTR, *Robo1* exon, and *Nfat5* intron) exhibited a convergent methylation change between pair bonding and aging. This study describes highly accurate DNA methylation-based estimators of age in prairie voles and provides evidence that pair bonding status modulates the methylome.

Disclosures: **L.L. Sailer:** None. **A. Haghani:** None. **J.A. Zoller:** None. **C.Z. Li:** None. **A.G. Ophir:** None. **S. Horvath:** None.

Digital Abstract Session

P360. Cognitive Natural Aging: Hippocampus and Network Mechanisms

Program #/Poster #: P360.05

Topic: H.12. Aging and Development

Support: NIH T32-AG000037
NIH R01-AG054617

Title: Age-related hearing loss is associated with lower functional connectivity

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Abstract: Age-related hearing loss was recently identified as a modifiable risk factor for dementia (Livingston et al., 2017) and is highly prevalent (>50%) among the older adult population (Lin et al., 2011). Previous studies examining how the brain may be impacted by hearing loss have largely used measures from the better ear without reporting which ear this represents. However, speech perception is typically poorer in the left ear. Thus, it is unclear if the effects of hearing loss on the brain may be driven by one ear. In the current study, we investigated how age-related hearing loss within each ear separately impacts brain functional connectivity between regions critical for auditory and higher-order cognitive processing.
We assessed resting-state fMRI functional connectivity in 1,000 participants (61.98±7.41 years, 50% women) from the UK Biobank. Functional connectivity was computed between left and right planum temporale as seed regions and the 1) default mode (DMN), 2) frontoparietal (FPN), and 3) cingulo-opercular (CON) network ROIs as target regions. Hearing was measured with a speech in noise reception threshold task (SRT), which was used to group participants into better performing ear groups (better left ear (56%): -5.97±1.50 dB; better right ear (n=44%): -6.07±.1.55 dB), in which a higher score indicates poorer hearing. ANCOVA models were used to predict functional connectivity from left and right ear hearing measures, controlling for age and sex, and separated into groups by their better ear ($p<.05$ FDR).
Poorer SRT scores in the left ear were associated with lower functional connectivity between left planum temporale and target regions: bilateral posterior parietal ROIs of the FPN and right posterior parietal ROI of the DMN, only in the group who had a better left ear. Right ear SRT scores were not significantly associated with functional connectivity. Secondary, voxel-wise analyses supported these findings, revealing that poorer SRT scores in the left ear were associated with lower functional connectivity in posterior and medial parietal regions, in the better left ear group only.
Our results show that age-related hearing loss in the left ear (but not the right) is associated with

reduced brain functional connectivity, specifically in individuals whose left ear is their better ear. We observed lower connectivity to the DMN, a network associated with memory and known to be impaired in Alzheimer's disease, and the FPN, a network associated with attentional resources. Thus, these findings suggest that age-related hearing loss may be a risk factor for cognitive decline due to reduced connectivity with higher-order cognitive networks.

Disclosures: M.C. Fitzhugh: None. **J. Pa:** None.

Digital Abstract Session

P360. Cognitive Natural Aging: Hippocampus and Network Mechanisms

Program #/Poster #: P360.06

Topic: H.12. Aging and Development

Support: Priority Program, SPP 1772 from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG), grants VO 1432/22-1
European Social Fund and the Free State of Saxony grant 100342331

Title: Prefrontal and parietal brain activation during dual-task walking in older adults - an fNIRS study

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Abstract: Walking while being engaged in an additional cognitive task is a common yet cognitively highly demanding behavior in daily life. Many neuroimaging studies investigated brain activity during dual-task (DT) walking by use of fNIRS or EEG. Most of them were limited to the prefrontal cortex (PFC), and found that PFC activity was increased during DT walking, and compared to single-task (ST) walking and ST cognition. This upregulation was typically more pronounced in older than younger adults, and was often explained by neural inefficiency or capacity limitation models. We used high-density multi-channel fNIRS to monitor an additional brain region, i.e., the parietal cortex (PC), aiming to better understand a potential interaction between frontal and parietal brain regions during DT walking. Data of 46 older adults (age: 68.57 ± 3.92; 26 females) were analyzed. Participants completed a virtual DT walking scenario that comprised two different DTs (walking + Serial's Threes; walking + Stroop), three STs (walking; Serial's Threes; Stroop), and a baseline task (standing). All tasks were carried out in a pseudorandomized order (5 trials per task, 30 s per trial) that was identic for all subjects. Brain activity was assessed using multi-channel continuous-wave fNIRS over the PFC and PC. HbDiff (the difference between oxygenated and deoxygenated hemoglobin concentrations) was computed as a marker of brain activation, and averaged within an interval of 10 to 30 s after trial onset. A 5 x 2 ANOVA (Task x Region) with follow-up pairwise estimated marginal means contrasts revealed higher brain activation in PFC and PC for both DTs compared to ST walking and ST cognition. Further, brain activity during DT was higher than the summed

activity of ST walking and ST cognition in PFC (overadditive), but slightly lower than that sum in PC (underadditive). Higher overadditivity in PFC was associated with smaller underadditivity in PC. Consistent with previous studies, PFC and PC upregulation in older adults under DT conditions suggests increased neurocognitive task demands during DT walking. Further, larger overadditive PFC activity during DT may indicate higher cognitive effort or ineffective task coordination processing in PFC (neural inefficiency). That higher PFC overadditivity was associated with smaller underadditivity in PC might be a further hint for ineffective task coordination and/or might highlight the allostatic interaction of both regions. Our findings provide new insights into the interplay of PFC and PC and highlight the importance of the application of multichannel fNIRS measures in multiple regions during DT walking.

Disclosures: **R. Stojan:** None. **M. Mack:** None. **O. Bock:** None. **C. Voelcker-Rehage:** None.

Digital Abstract Session

P360. Cognitive Natural Aging: Hippocampus and Network Mechanisms

Program #/Poster #: P360.07

Topic: H.12. Aging and Development

Support: U.S.-Israel Binational Science Foundation (BSF) 2017242

Title: Mapping structure to function and behavior with individual-level connectome embedding

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Abstract: Embedding of a graph or connectome embedding (CE), involves finding a compact vectorized representation of nodes that captures their topological attributes. Such embeddings would ideally represent not only the pairwise connections of a given node, but also its higher-order relations within the graph. Obtaining CE can be done using the word2vec algorithm adapted from linguistics. This algorithm finds a low-dimensional latent representation of words such that words that appear in the same context within a sentence would be closer in the embedded latent space. The approach can be adapted to study connectivity in the human brain, such that nodes that appear in a similar context in a random walk on a brain graph would be closer in the embedding space. Previous work was focused on characterizing the embedding of group averaged structural connectivity. This framework allowed inference of edges missing in the reconstructed structural connectivity matrix and improved structural to functional connectivity mapping. In the current work, we further develop and advance this framework by presenting a novel approach to align separately learned embeddings to the same latent space using a close form solution that utilizes parameters learned in the embedding fitting process. This enables us to apply the CE framework at the individual level, rather than the group level, to investigate the relation between individual differences and CE. To test this approach, we used

large lifespan datasets (NKI: n=542; Cam-CAN: n=601) that included diffusion-weighted imaging, resting-state fMRI, as well as physiological and behavioral measures. We empirically evaluated our approach for aligning CE within and between individuals. We demonstrate that CE substantially improves the mapping of structural connectivity to functional connectivity at the individual subject level as found previously in mapping of group-averaged connectivity. Furthermore, we found that important age-related individual differences in this structure to function mapping are preserved and even more pronounced when quantified using our new approach. Finally, as a proof of concept, we showed that CE captures individual differences in physiology and behavior by predicting age, intelligence, and gender. For age and intelligence, the predictive accuracy was substantially higher when using CE compared to the raw structural connectivity. Our novel alignment approach provides a substantial advancement in our ability to examine individual differences in structure-function correspondence as well as relating network topology to physiology and behavior.

Disclosures: **G. Levakov:** None. **O. Sporns:** None. **J. Faskowitz:** None. **G. Avidan:** None.

Digital Abstract Session

P360. Cognitive Natural Aging: Hippocampus and Network Mechanisms

Program #/Poster #: P360.08

Topic: H.12. Aging and Development

Support: NIMH Grant 1K01MH116098
ABRC ADHS16-0005488

Title: Adults with autism spectrum disorder show steeper age relationships with decreasing amygdala size: sex differences and associations with autism symptoms

Authors: ***B. A. PAGNI**, N. GUERITHAULT, M. J. M. WALSH, J. R. COOK, B. B. BRADEN;
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Abstract: While abnormal amygdala volumes have been observed in various age groups of individuals with autism spectrum disorder (ASD), little research has explored how volumes change across the adulthood lifespan, whether men and women have different aging trajectories, and what changes in symptomatology coincide. Children with ASD have enlarged amygdala which reach neurotypical size around adolescence and becomes smaller in adulthood, suggesting the developmental arc in ASD is altered compared to neurotypicals (NTs). Amygdala sizes strongly correlate with anxiety and social symptoms in ASD, however it is still unclear if age-related changes in volume have consequences on these symptoms. In this cross-sectional study of adults ages 18-71 years, amygdala volumes in men and women with ASD (n=143) were examined and compared to aging trajectories in NT men and women (n=86). Participants completed self-report measures for anxiety (State-Trait Anxiety Inventory, STAI) and ASD symptoms (Social Responsiveness Scale-2, SRS-2) and T1 MRI images were processed in

FreeSurfer and amygdala volume estimations were corrected by total intracranial volume. A MANOVA for left and right amygdala volume revealed a significant three-way diagnosis-by-sex-by-age interaction ($F(7,106)=2.147$, $p=0.045$). Post-hoc correlations showed a significant negative relationship between age and amygdala volume bilaterally for men with ASD (right: $r(98)=-0.517$, $p<0.001$; left: $r(98)=-0.481$, $p<0.001$) and in the right hemisphere for women with ASD ($r(34)=-0.343$, $p=0.021$). Age correlations in this age range did not reach significance for NT men or women. Behavioral correlations showed relationships between amygdala volumes and SRS-2 subscales 'Awareness' (right hemisphere: $r(128)=-0.187$, $p=0.034$), 'Cognition' (right hemisphere: $r(128)=-0.291$, $p=0.001$; left hemisphere: $r(128)=-0.261$, $p=0.003$), and 'Communication' (right hemisphere: $r(128)=-0.207$, $p=0.019$) for adults with ASD. Surprisingly, there were no significant correlations with anxiety symptoms in adults with ASD. These findings support a larger body of work which suggests an altered developmental arc in neuroanatomical structures in ASD and extends our knowledge by showing age-related volumetric changes in the amygdala are associated with ASD symptoms across the adult lifespan. Future work should seek to confirm these findings using longitudinal designs. Understanding age and sex effects may provide insights into the contribution of the amygdala in symptom presentation in ASD.

Disclosures: B.A. Pagni: None. **N. Guerithault:** None. **M.J.M. Walsh:** None. **J.R. Cook:** None. **B.B. Braden:** None.

Digital Abstract Session

P361. Learning and Memory in Aging: Medial Temporal Lobe and Neuropsychology

Program #/Poster #: P361.01

Topic: H.12. Aging and Development

Support: CIHR Grant #126105
Alzheimer's Society of Canada Grant #1435

Title: Medial temporal lobe volumetric trajectories across midlife and associations with episodic memory

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Abstract: Age-associated declines in episodic memory for contextual details (context memory) arise in early midlife and are associated with altered brain activity in prefrontal and medial temporal cortices (Ankudowich et al., 2017; Kwon et al., 2015). Yet, it remains unclear how memory circuitry in the medial temporal lobes (MTL) changes at midlife, and how this relates to declines in context memory. Importantly, women report increased memory deficits as they transition through menopause (Greendale et al., 2011), which may contribute to the greater prevalence of memory disorders in women in late life (Gao et al., 1998). To better understand these sex-specific changes in memory circuitry at midlife, we investigated sex-specific differences in the association between age and volume of MTL regions in young adults (YA: 19-

35 years old) and middle age adults (MA: 40-58). Additionally, we examined associations between MTL structure and episodic memory performance. We tested 81 participants (18 YA females, 15 YA males, 38 MA females and 10 MA Males) on context memory tasks. We employed a semi-automated segmentation protocol, by segmenting the perirhinal (PRC), entorhinal (ERC) and parahippocampal (PHC) cortices, and the anterior and posterior segments of the hippocampus (antHC and postHC). We modeled age-related changes in volume with quadratic regressions within each sub-group. Further, we predicted memory performance on the context memory tasks based on MTL volumes using multiple linear regression. Results from structure-age regressions demonstrate a positive quadratic relationship between PRC volume and age in YA females ($R2_{adj} = 43.93$, $p < 0.01$), and negative quadratic relationships between ERC ($R2_{adj} = 21.57$, $p < 0.01$) and PRC volumes ($R2_{adj} = 11.11$, $p < 0.05$) with age in MA females. We did not observe significant age-structure relationships in either of the male subgroups. Additionally, within the MA female subgroup, we found that volume of the anterior hippocampus significantly predicted performance on the hard component of the temporal context memory task ($R2_{adj} = 25.09$, $p < 0.01$). While midlife is understudied from a brain-imaging perspective, cognitive changes during this time may be especially important in women transitioning through menopause. Taken together, our results suggest differences in MTL structure across midlife, which decrease around 50 years old. By focusing on adults early in the aging process, and throughout the menopausal transition, we reveal sex-dependent characteristics underlying how regions involved in episodic memory processes change with age.

Disclosures: J. Snytte: None. **C. Beaudoin:** None. **K. Hooper:** None. **R.K. Olsen:** None. **N. Rajah:** None.

Digital Abstract Session

P361. Learning and Memory in Aging: Medial Temporal Lobe and Neuropsychology

Program #/Poster #: P361.02

Topic: H.12. Aging and Development

Support: Natural Sciences and Engineering Research Council of Canada Discovery Grant 2018-04401 to QY

Title: The Functional Roles of L-Type Calcium Channels in Odour Learning and Spatial Discrimination

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Abstract: L-Type calcium channels (LTCCs) have been shown to be intricately involved in the process of learning and memory. Calcium influx through LTCCs has been shown to be critical for learning and memory in several animal models. However, as we age, our ability to recall and form new memories deteriorates, leading to cognitive impairment. Interestingly, LTCC mediated

calcium current in neurons is also found to increase with ageing. We hypothesize that during the process of ageing, there is a paradoxical shift in the role of LTCCs in learning and memory. An increased amount of calcium entry through LTCCs results in a deviation from normal calcium homeostasis and contributes to cognitive decline that we see in an ageing population.We have set out to systematically investigate the potential effects of blocking LTCCs in respective brain areas associated with two types of learning in rats: an olfactory discrimination learning task in a food deprived model, in which the animals learn to associate one odour with an appetitive reward; as well as a spatial learning task in which the animals learn to discriminate between identical objects based on their location within an open field. We will use nimodipine at various age points (Young 6-10 months and Ageing animals >18 months), by first exploring the effects of systemic injection (5.5mg/kg), then, direct infusion to the olfactory cortex (Piriform Cortex (PC) and hippocampus (CA1) (10uM – 100uM), before undergoing either task.Preliminary results from the food retrieval paradigm suggests that when given an i.p. injection of nimodipine to young rats, it prevented their ability to learn the odor reward association. Moreover, direct infusion of nimodipine to the PC of the young animals showed the same trend as seen in the i.p injection group in the food retrieval task. In contrast, in the ageing animals, nimodipine permitted the animal's ability to learn the task, while control ageing animals showed little to no learning. When exploring the effects of nimodipine in the spatial discrimination, young animals given i.p injections of nimodipine, showed a drastic impairment in
their discrimination ability, strikingly, when examining ageing animals under the same condition, nimodipine showed a complete enhancement in the animal's ability to discriminate between the objects.This project will shed light on the differential roles that LTCCs play during development and support the potential for the investigation and development of therapeutics that target LTCCs and for age-related learning deficits as well.

Disclosures: T. Critch: None. **A. Ghosh:** None. **C. Reinhardt:** None. **C. Flynn:** None. **L. MacGowan:** None. **Q. Yuan:** None.

Digital Abstract Session

P361. Learning and Memory in Aging: Medial Temporal Lobe and Neuropsychology

Program #/Poster #: P361.03

Topic: H.12. Aging and Development

Support: Medical Scientist Training Program grant : T32GM107000
CART (Coins for Alzheimer's Research Trust) grant
Bright Focus Foundation

Title: The role of slow-wave-sleep in hippocampus-dependent memory

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Abstract: Our modern society allows us to live longer, however the increase in lifespan is associated with deficits such as decreased quality of sleep and cognitive function. Sleep deprivation/restriction studies indicate that excellent quality of sleep is necessary for optimal cognitive function, yet the evidence remains correlative and the mechanisms remain poorly understood. For example, deep non-rapid eye movement (NREM) sleep, also called slow-wave-sleep (SWS), a stage of sleep that is impaired with aging, has been implicated in hippocampus-dependent memory.
We are studying the role of SWS in hippocampus dependent memory function using, for the first time, gain-of-sleep experiments. We hypothesize that sleep deficits associated with aging are, at least in part, responsible for memory impairments and increasing the quantity and quality of SWS will reverse these memory deficits by enhancing synaptic plasticity and increasing protein synthesis necessary for memory.
Using chemogenetic activation of the sleep-promoting GABAergic neurons located in the parafacial zone (PZGABA), SWS is enhanced in adult (3-6months) and aged (18-24 months) mice. Mice are subjected to hippocampus dependent memory tests; spatial object recognition (SOR) and contextual fear conditioning (CFC). The same mice are also used for measuring protein synthesis in the cortex and hippocampus as a molecular mechanism by which SWS promotes memory. Finally, synaptic plasticity is measured in the hippocampus. Control groups include littermate non-SWS enhanced mice.
Our aged mice recapitulate the sleep and cognitive deficits associated with aging, phenotypes that are reversed by SWS enhancement. This is associated with an increase in protein synthesis and enhanced long term potentiation (LTP) in the hippocampus.
This study has the potential to not only definitively link SWS with hippocampus-dependent memory but also provide new molecular targets to promote cognition in aging and in neurological patients combining sleep disruption and memory consolidation, such as Alzheimer's disease.

Disclosures: O.E. Igiesuorobo: None. **L. Ferrari:** None. **H. Gompf:** None. **C. Anaclet:** None.

Digital Abstract Session

P361. Learning and Memory in Aging: Medial Temporal Lobe and Neuropsychology

Program #/Poster #: P361.04

Topic: H.12. Aging and Development

Support: DST-SERB, Govt. of India, New Delhi, India
The John G. Kulhavi Professorship in Neuroscience at CMU
The Filed Neurosciences Institute

Title: Carnosine counteracts age-related hypoactivity and cognitive dysfunction by reducing dysregulation of plasma corticosterone and secondary structure of amyloid-beta in the brains of aged rats.

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Abstract: Age-related stress leads to dysregulation of different biochemical pathways, leading to proteinopathies. Carnosine is an endogenous antioxidant that attenuates age-related stress and neurochemical pathologies. The goal of the present study was to assess the role of carnosine in modulating age-related: (a) plasma corticosterone status; (b) Aβ deposition in different brain regions (prefrontal cortex, hippocampus, hypothalamus, pons-medulla and cerebellum) and potential conformational changes as measured by Raman spectroscopy; (c) alterations in locomotor activity (rearing and locomotion) and (d) cognitive deficits. Young (4-month-old) and aged (18 and 24-month-old) male Wistar rats were given carnosine (2.0 μg/kg/day, i.t.) for 21 consecutive days and tested for spontaneous motor activity (rearing and locomotion) and cognitive (radial-arm-maze) abilities before being euthanized for plasma and histological analyses. Results indicated that in the aged rats: (a) the plasma corticosterone level was increased; (b) a graded deposition of Aβ in the examined brain regions (except for the cerebellum) was observed, with a maximum deposition in prefrontal cortex with the increase in abundance of β-sheet in the secondary structure of Aβ plaque; and (c) locomotor activity and cognitive abilities were reduced. Treatments of carnosine attenuated the age-related increase in plasma corticosterone levels, as well as the reduction in locomotor activity and cognitive abilities. Carnosine also reduced regional graded Aβ deposition with elevated β-sheet levels and improved the cognitive abilities in the aged rats. It can be concluded from the present study that: (a) age-related decreases in rearing and locomotor activities are associated with the increase of plasma corticosterone and a graded Aβ accumulation in the brain regions (except for the cerebellum) by increasing the abundance of β-sheets; (b) carnosine can attenuate these age-induced deficits by reducing (i) the abundance of β-sheet in the Aβ secondary structure and (ii) allostatic load.

Disclosures: S. Banerjee: None. **B. Mukherjee:** None. **M.K. Poddar:** None. **S. Raha:** None. **A.K. Singha:** None. **G.L. Dunbar:** None.

Digital Abstract Session

P361. Learning and Memory in Aging: Medial Temporal Lobe and Neuropsychology

Program #/Poster #: P361.05

Topic: H.12. Aging and Development

Support: NIA R01 AG068182-01
NIA AG062413-02S1
P01 AG062413
R37 AG013925

Title: Whole-body senescent cell clearance alleviates age-related brain inflammation and cognitive impairment in mice

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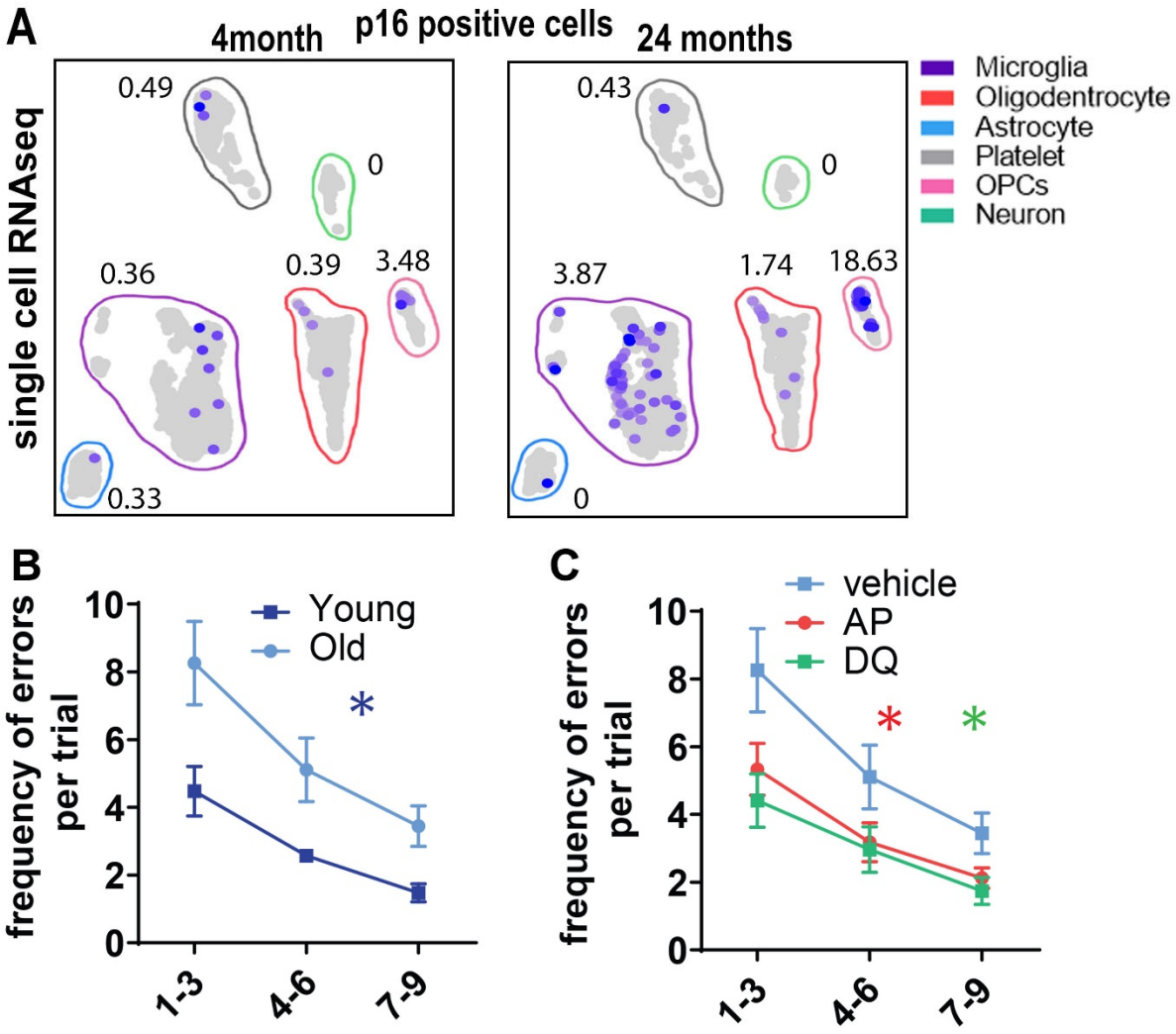
Abstract: Background and aims: Cellular senescence is characterized by an irreversible cell cycle arrest and a senescence-associated secretory phenotype. Recent studies have shown that clearance of senescent cells has beneficial effects in several neurodegenerative diseases and alleviates anxiety in obese mice. However, it is still not known if senescent cells influence brain aging and age-associated cognitive impairment. This study aimed to investigate the impact of senescent cell clearance in age-associated cognitive decline. **Methods:** INK-ATTAC mice were aged until ~27m of age and treated with vehicle, AP20187 (AP) or senolytic drug cocktail Dasatinib/Quercetin (DQ). Drugs were administered for 3 days per week, every two weeks (total 8 wks). Stone T-maze was used to measure cognition. For single-cell RNA-sequencing, the hippocampus was dissected from 4 and 24m old mice. Senescent cells and efficacy of senolytic interventions was assessed by IHC, IF and RNA-ISH. **Results:** We first conducted single-cell RNA-sequencing in the hippocampus from young and aged mice. We observed an age-dependent increase in p16+ cells, which was more pronounced in microglia and oligodendrocyte progenitor cells (**Fig A**). We then aged INK-ATTAC mice, in which p16+ cells can be eliminated with the drug AP and treated them with AP or DQ. We observed that both strategies resulted in a decrease in p16 exclusively in the microglial population, reducing microglial activation. Next, we used a modified Stone T-maze and confirmed that aged animals make more errors (**Fig B**) and need a longer time to complete the maze. We found that both AP and DQ resulted in a significant reduction in the frequency of errors made by aged mice (**Fig C**). **Conclusion**: Here, we show by scRNA-seq that p16 expression increases with age predominantly in microglia and oligodendrocytes. Importantly, we found that interventions targeting senescent cells are effective at removing p16+ microglia and improve cognitive function. Our data provide proof-of-concept for senolytic interventions' being a therapy to alleviate age-associated cognitive impairment.



Disclosures: **D. Jurk:** None. **M. Ogrodnik:** None. **S. Evans:** None. **S. Victorelli:** None. **T. Tchkonia:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Clinic, Unity Biotechnology. **J.F. Passos:** None. **N. Neretti:** None. **J.L. Kirkland:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Clinic, Unity Biotechnology.

Digital Abstract Session

P361. Learning and Memory in Aging: Medial Temporal Lobe and Neuropsychology

Program #/Poster #: P361.06

Topic: H.12. Aging and Development

Title: Sex differences in Memory Subtype Deficits in Parkinson Disease

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Abstract: Parkinson Disease (PD) disproportionately affects patients by sex; the incidence in males is 1.5 times higher than females. The Movement Disorder Society Task Force identified five domains for the diagnosis of mild cognitive impairment in PD: attention and working memory, executive function, language, episodic memory and visuospatial function. However, while most studies agree that in PD females have superior cognitive performance, sex differences in visuospatial performance have yet to be evaluated. Interestingly, in healthy populations, females perform better in verbal based tasks of memory while males show superior performance in visuospatial tasks; however, we tested the hypothesis that females with PD would have superior performance compared to males on measures of verbal memory and visuospatial processing. A total of 146 participants (52 female) diagnosed with PD ages of 50 and 82 years were recruited. Sex was determined by self-report and only those entered as male or female were included. Demographic/disease information and a large battery of neuropsychological tests including measures verbal and auditory memory and visuospatial function using the Wechsler Memory Scale-third edition (WMS-III), were collected from each patient. All patients were tested in his or her best ON state. One-way ANOVA revealed no significant differences between sexes in age ($F(1,145) = 0.073$, $p = 0.788$), global cognitive status as indicated by MMSE performance ($F(1,139) = 0.057$, $p = 0.811$), nor disease descriptive variables such as illness duration ($F(1,56) = 0.103$, $p = 0.750$), disease stage ($F(1,88) = 0.589$, $p = 0.445$), or motor evaluation $F(1,95) = 0.021$, $p = 0.886$). There was a significant difference in years of education favoring males ($F(1,145) = 12.240$, $p = 0.001$), thus years of education was used as a covariate in all analyses of cognitive measures. Results from the MANCOVA using years of education as a covariate revealed significant differences across sex in immediate auditory memory index ($F(3,135) = 4.313$, $p = 0.040$, Cohen's $d = 0.13$), and immediate ($F(3,135) = 8.739$, $p = 0.004$, Cohen's $d = 0.51$) and delayed visual memory index ($F(3,135) = 5.183$, $p = 0.024$, Cohen's $d = 0.40$). We see significant and consistent sex differences in measures of cognitive function with superior performance in females compared to males. While our results are consistent with superior verbal performance in females, we found that females also outperformed males on visuospatial tasks inconsistent with results in healthy populations. Therefore, PD may have sex specific mechanisms that disproportionately affect memory in males.

Disclosures: T.H. Reekes: None. **C.I. Higginson:** None. **K.A. Sigvardt:** None. **D.S. King:** None. **D. Levine:** None. **V.L. Wheelock:** None. **E.A. Disbrow:** None.

Digital Abstract Session

P361. Learning and Memory in Aging: Medial Temporal Lobe and Neuropsychology

Program #/Poster #: P361.07

Topic: H.12. Aging and Development

Support: Sierra Pacific Mental Illness Research, Education, and Clinical Center (MIRECC) at the VA Palo Alto Health Care System
Alzheimer's Association New Investigator Research Grant [NIRG-15-362171 to JKF]
Department of Defense Telemedicine and Advanced Technology Research Center [W81XWH-12-1-0584 to JKF]
VA Rehabilitation Research and Development Service [I21RX001884 to JKF]

Title: Mindful awareness attenuates the negative effect of worry on processing speed

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Abstract: Introduction: Worry has been shown to have a negative impact on many aspects of neurocognitive performance. Interestingly, research indicates that mindfulness both improves aspects of cognitive ability and also reduces worry symptoms. Yet, the impact of mindfulness on the relationship between worry and cognition has yet to be explored. The present study investigates the potential moderating influence of mindfulness on the relationship between worry and cognitive performance. **Method:** The sample included 113 older Veterans who were screened at the VA Palo Alto Health Care System (94.7% male, mean age = 71.20±9.05). Domains of interest included episodic memory (Rey Auditory Verbal Learning Test; RAVLT), processing speed (Trail Making Test Trial A; TMT A), and executive function (Trail Making Test Trial B; TMT B). Mindful awareness was assessed with the Five Facet Mindfulness Questionnaire (FFMQ). Worry symptoms were assessed using the Penn State Worry Questionnaire (PSWQ). Multiple linear regression was used to evaluate the relationship between mindful awareness, worry, and cognitive function. Due to its relationship with cognitive performance, age was included as a covariate in all regression models. Post-hoc simple slopes analysis was conducted to further evaluate the moderating effects of mindful awareness. **Results:** Mindful awareness significantly moderated the relationship between worry and processing speed. Older Veterans with low mindful awareness and high worry performed worse compared to those with low mindful awareness and low worry. In those older Veterans with high mindful awareness, level of worry did not impact processing speed performance. Mindful awareness did not significantly impact the relationship between worry and executive functioning or episodic memory. **Conclusions:** Mindful awareness attenuates the negative impact of worry on processing speed in older Veterans. Importantly, mindful awareness is only one aspect of mindfulness. Future research should explore the impact of other facets of mindfulness on the relationship between worry and cognition.

Disclosures: **N. Harper:** None. **M. Delgadillo:** None. **A. Erickson:** None. **J.K. Fairchild:** None.

Digital Abstract Session

P362. Mechanisms of Age-Related Cognitive Decline

Program #/Poster #: P362.01

Topic: H.08. Learning and Memory

Support: R01 AG003376, McKnight Brain Research Foundation

Title: Effects of chronic, high-dose minocycline treatment on cognitive performance in aging rats

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Abstract: The antibiotic minocycline is a promising therapeutic intervention in multiple age-associated diseases that share inflammation as a symptom. Its neuroprotective effects have been attributed to anti-inflammatory properties exerted through interaction with T-cells and microglia. Minocycline crosses the blood-brain-barrier and can ameliorate cognitive deficits in experimental models of ischemic stroke, traumatic brain injury, and neurodegenerative conditions like Alzheimer's, Parkinson's, and Huntington's diseases. To address the lack of research into the effects of minocycline on normative aging in the absence of brain trauma or disease, we investigated whether minocycline might affect age-related cognitive decline in rats. Middle-aged (16mo) male Fischer rats were divided into control and treatment groups, with treated rats receiving 70mg/kg/day of minocycline via drinking water (a high dose chosen for its minimal effect on water consumption). After 8 weeks of treatment, rats (18 mo) were administered a behavior test battery, with a retest 10 weeks later (20mo) to assess the impact of continued minocycline treatment on cognitive performance. The battery includes spatial and cued versions of the Morris watermaze, a spontaneous object recognition (SOR) task, and a delayed matching-to-place working memory task. Analysis of the 1st battery shows that while treated rats trend towards learning the spatial watermaze slower than controls (2WAY RM ANOVA: treatment: $F(1, 21) = 4.049$, $p = 0.057$), both groups perform similarly at the end of the task, and did equally well on the perirhinal cortex-dependent SOR task and prefrontal cortex-dependent working memory task. When retested 10 weeks later, treated rats learn the spatial watermaze at the same rate as controls, and both groups display age-related decline in performance on the SOR task. On the working memory retest, both treated and control rats achieve a shorter pathlength on the retention trial. The present data suggest minocycline is not effective for modifying cognition in normative aging. While minocycline has been successful

treating cases of severe brain injury or neurodegenerative disease, the degree of neuroinflammation in the aging brain may be below threshold for such beneficial effects.

Disclosures: **K. Young:** None. **M.A. Zempare:** None. **A.L. Dalmendray:** None. **A. Gregolynskyj:** None. **M.K. Chawla:** None. **J.F. Guzowski:** None. **C.A. Barnes:** None.

Digital Abstract Session

P362. Mechanisms of Age-Related Cognitive Decline

Program #/Poster #: P362.02

Topic: H.08. Learning and Memory

Support: McKnight Brain Research Foundation
NIH/NIA 1R01AG049722
NIH/NIA R01AG055544

Title: Cortical and hippocampal activity altered with loss of perforant path fibers

Authors: *T. L. COOPER[1], S. M. TURNER[1], K. N. LUBKE[1], J. J. THOMPSON[1], S. A. JOHNSON[3], A. P. MAURER[1], S. N. BURKE[2];
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Abstract: Discrimination between objects that share similar features is a cognitive function often impaired with normal aging and exacerbated with progressive neurodegenerative conditions such as Alzheimer's Disease (AD). The perforant path, a connection that plays a crucial role in mnemonic discrimination, is also highly vulnerable to aging, with extensive fiber loss associated with Mild Cognitive Impairment and episodic memory deficits. In a prior publication from our lab, animals with a unilateral perforant path transection were tested on a mnemonic discrimination task using LEGO object pairs with 90%, 70%, or 50% feature overlap and additional lure trials using a LEGO and standard object for 0% similarity. Animals with loss of perforant path fibers showed impaired performance on a mnemonic discrimination task, and this difference from sham animals exacerbated as similarity between objects increased. The results of that study mimic findings from aged rats on the same task in comparison to young animals, though there was a greater magnitude of age-related deficits, supporting the idea that loss of perforant path fibers may be one factor of many involved in the progression of AD and age-related cognitive decline. This study elaborates upon those behavioral deficits by analyzing neuronal activity in cortical and hippocampal sub-regions involved in mnemonic discrimination following unilateral perforant path transection. As dentate gyrus disruption did not produce a behavioral deficit, we hypothesize that the dysfunction may arise from reduced connectivity between CA1/CA3 subregions of the hippocampus and the perirhinal (PER) and lateral entorhinal (LEC) cortices. Sections of CA1, CA3, PER, and LEC were stained for immediate-early gene (IEG) *Arc* to visualize neuronal activity associated with behavioral testing. Cells were counted as being positive for cytoplasmic *Arc*, nuclear *Arc*, both cytoplasmic and nuclear *Arc*, or

negative for *Arc* expression. Only pyramidal cells, not glial cells, were included in the analysis. Cells positive for nuclear *Arc* indicate principal neuron activity 5 minutes prior to brain collection, and *Arc* in the cytoplasm indicates activity approximately 30 minutes prior. Analysis of *Arc*-positive neurons in each of the four regions reveals the pattern of activity associated with a mnemonic discrimination task as well as how cognitive impairment due to perforant path transection manifests in neuronal activity. Preliminary analysis indicates increased cells in the LEC positive for both cytoplasmic and nuclear *Arc* and decreased cytoplasmic-*Arc* positive cells in the CA3 for animals with loss of the perforant path.

Disclosures: T.L. Cooper: None. **S.M. Turner:** None. **K.N. Lubke:** None. **J.J. Thompson:** None. **S.A. Johnson:** None. **A.P. Maurer:** None. **S.N. Burke:** None.

Digital Abstract Session

P362. Mechanisms of Age-Related Cognitive Decline

Program #/Poster #: P362.03

Topic: H.08. Learning and Memory

Support: NIH Grant AG047334
NIH Grant AG054804

Title: White matter tract integrity mediates relationships between learning-related activity in younger and older adults

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Abstract: Healthy aging is accompanied by declines in our ability to learn associations between events without awareness, termed implicit associative learning (IAL). Functional magnetic resonance imaging (fMRI) studies have attributed these age-related IAL deficits to over-recruitment of the hippocampus and under-recruitment of the basal ganglia and prefrontal cortex. Our recent fMRI work further showed age-related decreases in relationships between IAL-related activity from these regions, which may be due to the negative effect of aging on their white matter connections disrupting the propagation of neural signals. Here, we tested this hypothesis in healthy younger (n = 28; 20.8 ± 2.3 years) and older (n = 22; 73.6 ± 6.8 years) adults who completed the Triplet Learning Task (TLT) during fMRI and diffusion tensor imaging (DTI). The TLT measures IAL as the difference in reaction time to cue-cue-target "triplets" that occurred more versus less frequently. IAL-related activity was modeled as the difference between high and low frequency triplets within four functionally defined regions of interest from our prior voxel-wise fMRI work: the prefrontal cortex, hippocampus, and basal ganglia (putamen, globus pallidum). Tract integrity (fractional anisotropy; FA) was extracted from four skeletonized standard white matter tracts connecting the hippocampus (fornix, hippocampal cingulum) or basal ganglia (external capsule, and internal capsule) to prefrontal

cortex. As expected, results revealed age group differences in IAL performance, hippocampus and prefrontal IAL-related activity, and fornix and external capsule white matter integrity. Independent of age group, IAL-related prefrontal activity was significantly related to activity in the hippocampus and basal ganglia. Moreover, these prefrontal-hippocampus relationships were mediated by integrity of the fornix and hippocampal cingulum in both younger and older adults. However, the prefrontal-basal ganglia relationships were mediated by internal capsule integrity in younger, but not older, adults. Together, these results provide minimal support for the hypothesis that age-related differences in white matter integrity affect the coordination of neural activity between interconnected brain regions and instead suggest that individual differences in brain structure constrain inter-region relationships between brain function in both younger and older adults.

Disclosures: J.L. Merenstein: None. **I.J. Bennett:** None.

Digital Abstract Session

P363. Cognitive Disorders: Cerebellum

Program #/Poster #: P363.01

Topic: H.12. Aging and Development

Support: NIH P50 MH0942581
NIH U01 NS103780
Kiwanis International Neuroscience Research Foundation

Title: Improvement in Cognitive and Psychological Functioning after Surgical Decompression in Chiari Malformation Type I - A Prospective Study

Authors: *S. C. SEAMAN[1], C. DEIFELT STREESE[3], K. MANZEL[2], J. KAMM[6], D. TRANEL[4], B. J. DLOUHY[5];
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Abstract: Introduction: Chiari Malformation Type I (CM-I) is a congenital and developmental abnormality in which the cerebellar tonsils are displaced more than five millimeters below the foramen magnum. This can result in cerebrospinal fluid obstruction, brainstem compression, and syringomyelia. Symptoms attributed to CM-I include Valsalva-induced headaches and bulbar symptoms, and these typically improve after surgery. However, little is known about the cognitive profile in CM-I and whether surgery has any impact. We therefore sought to perform a comprehensive analysis. **Patients/Methods**: Patients age ≥7 with classic symptoms and imaging of CM-I were included; patients with any cognitive confounders were excluded. Assessments were made preoperatively and at 6-18 months postoperatively. Evaluators blinded to the clinical severity assessed subjects using 26 neuropsychological and psychological tasks with data converted to Z scores based on normative data. Analysis was conducted using mixed fixed effect

modeling for preoperative assessment and change in performance after a standard suboccipital craniectomy and decompression. Overall performance change between time points was assessed with the Wilcoxon match-pair sign testing. **Results**: Thirty-six patients met criteria, with 25 tested at both time points. The mean age was 28, mean education 12 years, and a majority were female (75%) and right-handed (78%). Mean tonsillar descent was 14 mm. Adjusted for preoperative motor performance, baseline performance in tasks of visual perception/visuospatial construction and memory were significantly different from the normative mean (complex figure copy $z = -0.86$, $p = 0.002$; complex figure recall [CFT-Recall] $z = -1.09$, $p < 0.0001$). No mean z scores showed above-normal performance. Adjusted for mood and motor performance, postoperative improvement was noted in tasks of construction and memory (CFT-Recall, $p = .004$). The cumulative postoperative performance across all measures demonstrated improvement ($p = 0.002$). **Conclusion**: CM-I patients had mildly impaired baseline (preoperative) performance compared to normalized data in visuospatial and visuoconstructional abilities. Importantly, CM-I patients had general improvement in cognitive and psychological performance after surgery, with specific improvements in visuospatial memory. We cautiously interpret these findings to suggest the cerebellum's involvement in cognitive function.

Disclosures: S.C. Seaman: None. **C. Deifelt Streese:** None. **K. Manzel:** None. **J. Kamm:** None. **D. Tranel:** None. **B.J. Dlouhy:** None.

Digital Abstract Session

P363. Cognitive Disorders: Cerebellum

Program #/Poster #: P363.02

Topic: H.12. Aging and Development

Support: CNAP -GOPC504007
CNAP-NIGMS- GM113109 NIH
start up funds to BP

Title: Differential impacts of VPA (Valproic acid) exposure on male and female cerebellar volumes in Long-Evans rats.

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Abstract: Autism spectrum disorder (ASD) is a developmental disorder effecting communication, social interactions, and repetitive behaviors. In order to develop better treatment strategies, it is important to understand the underlying neurobiology of the disorder. The VPA model is a well- known animal model of autism-like behavior with both construct and face validity (Mabunga et al., 2015; Roullet et al., 2013). Women who were prescribed medications containing VPA to treat seizures and bi-polar disorder had increases in the number of children

that developed ASD compared to the regular population (Chomiak et al., 2013; Dean et al., 2002). The VPA model mimics this by introducing VPA into the system of a pregnant dam and then the offspring are raised and tested. This study used the VPA model to examine behavioral deficits and related brain changes between treated and untreated offspring. Pregnant dams were injected with VPA or saline and the offspring were reared to adulthood. Our prior work examined performance on social interactions, marble burying behavior and attentional set-shifting performance. This study used those same animals (males and females) and measured brain volumes of the cerebellum via MRI (magnetic resonance imaging). After 3D scans were obtained, volumes of lobules and crus regions were taken using a region of interest (ROI) approach. ROIs were hand segmented by blind to condition researchers. Preliminary results indicate that female VPA offspring had significant differences in brain volume in several lobules of the cerebellum compared to control females. Female VPA rats had decreased volumes of lobules I, IV and X, whereas male VPA rats had increased volumes of lobule VI. Female humans with ASD frequently have hypoplasia of the cerebellum whereas, males with ASD can have hypoplasia or hyperplasia of the cerebellum (Chen et al., 2017). Thus, this study highlights sex differences found in ASD are also occurring in a rodent model of ASD, and provides future hypotheses for testing cerebellar function in relation to behavioral deficits.

Disclosures: **B. Plakke:** None. **Z.E. McKinnell:** None. **M. Payne:** None. **I. Mali:** None. **S.H. Bossmann:** None. **T.B. Shrestha:** None.

Digital Abstract Session

P364. Animal Models: Mechanisms of Cognitive and Behavioral Aging

Program #/Poster #: P364.01

Topic: H.12. Aging and Development

Support: NIH Grant AG046266
NIH Grant AG046266-5S1
NIH Grant AG064925

Title: Longitudinal cognitive decline and neuropathology in aging marmosets (Callithrix jacchus).

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Abstract: Nonhuman primate (NHP) models of human cognitive aging are critical to understand vulnerabilities to neurodegenerative diseases such as Alzheimer's disease (AD). However, longitudinal designs which evaluate cognitive change within the same individual are difficult to implement in long-lived NHPs. Common marmosets (*Callithrix jacchus*) are ideally suited for longitudinal studies of cognitive aging, as they have a naturally short lifespan of about 10 years

and develop AD-relevant neuropathology during aging. We studied 27 marmosets (14 F, 13 M) from middle ($\bar{X}$ age=5) to old age across 4 years. Each year, marmosets completed a reversal learning task on a touchscreen interface. Cognitive performance improved during the first three years due to practice effects but declined in the fourth year with the onset of old age. Importantly, this decline was more prominent for females than males. Female cognitive impairment was not explained by sex differences in motivational factors, motor competence, stress reactivity or differences in overall behavioral profiles. Trajectories of cognitive decline were highly variable among both male and female marmosets, suggesting pathological aging and neurodegenerative processes in some individuals. Indeed, we observed different degrees of β-amyloid burden in these aged marmoset brains along with microglial activation and atrophy in the prefrontal cortex and hippocampus. Analyses of neuronal dendritic spine density and morphology in these areas will also be characterized. Future studies in this valuable model of preclinical AD will help identify risk factors, clarify the mechanisms leading to neuropathology and design interventions to prevent or delay disease progression.

Disclosures: E. Rothwell: None. **C. Freire Cobo:** None. **K.P. Workman:** None. **A. Lacreuse:** None. **P.R. Hof:** None. **M. Edwards:** None. **W. Janssen:** None. **M. Varghese:** None.

Digital Abstract Session

P364. Animal Models: Mechanisms of Cognitive and Behavioral Aging

Program #/Poster #: P364.02

Topic: H.12. Aging and Development

Support: NeuroNetwork for Emerging Therapies
The Robert E. Nederlander Sr. Program for Alzheimer's Research

Title: Inflammation and innate inflammatory cGAS/STING mechanisms may play a role in obesity- and prediabetes-mediated cognitive decline in aged mice

Authors: S. E. ELZINGA, R. E. HENN, E. GLASS, F. E. MENDELSON, J. M. HAYES, G. G. MURPHY, *E. L. FELDMAN;
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Abstract: Obesity, prediabetes, the metabolic syndrome, and diabetes are growing in prevalence around the world. These conditions also predispose patients to the development of neurological complications, including cognitive decline. This is especially true for patients with midlife obesity and diabetes. A potential shared mechanism that may be responsible for this increased risk is chronic inflammation. One innate inflammatory pathway that has received recent attention is the intracellular double stranded DNA sensing cGAS/STING pathway. Therefore, we performed a pilot study using our non-transgenic murine model, which develops insulin resistance and nervous system complications in response to high fat feeding, to investigate the potential role of cGAS/STING in midlife obesity and prediabetes-mediated cognitive decline. At 1 yr of age, male C57/BL6 mice were started on either standard diet (SD; n=10) or a 60% high

fat diet (HFD; n=10) composed primarily of lard. Animals were fed their respective diets for 12 weeks, at which time they were assessed for cognitive changes using social recognition testing. After 13 weeks on diet, metabolic phenotyping was performed with glucose tolerance testing and general inflammatory phenotyping was carried out via ELISA. Hippocampi were isolated and assessed by Western blotting for cGAS/STING pathway protein expression. As expected, animals fed HFD had impaired glucose tolerance compared to SD controls. Additionally, they had increased circulating levels of the cytokine tumor necrosis factor alpha and the chemokine monocyte chemoattractant protein-1, indicating a pro-inflammatory phenotype. When looking at central nervous system specific hippocampal changes, STING protein expression was lower in HFD animals compared their SD counterparts. Interestingly, HFD mice also had deficits in short and long term memory. Taken together, these data indicate that HFD-induced obesity and prediabetes in mice promote cognitive decline and that this may be, at least in part, due to changes in inflammatory profiles and specifically regulation of the cGAS/STING pathway. However, more studies are warranted to fully understand these potential links and to understand underlying mechanisms of cGAS/STING involvement in obesity, prediabetes, and cognitive decline.

Disclosures: S.E. Elzinga: None. **R.E. Henn:** None. **E. Glass:** None. **F.E. Mendelson:** None. **J.M. Hayes:** None. **G.G. Murphy:** None. **E.L. Feldman:** None.

Digital Abstract Session

P364. Animal Models: Mechanisms of Cognitive and Behavioral Aging

Program #/Poster #: P364.03

Topic: H.12. Aging and Development

Title: Late-life social isolation in female mice leads to increased exploratory behaviors

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Abstract: This study aims to investigate the effects of late-life social isolation in a female mouse model. All mice (n = 40) were female and 18-months of age at the beginning of the study and had been group housed during that rearing period. At 18-months of age mice were randomly assigned to either continue group housing for one month (n = 20) or undergo social isolation (n = 20) for one month. After the experimental rearing period mice underwent a battery of tests to assess depressive-like symptomology, social behaviors, exploratory behaviors, and contextual fear conditioning. The testing battery lasted one week. Tests were conducted only one per day in least-to-most aversive order, and isolated mice remained isolated throughout the testing. Hippocampal tissue was collected for examination of inflammatory cytokines (*IL-6*, *IL1β*, *Tnf-α*) and microglia activation (*Itgam*). We found increases in hyperactivity and exploratory behaviors after isolation, but no alterations in depressive-like symptomology, social interaction, fear memory, or inflammatory cytokines and microglia activation. Previous research has shown that humans, and young rodents, may engage in enhanced exploratory and social behavior following

a period of social isolation. This is the first model of female late-life social isolation which can be used to inform future research and interventions for this population.

Disclosures: G. Sullens: None. **K. Gilley:** None. **K. Jensen:** None. **H. Rodgers:** None. **M. Sekeres:** None.

Digital Abstract Session

P365. Schizophrenia: Micro-RNA Signaling

Program #/Poster #: P365.01

Topic: H.13. Schizophrenia

Support: NHMRC Senior Research Fellowship 1121474

Title: Mirna expression signature of cognitive dysfunction in the peripheral blood of patients with schizophrenia

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Abstract: Schizophrenia (SZ) is a neurodevelopmental psychiatric disorder affecting 1% of the population. While the diagnosis is currently based on signs and symptoms, by understanding the molecular determinant of SZ, we may be able to identify biomarkers and better direct treatment to the underlying causal factors. MicroRNAs (miRNAs) are a class of small non-coding RNAs which are already known to be associated with the disease. The aim of the current pilot study was to investigate the plausible association of miRNA expression with the severity of SCZ cognitive symptoms. We conducted small RNA-Seq on peripheral blood mononuclear cells (PBMCs) of five male patients with severe cognitive deficits and compared it to ten male patients with moderate impairments (CS group), collected by the Australian Schizophrenia Research Bank (ASRB). Of the 15 differentially expressed miRNAs with PValue<0.05, only hsa-miR-3175, which was 12-fold down-regulated in CD cases, survived multiple testing correction (FDR<0.05). TargetScan database and ToppFun online tool revealed that miR-3175 predicted targets are involved in neurogenesis, synaptic signalling, and regulation of synaptic plasticity, and associated with several neurodevelopmental disorders, including SZ. Transfecting SH-SY5Y-derived neuron-like cells with the miRNA mimic and inhibitor oligonucleotides led to the expression dysregulation of 68 and 895 genes, respectively, with 56 genes common between the two conditions. Among these 56 genes, 16 are miR-3175 predicted targets, of which 14 are related to the immune system, and 12 are known to be associated with psychiatric disorders. In addition, Gene Set Enrichment Analysis (GSEA) by ToppFun tool showed that they are involved in many processes and pathways related to the immune system, and are linked to autoimmune disorders, which is an important observation considering the well-established link between immune dysfunction and psychiatric disorders, including SZ. Collectively, our results suggest

that miR-3175 might be an interesting candidate biomarker for cognitive deficit subtype of SZ worthy of further investigation.

Disclosures: B. Khavari: None. **M.P. Geaghan:** None. **E. Mahmoudi:** None. **V. Carr:** None. **M.J. Green:** None. **M.J. Cairns:** None.

Digital Abstract Session

P366. Schizophrenia and Bipolar Disorder: Neural Correlates

Program #/Poster #: P366.01

Topic: H.13. Schizophrenia

Title: Effects of anxiety on p50 sensory gating and cognition in schizophrenia and bipolar disorder

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Abstract: Schizophrenia and bipolar I disorder (BPI) are characterized by severe symptoms like psychosis and debilitating mood swings between depression and mania, respectively. Anxiety is another symptom frequently experienced but oftentimes is not properly acknowledged and diagnosed during treatments for these psychiatric disorders. The goal of this exploratory research was to bring more notability to the impact of anxiety on schizoaffective (SczA), BPI and schizophrenia chronic paranoid type (SczP) patients in their daily lives by focusing on its effect on sensory gating and neurocognition. Subjects diagnosed with SczA (n=40), SczP (n=30), and BPI (n=55) were recruited along with controls (n=41) from the UCI Medical Center and the community. Selected item scores from the Positive and Negative Syndrome Scale (PANSS) and the Hamilton Depression Rating Scale (HAM-D) were taken to measure levels of anxiety. Using a paired click (S1 and S2) task, sensory gating was measured focusing on the positive evoked potential occurring around 50 msec (P50). Peak amplitudes were measured and used to calculate the S1-S2 difference and S2/S1 ratio. A neurocognitive test battery was also conducted, and the relationships among the anxiety, cognitive, and evoked potential measures for each group were studied, as well as differences between groups on these measures. Results from this study showed that anxiety scores clearly separated the patient groups from controls. Neurocognitive performance consistently distinguished the patient groups, with SczA performing worse than the others in the domains of attention, delayed memory, and short memory. Specifically, high anxiety in SczA correlated with deficiencies in sensory gating and in performances on various domains of neurocognition. BPI performed better in most domains than the other patient groups, but SczA was affected by anxiety more than both the BPI and SczP groups. High PANSS Gen Anxiety significantly correlated with decreased S1-S2 differences, while high HAM-D Anxiety significantly correlated with increased S2/S1 ratios. Both of these are characteristics of deficiency in sensory gating.

Disclosures: D. Cao: None. **W.E. Bunney:** None. **J.V. Patterson:** None.

Digital Abstract Session

P366. Schizophrenia and Bipolar Disorder: Neural Correlates

Program #/Poster #: P366.02

Topic: H.13. Schizophrenia

Support: Lycaki-Young Funds (State of Michigan)
DMC Foundation
Ethel and James Flinn Foundation
Mark Cohen Neuroscience Endowment
National Institutes of Mental Health (MH111177)

Title: Effective connectivity analyses of the plasticity of brain networks in schizophrenia during learning

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Abstract: Schizophrenia is suggested to be a disorder of brain network plasticity, and the process of learning is characterized by the plasticity of neural circuits. Ostensibly, patients with schizophrenia demonstrate poor learning performance in comparison to healthy individuals. Understanding the network plasticity of schizophrenia patients has rarely been attempted at the macroscopic network scale. Thus, we used a learning paradigm that induced negatively accelerated associative learning, and then utilized Dynamic Causal Modeling (DCM) to investigate the plasticity of ascending and descending pathways in the brain networks in schizophrenia. The early and late stages of encoding and retrieval externally modulate pathways in the brain during associative learning tasks. The associative learning task required engagement from ventral and dorsal visual pathways, the hippocampus (HPC), and frontal regions. We hypothesized two possible models to test for whether descending pathways from frontal regions were being modulated by the conditions and found that in the most probable model this was not the case. We implemented DCM to estimate effective connectivity of pathways and how their degree of modulation changed in response to the conditions. This allowed us to investigate altered plasticity in schizophrenia. The object-location association paradigm required subjects to associate the name of a unique object with a location in space and recall it later. The paradigm consisted of eight cycles of encoding, rest, and retrieval to induce asymptotic performance. All subjects (n=90) were provided informed consent and were compensated for their participation. Subjects in the control (n=38; nine females; mean age: 28.20; range: 18-45) group (HC) are those who are free from any neurological or psychological evaluation. Subjects in the patient (SCZ) group (n=52; ten females; mean age: 31.96; range: 18-50) were stabilized on anti-psychotics. The fMRI data was acquired using a 3T Siemens Verio scanner (32-channel volume head coil).

Images were pre-processed for spatial irregularities and analyzed using SPM 12. Our findings demonstrate that both SCZ and HC network pathways had a similar increase in the degree of modulation from early to late encoding, displaying plasticity of their networks. The results during the condition of retrieval differ, displaying evidence for altered plasticity in schizophrenia patients. The ascending pathway from the inferior temporal gyrus to the HPC had a time by group difference of significance 0.009, signifying contrasting effects of retrieval on this pathway. Overall impairment of frontal regions was also observed in patients.

Disclosures: K.G. Nanaaware: None. **A. Chowdury:** None. **S. Baajour:** None. **J.A. Stanley:** None. **V.A. Diwadkar:** None.

Digital Abstract Session

P366. Schizophrenia and Bipolar Disorder: Neural Correlates

Program #/Poster #: P366.03

Topic: H.13. Schizophrenia

Title: Gender Differences in Neurocognition and Symptom Severity in Schizophrenia and Bipolar I Disorder

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Abstract: Schizophrenia (Scz) and bipolar I disorder (BPI) can have symptom overlap, making diagnosis difficult, and profound cognitive impairments that greatly affect clinical outcomes and quality of life. Previous studies have shown a gender difference in symptom severity (SS) for Scz; it remains poorly studied in BPI. Little research has been done on gender differences in neurocognition for Scz or BPI, and none have explored the role of SS on these differences. This study investigated whether there are gender differences in neurocognition and/or symptom severity in BPI and two Scz subtypes: schizoaffective disorder (SczA) & schizophrenia paranoid type (SczP). The BPI group was sub-categorized into “with psychosis” (BPI Psy) and “without psychosis” (BPI woPsy). Groups were: SczA (F=26; M=24); SczP (F=9; M=23); BPI Psy (F=11; M=13); BPI woPsy (F=21; M=17). Subjects (14-78 years old) were interviewed to establish diagnosis, completed a series of clinical rating scales (PANSS, Hamilton Depression Scale, Beck Depression Inventory, MADRS, etc.) to evaluate SS, and then completed a neurocognitive assessment that included tests for memory, attention, executive function, fine motor control, language, and emotional perception domains. Domain z-scores were calculated using control score means & standard deviations. SS was assessed using each clinical scale’s scoring rubric. ANOVA (Diagnostic Group by Gender) and Newman-Keuls method were used to investigate Group and Gender differences. ANOVA demonstrated multiple significant results ($p<0.05$) due to diagnosis or gender; there was no significant interaction between diagnosis and gender. Females had significantly worse scores for depression scales (Beck, Hamilton, MADRS) and the Mee-Bunney psychological pain scale than males. Males scored significantly higher in the attention and fine motor control domains, but lower for immediate memory. Significant Pearson

correlations existed between many SS measures and neurocognitive domain scores for all gendered subgroups except SczA Males. BPI Psy males had a correlation between CGI and executive function (r= -0.537, p=0.058); SczP females had a correlation between the Hamilton score and attention domain (r= -0.794, p=0.011). BPI Psy females had a correlation between the Hamilton score and visual memory (r= -0.739, p=0.009); SczA females had one between CGI and emotion perception (r= -0.429, p=0.037). These results and others suggest that females with Scz or BPI have more neurocognitive impairments and more severe symptoms (especially for depression) than males, and that worse neurocognitive performance may be associated with increased symptom severity.

Disclosures: T.A. Chu: None. **S.L. Ferretti:** None. **S.T. Siddiqi:** None. **W.E. Bunney:** None. **J.V. Patterson:** None.

Digital Abstract Session

P366. Schizophrenia and Bipolar Disorder: Neural Correlates

Program #/Poster #: P366.04

Topic: H.13. Schizophrenia

Support: NIH Grant

Title: Correlation Between Sensory Gating Deficits and Smoking in Patients with Schizophrenia and Bipolar Disorder

Authors: *R. VERMANI, J. V. PATTERSON, W. E. BUNNEY, Jr.;
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Abstract: The prevalence of smoking is 22.5% in the U.S. general population. For those with mental illness such as schizophrenia (Scz) and bipolar disorder (BPI), the prevalence values are almost 3 times the norm. The prevalence of smoking in these groups highlights the need to understand the role of smoking in Scz and BPI, as it can lead to high morbidity and mortality. The purpose of this research was to investigate the effects of chronic smoking on sensory gating, a measure of brain inhibitory function. Sensory gating is the ability to attend to selective stimuli and ignore redundant or irrelevant information, preventing the brain from being exposed to an overflow of information. When paired auditory clicks are presented to normal controls, the P50 evoked potential (EP) response to the second click (S2) is attenuated in amplitude compared to the first (S1), measured as the S2/S1 ratio or S1-S2. In Scz, and often BPI, sensory gating is usually impaired. A previous study assessing the immediate effect of smoking on sensory gating in Scz found impaired gating before, but temporarily improved gating after smoking. In contrast, another study showed that chronic smoking impaired gating in Scz smokers compared to Scz nonsmokers. However, little research has been done on the effects of smoking on sensory gating in BPI or SczA specifically. This project studied the smoking effects on sensory gating in 3 patient groups: Scz paranoid type (SczP, n=32), schizoaffective disorder (SczA, n=35), and bipolar I disorder (BPI, n=60). Subjects participated in a 64-channel EEG during an auditory

paired click test. Ninety paired clicks (S1 and S2) were separated by 10 seconds with 500 ms between pairs. Neuroscan software was used to record EEG data, with channels referenced to linked mastoids. Electro Magnetic Source Estimation Suite (EMSE) was used to process the raw data and measure the P50 peaks. Results indicated a significant difference in pack-yrs between the three groups, with BPI having a lower mean pack-years than SczA and SczP. Analysis of variance showed a significant difference in P50 peak amplitude between smokers and nonsmokers at S1 in all three groups, with reduced peak amplitude in smokers, but not at S2. For the SczA and BPI groups, but not for SczP, there was a larger S2/S1 ratio and smaller S1-S2 difference, both indicating worse gating, in smokers compared to nonsmokers. The conclusion to be drawn from these results is that chronic smoking impairs sensory gating. While previous studies have shown that smoking can temporarily improve function in these groups, there is a long-term negative effect. This underscores the need for the development of effective cessation therapies for these patients.

Disclosures: R. Vermani: None. **J.V. Patterson:** None. **W.E. Bunney:** None.

Digital Abstract Session

P366. Schizophrenia and Bipolar Disorder: Neural Correlates

Program #/Poster #: P366.05

Topic: H.13. Schizophrenia

Title: Effect of age of onset of schizophrenia and duration of illness on sensory gating mechanisms and neurocognitive performance

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Abstract: Earlier onset of schizophrenia has been associated with poor neuropsychological functioning and typically more symptom severity, but there are few studies regarding the effect of illness duration. There also is a paucity of evidence about the relationship of age of onset and duration of illness with sensory gating or with neurocognitive function in subgroups of schizophrenia(Scz). This study investigated whether illness duration and age of onset of Scz can predict deficits in sensory gating and neurocognitive performance. Subjects diagnosed with schizoaffective disorder (SczA; N=44; F=23; M=21) and paranoid schizophrenia (SczP; N=30;F=8; M=22) ranging in age from 15-60 years were recruited. Patients completed clinical surveys and interviews to quantify severity of their symptoms. CMINDS ® software was used to evaluate neurocognitive function using performance in 6 domains (attention, executive, immediate, short-term, delayed, and visual memory) calculated in standard deviation units from average performance for controls (N=35). Sensory gating was assessed using P50 evoked potentials, elicited using the paired click paradigm, and measured with an automated software. SczA and SczP groups were also studied by gender. Correlation and multiple regression analyses were performed with significance levels at $p \leq 0.05$. Pearson correlations for age of onset and

illness duration were significant in SczA and SczP males but not in females. Duration of illness and S1amplitude correlated in the SczA males and age of onset and S2/S1 ratio were correlated in SczP females. In SczA females, onset age was correlated with attention and short-term memory, and illness duration was correlated with delayed memory. In SczP males, illness duration was correlated with both executive and short-term memory. Regression analysis using age of onset and illness duration as independent variables showed that duration of illness predicted S1 peak amplitude and the S1-S2 difference in SczA males. As duration of illness increased, S1amplitude and the S1-S2 difference decreased, showing a comparatively less effective sensory gating mechanism. In SczA females, duration of illness predicted performances in executive and delayed memory domains, and age of onset predicted performances in attention and short-term memory domains. In SczP males, duration of illness predicted performance on short-term memory. These relationships show that as duration of illness increased or age of onset decreased, neurocognitive performance decreased. These results supported a conclusion that age of onset and duration of illness can influence both sensory gating and neurocognitive performance in Scz.

Disclosures: **A. Yaseen:** None. **W.E. Bunney:** None. **J.V. Patterson:** None.

Digital Abstract Session

P366. Schizophrenia and Bipolar Disorder: Neural Correlates

Program #/Poster #: P366.06

Topic: H.13. Schizophrenia

Support: NIMH R01 MH118500

Title: Effects of distinct excitatory cortical and inhibitory reticular and local thalamic inputs on spindle dynamics

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Abstract: Based on two distinct thalamocortical (TC) circuits with reciprocal components in primates, we developed models of core, matrix, and mixed TC loops. The core TC circuit, prevalent in sensory thalamus, drives activity focally in the middle cortical layers and gets feedback through small modulatory cortical axon terminals from pyramidal neurons in layer 6 (L6). The matrix TC circuit, can drive activity in high-order thalamus, through large axon terminals from cortical layer 5 (L5) pyramidal neurons and includes broad thalamic feedback to the superficial cortical layers. The inhibitory thalamic reticular nucleus (TRN) intercepts all TC communication and is situated strategically between the thalamus and cortex. We used distinct core and matrix TRN components to engage cortico-TRN and thalamo-TRN loops in pure core, pure matrix or mix TC loops to investigate the functional consequences of different ratios of core and matrix node connectivity contribution to spindle dynamics. Our models comprised more numerous projections from cortical L6 pyramidal neurons to TRN and thalamus, but we also

included direct L5 projections to matrix TRN (L5-TRN) and thalamus with a range of density of L5-TRN, starting from zero. Based on our rate-based model circuit we found: a) increased local inhibition in the thalamus or b) increased TRN inhibition of core and matrix thalamic neurons enhances spindle generation and sustains spindle activity for longer periods; c) a more diffuse nature of spindles in matrix compared to core, with the mix type showing intermediate properties in agreement with hypotheses that spindles can be classified in core-generated, matrix-generated or mixed types, depending on the neuroanatomy of pathways involved in their generation; d) the involvement of L5-TRN projection enhances the spindle generation and propagation; and e) spindle power can be modulated based on the level of cortical feedback and involvement in model core vs. matrix. Our rate-based model tested the impact of different ratios and specializations of neuroanatomical connectivity at multiple nodes of the TC circuit in spindle dynamics. Our simulations provide detailed metrics for shifts in the engagement of distinct TRN, core, and matrix circuits underlying typical sleep spindle generation and states of vigilance. This work can help establish a framework to study disruption of TC-TRN circuit balance in seizures, atypical sensory reactivity, and deficits in sleep and attentional gating seen in autism and schizophrenia.

Disclosures: A. Yazdanbakhsh: None. **B. Zikopoulos:** None.

Digital Abstract Session

P366. Schizophrenia and Bipolar Disorder: Neural Correlates

Program #/Poster #: P366.07

Topic: H.13. Schizophrenia

Support: MEXT, Japan; 17H05735
MEXT, Japan; 18H05428
MEXT, Japan; 18H05433
the project Brain Mapping by Integrated Neurotechnologies for Disease Studies
the PRESTO program
Support Unit for Bio-Material Analysis, Research Resource Division, RIKEN Center for Brain Science

Title: Extra-large spines distort neuronal computation in synaptic disorders

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Abstract: The spatiotemporal organization of neuronal firing is crucial for information processing, but how thousands of inputs to the dendritic spines drive the firing remains a central question in neuroscience. Despite the fact that the distribution of spine sizes, an index of synaptic weight, is strongly skewed with a heavy tail, the (patho)physiological significance of large spines

remains entirely unknown. Here, we found that extra-large (XL) spines (more than three standard deviations from the average) supralinearly boosted the firing triggered by NMDA spikes within these spines. The resulting synaptic amplification in a few XL spines was sufficient to drive neuronal firing in the absence of the normally required Ca^{2+} spike. Interestingly, mice with knockdown of DISC1, a molecule implicated in psychiatric conditions, exhibited ten-fold more XL spines and markedly increased firing. We experimentally and theoretically observed that XL spines negatively correlated with working memory, which can contribute to psychiatric pathophysiology.

Disclosures: K. Obi-Nagata: None. **F. Shirai:** None. **N. Suzuki:** None. **T. Okimura:** None. **S. Tanaka:** None. **A. Hayashi-Takagi:** None.

Digital Abstract Session

P366. Schizophrenia and Bipolar Disorder: Neural Correlates

Program #/Poster #: P366.08

Topic: H.13. Schizophrenia

Support: NIMH grants R21MH082417
NIMH grants R01MH084038

Title: Phencyclidine (PCP) causes cognitive control failures by rapidly dysregulating translation, and discoordinating gamma-timescale neural discharge

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Abstract: We previously reported that the psychotomimetic phencyclidine (PCP) causes hyperlocomotion and theta (8-Hz)-modulated 70-Hz mid-frequency gamma oscillations to dominate 30-Hz slow gamma in hippocampus CA1 local field potentials, which consequently discoordinates the timing of discharge amongst CA1 place cells, which make ensemble discharge representations of a familiar space unrecognizable, without disturbing individual place fields (Kao et al., J. Neurosci., 2017). Here we investigate in mice, the mechanisms of the PCP effects. Although PCP is a noncompetitive NMDA receptor antagonist, we observed that, not only does 8mg/kg PCP cause hyperlocomotion, but the systemic administration of PCP also impairs a familiar active place avoidance task on a rotating arena that requires cognitive control to avoid the stable location of shock while ignoring irrelevant locations on the rotating arena. Pretreatment with either of the negative regulators of translation anisomycin (60 mg/kg) or MPEP (70 mg/kg), prevents both the sensorimotor and the cognitive impairments. Next, using acute hippocampus slices, we directly determined that PCP unbalances translation that engages the AKT, mTOR and 4EBP1 translation machinery and increases ARC protein. These effects are mimicked by NR2A blockade, confirming that NMDAR-antagonism can dysregulate translation.

Based on these findings, we suggest a translation dysregulation hypothesis that PCP causes cognitive impairments by dysregulating translation. This hypothesis predicts that pretreatment with translation inhibitors will also prevent the PCP-induced discoordination of sub-second neural ensemble discharge. We are testing this prediction by recording the temporally coordinated discharge within large ensembles of hippocampus cells in head-fixed mice before and after MPEP, PCP, and their control administrations, blinded to the treatment. These findings identify a molecular mechanism for the cognitive effects of PCP: NR2A-mediated antagonism of a subset of NMDA receptors that dysregulate translation and discoordinate the sub-second temporal organization of hippocampal discharge, ultimately impairing cognitive control.

Disclosures: **E. Park:** None. **D. Dvorak:** None. **K.W. Tunnell:** None. **J. Gutierrez:** None. **H. Kao:** None. **H. Jourdi:** None. **E. Wallace:** None. **A.A. Fenton:** None.

Digital Abstract Session

P366. Schizophrenia and Bipolar Disorder: Neural Correlates

Program #/Poster #: P366.09

Topic: G.06. Anxiety Disorders

Title: Phenome-wide association study of complement component 4 variants with neuropsychiatric disease and beyond

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Abstract: The Major Histocompatibility Complex (MHC) locus is critical to immune response, and also contains the strongest genetic association with Schizophrenia. Of particular interest is Complement Component 4 (C4), which is involved in synapse elimination, and thus contributes to the “excessive pruning” hypothesis of Schizophrenia. By utilizing novel catalogues of C4 variants, we impute C4 isoforms and perform a Phenome-Wide Association Study (PheWAS) to identify their phenotypic effects in the Million Veteran Program (MVP). We are particularly interested in neuropsychiatric disorders, but given the broad range of effects of the Complement System, we also hope to contribute to a greater understanding of C4 dynamics. Moreover, the large sample size of the MVP will help elucidate effects associated with rare C4 isoforms. Finding biologically relevant phenotypes associated with C4 variants will open greater avenues for understanding MHC locus dynamics with neuropsychiatric diseases. We use Beagle and the Sekar lab’s C4 reference panel to impute C4 isoforms in the MVP. Then, we perform PheWAS to query the association of C4 variants with phenotypes and examine interesting relationships further. Across the phenome, we see significant hits for a variety of diseases. Interestingly, the directions of these effects seem to be common among broad categories of phenotypes but often opposite across different phenotypic categories, indicating a possible mechanism driving differential effects across the phenome with possible evolutionary trade-offs. There are also numerous significant phenotypes for other C4 isoforms outside the realm of neuropsychiatric

diseases. Initial results indicate C4 haplotype BS is neuroprotective for several neuropsychiatric disorders including Schizophrenia, mood disorders, and anxiety disorders. Particularly interesting is the fact that upon controlling for Schizophrenia PRS score, haplotype BS now increases risk for Schizophrenia. This suggests strong population effects that correlate with the presence of haplotype BS. Given the arcane dynamics of the MHC locus, C4 imputation is a useful tool to understand a complicated region involved in many different traits. Our results here show that understanding an individual's C4 isoform can aid in explaining the risk for a variety of diseases.

Disclosures: S. Venkatesh: None. **M. Kim:** None. **M. Gandal:** None. **G. Voloudakis:** None. **P. Roussos:** None.

Digital Abstract Session

P367. Modeling Schizophrenia in Rodents

Program #/Poster #: P367.01

Topic: H.13. Schizophrenia

Support: CONACYT (Mexico) PhD Scholarship 481316
MINECO-EU-FEDER (Spain) Grant SAF2016-75500-R
CONACYT (Mexico) Grant 252808
PRODEP (Mexico) Grant CA-BUAP-120

Title: Risperidone attenuates structural neuroplasticity and inflammatory alterations in the nucleus accumbens in a schizophrenia-related neurodevelopmental model in the rat

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Abstract: Hyperactivity of the mesolimbic pathway (dopaminergic axons from the ventral tegmental area to the ventral striatum or nucleus accumbens: NAcc) underlies the neurobiology of psychotic symptoms in schizophrenia. The increase of the dopaminergic tone in the NAcc compromises the structure and function of the spiny projection neurons (SNPs), which consequently disrupts the connectivity among limbic system areas, and also could produce inflammation and oxidative/nitrosative stress (O/NS). It has been suggested that atypical antipsychotic drugs attenuate psychosis beyond their modulatory activity on the mesolimbic pathway, since these drugs have anti-inflammatory and antioxidant effects. Therefore, the effects

of the atypical antipsychotic risperidone (RISP) on the structural neuroplasticity and the biochemistry of the NAcc of adult male rats with neonatal ventral hippocampus lesion (NVHL) were evaluated, which is a neurodevelopmental schizophrenia-related model. For this purpose, using the Golgi-Cox technique, the following morphological parameters of SPNs of the NAcc were evaluated: arborization, length by dendritic order, total dendritic length, number of dendritic spines and morphological classification of dendritic spines; neuronal counting by stereology, and inflammatory mediators and O/NS markers were evaluated by colorimetric reactions and Western-blot.The 21-day treatment with RISP (0.25 mg/kg, ip) ameliorates neuronal atrophy and improves dendritic spine dynamics by increasing the number of functional spines of the SPN in the NAcc of rats with NVHL. Furthermore, RISP normalizes alterations in inflammatory pathways regulated by nitric oxide, nuclear factor-κB (NF-κB), and mitogen-activated protein kinases (MAPK); and consequently, reduces O/NS. Also, RISP stimulated the antioxidant pathway regulated by the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) by increasing the concentration of the antioxidant enzyme metallothionein I-II (MT I-II). These RISP effects in the NAcc together with its neurotrophic, anti-inflammatory, and antioxidant effects on the prefrontal cortex in rats with NVHL can be related to the behavioral improvement of these animals in motor activity and social interaction tests.These results highlight the importance of studying the alternative mechanisms of atypical antipsychotics such as RISP and establish the usefulness of this animal model to explore links between neurodevelopment and the immune response relevant to the pathophysiology of schizophrenia.

Disclosures: **H. Tendilla-Beltrán:** None. **H. Coatl-Cuaya:** None. **R.A. Vázquez-Roque:** None. **D. Martín-Hernández:** None. **L. Garcés-Ramírez:** None. **J.L.M. Madrigal:** None. **J.C. Leza:** None. **G. Flores:** None.

Digital Abstract Session

P367. Modeling Schizophrenia in Rodents

Program #/Poster #: P367.02

Topic: H.13. Schizophrenia

Support: SEP-CINVESTAV #141
CONACYT GRANT 458209
CONACYT GRANT 485970

Title: Long-term behavioral and neuroanatomical effects induced by the schizophrenia animal model of neonatal administration of MK-801 in male Wistar rats

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Abstract: Schizophrenia is a psychiatric disorder that affects around 1% of the adult population worldwide; it is characterized by a wide range of symptoms known as positive (hallucinations and delusions), negative (social isolation), and cognitive. Due to its complexity, schizophrenia neurobiology remains unknown. A theory about the origin of schizophrenia is the hypofunction of the NMDA receptors in early neurodevelopmental stages. Neonatal administration of the non-competitive NMDA receptor antagonist (MK-801) is an animal model that mimics negative and cognitive symptoms of schizophrenia. On the other hand, the hippocampus, a brain region crucial for memory and cognitive processing, has been implicated in the neurobiology of schizophrenia. However, the neuroanatomical effects induced by neonatal administration of MK-801 in this brain region are unknown. Therefore, we aimed to evaluate the long-term (up to postnatal day 90) behavioral effects (locomotion, social interaction, and cognitive performance) and the neuroanatomical effects induced by neonatal administration of MK-801. To achieve this aim, male Wistar rats were injected during five days with MK-801 (0.2 mg/kg) or vehicle (saline solution 0.9%), starting at the postnatal day (PND) 7. Thereafter, rats were housed in groups until the beginning of adulthood (PND 90). At the PND 90, independent groups (n=12) were evaluated in five behavioral tests: open field, social interaction, novel object recognition, Barnes maze, and pre-pulse inhibition, or were euthanized by decapitation to obtain their brains, which were stained with Golgi-cox solution. Our results show that the neonatal administration of MK-801 has long-term behavioral effects such as hyperlocomotion, reduced social interaction, learning and memory impairments, and alterations in sensory gating and motor response. Moreover, neonatal administration of MK-801 also induced alterations in the dendritic morphology of the pyramidal neurons of the dorsal hippocampus CA3 area. Specifically, pyramidal neurons in the MK-801 group presented a significant increase in the total dendritic length compared to the control group, but only apical dendrites contributed to this effect. However, despite its major length, the pyramidal neurons' arborization in the MK-801 group was less complex than the control group. Altogether, we found that the hypofunction of NMDA receptor in early stages of neurodevelopment induced by neonatal administration of MK-801 produces behavioral and neuroanatomical alterations that resemble those observed in schizophrenic patients, which adds more construct and appearance validities to the model.

Disclosures: H. Mancha Gutiérrez: None. **C. Lopez-Rubalcava:** None. **M. Lopez-Pacheco:** None.

Digital Abstract Session

P368. Biochemical and Molecular Techniques

Program #/Poster #: P368.01

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: National Science Foundation Graduate Research Fellowship, Grant No. 1745302
NIH K99/R00 Pathway to independence Award
Open Philanthropy Project
John Doerr

Hock E. Tan and K. Lisa Yang Center for Autism Research
U.S. Army Research Laboratory and the U.S. Army Research Office under contract/grant number W911NF1510548
NIH Director's Pioneer Award 1DP1NS087724

Title: Multiplexed Expansion Revealing of 3D multi-protein synaptic nanostructures

Authors: J. KANG[1], ***M. SCHROEDER**[2], A. WASSIE[3], M. ZENG[1], G. FENG[4], D. SARKAR[3], E. S. BOYDEN[5];
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Abstract: The ability to study the crowded and complex three-dimensional molecular architecture of the synapse remains a challenge, as electron microscopy lacks molecular specificity, and the resolution of optical microscopy is diffraction-limited. Existing optical super-resolution methods are limited by the crowdedness of the biomolecules themselves, which constrains access to epitopes by tags such as antibodies. We recently developed Expansion Revealing (ExR; Sarkar et al., *bioRxiv* 2020), a novel expansion-based technology that enables super-resolution imaging (~20 nm resolution) of brain tissue using conventional antibodies and that reveals previously hidden brain nanostructures. Here, we report multiplexed Expansion Revealing (mExR), a complementary tool to ExR, which enables immunostaining of tens of synaptic proteins in the same tissue sample using conventional antibodies without signal deterioration or bleed-through between rounds. Multiplexing has the potential to facilitate discovery of previously uncaptured high-dimensional protein organizations and even complex multi-protein interactions. Using this technology, we revealed the three-dimensional nanoscale architecture of ten synaptic proteins in the mouse primary somatosensory cortex, including Bassoon, $Ca_V2.1$, CamKII , Gephryn, GluR1, Homer1, PSD-95, RIM1/2, SynGAP, and Shank3 (**Fig. 1**). We deployed custom-written MATLAB software to register serial imaging rounds with high precision and extracted 700+ features of synaptic puncta in each channel, including measures of colocalization and inter-puncta distance. We further demonstrate mExR in Thy1-YFP and Dlx5/6-GFP mice, enabling cell-type specific labeling of neuronal spines and boutons alongside multiplexed synaptic proteins. mExR is the first technology to facilitate high-throughput, low-cost super-resolution imaging of tens of synaptic proteins in a single sample. mExR enables unprecedented access to “molecular connectomes” and will facilitate the discovery of previously undiscovered synapse types.

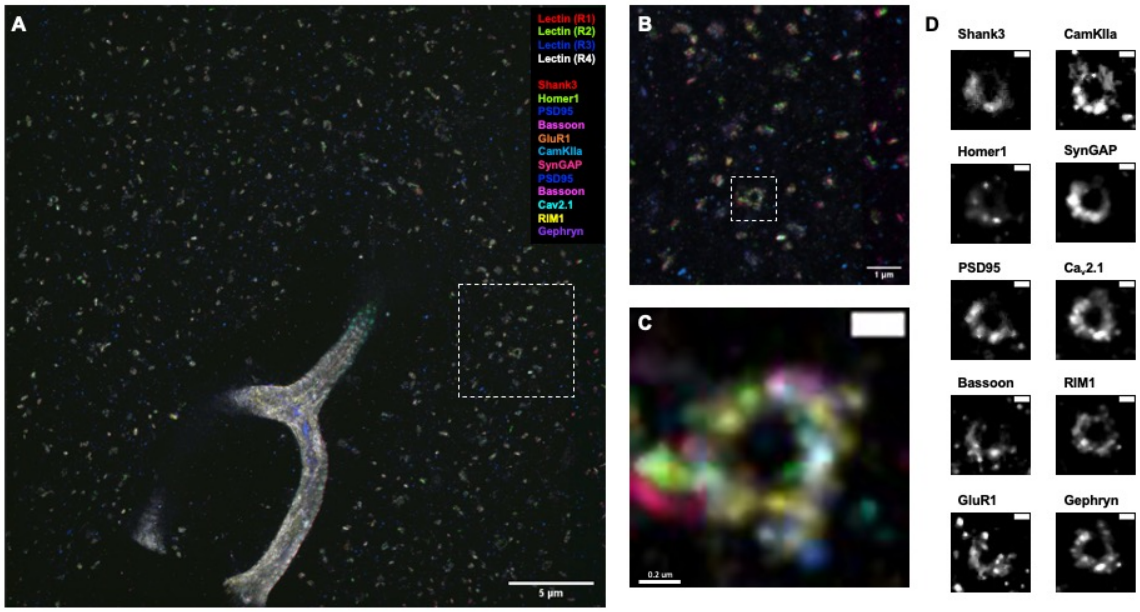

Figure 1. Ten-color mExR image of ten synaptic proteins in the mouse somatosensory cortex. **A)** Illustrative field of view (35x35x11 um^3 biological units) showing synapses of various shapes, sizes, and protein compositions, and a blood vessel. **B)** Inset from **(A)**, showing more detailed synaptic structures. **(C** Inset of **B)**, showing a 3-dimensional, 10-color rendering of a perforated synapse. Lectin was used to register images across rounds. **D)** Grayscale images of each channel for the synapse shown in **(C)**, labeled by protein. Scale bars: 5um in **(A)**, 1 um in **(B)** 0.2um in **(C)** and **(D)**.

Disclosures: **J. Kang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on a patent application for ExR. **M. Schroeder:** None. **A. Wassie:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on a patent application for ExR. **M. Zeng:** None. **G. Feng:** None. **D. Sarkar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on a patent application for ExR. **E.S. Boyden:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on a patent application for ExR, Co-founder of a company seeking to deploy medical applications of ExM-related technologies.

Digital Abstract Session

P368. Biochemical and Molecular Techniques

Program #/Poster #: P368.02

Topic: F.02. Behavioral Neuroendocrinology

Support: CIHR Grant 133606
CIHR Grant 426405
CFI Grant 32631
ANII fellowship POS_EXT_2016_1_134441

Title: Estrogen Profiling in Microdissected Brain Tissue using a Novel Ultra-Sensitive LC-MS/MS Assay

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Abstract: Estrogens are steroid hormones that affect many aspects of brain function, including cognition, social behavior, and neuroprotection. It is well-known that estrogens are synthesized in the ovaries. Estrogens are also synthesized in the brain, where aromatase is expressed in specific regions. Steroids synthesized within the brain are called neurosteroids. Importantly, estrogens play crucial roles in the brain, even at extremely low levels. Current assays lack the necessary sensitivity and/or specificity to measure brain-synthesized estrogens. Furthermore, current methods focus on only 17β-estradiol and generally disregard other estrogens that are synthesized in the brain. Here, we developed a method to measure several estrogens simultaneously, with high sensitivity and specificity. To improve sensitivity, we derivatized estrogens with 1,2-dimethylimidazole-5-sulfonyl-chloride (DMIS). We used liquid chromatography tandem mass spectrometry (LC-MS/MS) to examine a panel of eight estrogens: 17β-estradiol, 17α-estradiol, estrone, estriol, 2-hydroxyestradiol, 4-hydroxyestradiol, 2-methoxyestradiol, and 4-methoxyestradiol. After derivatization, we have improved sensitivity 20-fold, detecting as little as 0.01 pg per sample, demonstrating that our method is extremely sensitive. For each analyte, we have identified a distinct retention time as well as 2 scheduled multiple reaction monitoring (sMRM) transitions that were used as quality control criteria for clear identification. Therefore, we are able to distinguish each estrogen (even stereoisomers) by the chromatographic separation and the sMRM, demonstrating that our method is highly specific. This method has been applied to microdissected brain samples. Initially, we used a songbird model because songbirds have high levels of aromatase and 17β-estradiol in specific brain regions. We were able to simultaneously quantify multiple estrogens in small amounts of brain sample (1-2 mg) in brain areas that regulate aggression such as the hypothalamus and the nucleus taeniae (homologous to mammalian amygdala). We examined seasonal changes of estrogens in the brain and blood. Future work will apply this method to mouse, rat, and human samples and expand the panel of estrogens examined. Our ultra-sensitive assay is essential for small animal models, where estrogen measurement is extremely challenging because of the limited amount of brain tissue. This novel technique will also have wide-ranging applications for basic research and clinical testing, including estrogen measurement in humans with low estrogen levels, such as men, pre-pubertal children, and post-menopausal women.

Disclosures: C. Jalabert: None. **M.A. Shock:** None. **C. Ma:** None. **K.K. Soma:** None.

Digital Abstract Session

P368. Biochemical and Molecular Techniques

Program #/Poster #: P368.03

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant 1-DP1-DA-044250-01
NIDA Training Grant T32DA028874
Whitehall Foundation, Inc.
Penn's At-Large Epigenetics Pilot Grant

Title: Novel approach for cell-type specific profiling of HPTMS and corresponding gene expression

Authors: *D. K. FISCHER, M. D. CARPENTER, K. S. CZARNECKI, E. A. HELLER; Univ. of Pennsylvania, Philadelphia, PA

Abstract: Recent evidence shows that histone post-translational modifications (HPTMs) regulate gene expression via chromatin accessibility and transcription factor recruitment. However, the functional relevance of these HPTMs remains elusive. Here, we applied novel methods to establish a methodology for transcriptional and epigenetic profiling of specific neuronal cell types in mouse brain. Transcription differs in the two distinct subtypes of GABAergic medium spiny neurons (MSNs) within the nucleus accumbens. MSNs express either D1 dopamine receptor (Drd1) or adenosine 2A receptor (A2a). Drd1 and A2a expression define the direct and indirect motor output pathways, respectively, and express distinct genes in response to rewarding stimuli. We established transgenic mouse lines that express an affinity tagged nuclear receptor (GFP- Sun1 fusion) in dopamine Drd1- or A2a-containing MSNs. To initiate the protocol, the bilateral caudate/striatum of male and female mice from these mouse lines were subjected to affinity isolation of nuclei tagged in specific cell types (INTACT) protocol. Nuclei were then subjected to cleavage under targets and release using nuclease (CUT&RUN) or quantitative PCR (qPCR). Using this approach, we were able to bioinformatically profile multiple HPTMs and gene expression in specific cell-types of a single bilateral mouse striatum. Here, we present data from our INTACT into CUT&RUN or qPCR optimization protocols, unveiling a robust method of cell-type specific quantification of HPTMs and corresponding gene expression.

Disclosures: D.K. Fischer: None. **M.D. Carpenter:** None. **K.S. Czarnecki:** None. **E.A. Heller:** None.

Digital Abstract Session

P368. Biochemical and Molecular Techniques

Program #/Poster #: P368.04

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Novel mitochondria marker for staining formaldehyde fixed cells and tissue sections

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Abstract: There are many mitochondrial-targeted fluorescent proteins and fluorescent dyes available to researchers to study the function of mitochondria in live cells. Unfortunately, live-cell probes tend to lose their fluorescence after fixing cells with chemical fixatives including formalin and formaldehyde, making these mitochondria trackers incompatible with immunohistochemistry and in situ hybridization. To overcome this problem, we have developed a novel mitochondria marker, MT-5, for labeling mitochondria in fixed cells and tissues. MT-5 is a bright, far-red fluorescent probe (655 nm excitation/669 nm emission) that localizes in the mitochondria and is highly photostable: it retains brightness in stained samples stored for several weeks and after prolonged fluorescence imaging. MT-5 works equally well on frozen and paraffin-embedded tissue sections and does not cross-react with cell nuclei or the plasma membrane. In addition, MT-5 can be combined in multiplex immunohistochemistry experiments using fluorescent antibody-conjugates with non-overlapping excitation and emission spectra. We present data demonstrating the use of MT-5 on rat and mouse brain tissue sections using different concentrations and incubation time. This optimized protocol provides researchers a fast, one-minute incubation at 200nM resulting in strong, long-lasting mitochondrial staining.

Disclosures: **A.E. Kalyuzhny:** None. **J. Taylor:** None. **A. Ptak:** None. **P. Wood:** None. **D. Miller:** None. **H. Maple:** None.

Digital Abstract Session

P368. Biochemical and Molecular Techniques

Program #/Poster #: P368.05

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIA AG065682

Title: A Genetically Encoded Fluorescent Sensor for monitoring Sonic Hedgehog (Shh) Signaling

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Abstract: Shh signaling is critical for the development, maintenance and function of the central nervous system. Shh acts in a concentration dependent manner and 1.8 fold changes in Shh signaling strength can result in vastly different biological outcomes as evidenced by Shh dependent cell fate determination during development. Shh signaling is spatially highly restricted and affects cellular physiology within seconds to hours. For example, we have shown previously that the precise spatial and temporal integration of multiple sources of Shh in the pMN domain of the developing spinal cord is critical for the switch from neurogenesis to oligogenesis (Starikov et al., bioRXiv; doi: https://doi.org/10.1101/534750). Further, we have shown that

mesencephalic dopamine neurons (DAN) in the adult brain engage in neuronal co transmission using Shh. Here, activity dependent release from of Shh from DAN impinge on cholinergic neurons of the striatum and affect the rate of reinforcement learning in the normal brain and the formation of dyskinesia in parkinson's models (Malave et al.; bioRXiv: https://doi.org/10.1101/2020.03.09.983759). To determine how moment to moment changes in Shh signaling strength are interpreted by Shh receiving cells and integrated with other extra cellular signals, tools need to be generated that allow quantifying Shh signaling strengths with subcellular spatial and millisecond temporal resolution. We have developed an activation-based biosensor for Smoothened, the G-protein coupled receptor (GPCR) downstream effector of Shh signaling in target cells. Guided by the prior design of GPCR-Activation Based (GRAB) sensors and analysis of their sequence homology, we inserted a circularly permutated (cp) GFP module into the third intracellular loop of Smoothened via HiFi assembly. The sensor was characterized in HEK293 cells with Smoothened pharmacology, using a vector that produces a Smoothened receptor with a non-fused GFP as a control. Our results demonstrate the sensor achieves physiologically relevant affinity and specificity, with sub-second kinetics. My poster will present the biophysical characterization of this novel recombinant SmoGRAB sensor and validation of its ability to detect physiological relevant dynamics of Shh signaling in vitro.

Disclosures: S.L. Bernal: None. **A.H. Kottmann:** None. **S. Uribe-Cano:** None.

Digital Abstract Session

P368. Biochemical and Molecular Techniques

Program #/Poster #: P368.06

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Using microRNA-target chimeras to study post-transcriptional gene regulation in the mammalian brain

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Abstract: Using microRNA-target chimeras to study post-transcriptional gene regulation in the mammalian brain

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One of the most intriguing and complex questions in neuroscience concerns the molecular mechanisms by which synapses participate in learning and memory. Beginning as early as the 1960s, a growing body of evidence demonstrates that new protein synthesis is required for learning and long-term memory formation.[1] Further, local protein synthesis at synapses has been shown to participate in plasticity and perturbations in translation are associated with disorders of cognitive function such as autism.[2,3] The realization of the proximity of protein synthesis to the sites of synaptic transmission, and its disruption in disease, has underscored the importance of

understanding the nature of post-transcriptional regulatory mechanisms that shape the complement of proteins at synapses.Since their discovery in 1993, microRNAs (miRNAs) have been appreciated for their breadth of function as post-transcriptional regulators by translation inhibition and transcript destabilization.[4] miRNAs regulate neuronal plasticity and dendritic spine morphogenesis, are implicated in higher-order brain functions such as memory and cognitive dysfunction, and proteins involved in miRNA biogenesis and function are found near synapses.[1] The evolutionarily conserved let-7 family of miRNAs has emerged as a critical mediator of post-transcriptional gene regulation in many growth-related processes and is highly abundant in mature differentiated neurons.[5-6] Work from our lab and others has shown that let-7 miRNA levels can be regulated by neuronal activity,[7-9] however, an unambiguous determination of the critical *in vivo* mRNA targets of let-7 and other miRNAs has not been previously possible. This project employs a modified version of the Argonaute-Crosslinking and Immunoprecipitation (Ago-CLIP) technique to generate miRNA-target chimeric RNAs to investigate critical mRNA targets and small RNA regulatory mechanisms governing protein synthesis in the mammalian brain.

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Disclosures: W.T. Mills: None. **M.K. Meffert:** None.

Digital Abstract Session

P368. Biochemical and Molecular Techniques

Program #/Poster #: P368.07

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Beijing Municipal Science & Technology Commission Z181100001318002
Beijing Brain Initiative of Beijing Municipal Science & Technology Commission Z181100001518004
Guangdong Grant “Key Technologies for Treatment of Brain Disorders” 2018B030332001
the General Program of National Natural Science Foundation of China projects 31671118, 31871087, and 31925017
NIH BRAIN Initiative NS103558
Shanghai Municipal Science and Technology Major Project 2018SHZDZX05
Shanghai Pujiang Program 18PJ1410800

Title: A series of genetically encoded GRAB sensors for measuring serotonin dynamics in vivo

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Abstract: Serotonin (5-HT) is a phylogenetically conserved monoamine neurotransmitter, critical for mood control, reward processing, and sleep-wake homeostatic regulation. Malfunction of the 5-HT system is also involved in depression, addiction, compulsivity, and other neurological diseases. Indeed, drugs targeting central serotonergic activity have been used to treat virtually every psychiatric disorder, with the best example being the use of selective serotonin reuptake inhibitors (SSRIs) for depression. Yet, despite the importance of 5-HT, our understanding of cell-specific 5-HT signaling during behaviors is very much lacking, in part due to our inability to measure 5-HT *in vivo* with high sensitivity and spatiotemporal resolution. Here, using molecular engineering, we developed genetically-encoded 5-HT sensors by coupling cpEGFP or cpmApple with 5-HT receptors. Upon 5-HT binding, the chimeric receptor changes its conformation which leads to a subsequent fluorescent signal increase from the embedded cpEGFP or cpmApple, therefore reporting the 5-HT level. We named this kind of sensors "the GPCR Activation Based sensors for 5-HT'', short for $GRAB_{5\text{-}HT}$. With iterative engineering, we developed a series of $GRAB_{5\text{-}HT}$ sensors with high specificity and sensitivity. Furthermore, we demonstrated $GRAB_{5\text{-}HT}$ sensors could be applied in multiple organisms, including *Drosophila* and mice for reliable detection of 5-HT *in vivo*. Thus, these series of tools would enhance our understanding of the 5-HT signaling dynamics and mechanisms in the complex nervous system.

Disclosures: J. Wan: None. **W. Peng:** None. **X. Li:** None. **T. Qian:** None. **K. Song:** None. **J. Zeng:** None. **F. Deng:** None. **S. Hao:** None. **J. Feng:** None. **P. Zhang:** None. **Y. Zhang:** None. **J. Zou:** None. **S. Pan:** None. **M. Shin:** None. **J. Venton:** None. **J. Zhu:** None. **M. Jing:** None. **M. Xu:** None. **Y. Li:** None.

Digital Abstract Session

P368. Biochemical and Molecular Techniques

Program #/Poster #: P368.08

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH2092TN

Title: In-vitro and ex-vivo detection of biomolecules through fast scan cyclic voltammetry

Authors: *H. RAFI, A. G. ZESTOS;
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Abstract: FSCV, or fast scan cyclic voltammetry, is a novel electrochemical technique that can measure the oxidation and reduction of various molecules and proteins. They are oxidized/reduced at very specific voltages which serve as a "molecular fingerprint" for detection. Used in FSCV are carbon fiber microelectrodes, or CFMEs; in-house made electrodes composed of a glass capillary tube and 7-micron thin carbon fiber. Not only are these electrodes biocompatible and able to detect molecules and neurotransmitters with high spatiotemporal resolution but can be coated with polymers to enhance said detection. Previous studies from our lab have shown that coating the electrode with synthetic polymers, can increase sensitivity to a desired transmitter. In the current project, we look at a novel carbon fiber, multiarray electrode manufactured by our colleagues at University of Michigan. This novel electrode can simultaneously detect neurotransmitters through four channels, enabling use in multiple locations. We determine if there is improved detection for dopamine between the multi array and CFMEs and whether multiplexing can be achieved via the multiarray for biomolecules such as dopamine, serotonin, and adenosine. Results from these experiments will not only determine if the multiarray is superior to CFMEs, but if simultaneous detection and identification of biomolecules is possible. It is crucial to have highly sensitive electrodes for accurate measurements which allow for applications in in-vivo, for example, with measuring elicited release of dopamine in an animal model.

Disclosures: H. Rafi: None. **A.G. Zestos:** None.

Digital Abstract Session

P369. Data Analysis and Statistics: Neuronal Networks

Program #/Poster #: P369.01

Topic: I.07. Data Analysis and Statistics

Title: Structural Properties of the Ciona intestinalis (L.) Connectome

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Abstract: The ascidian tadpole larva *Ciona intestinalis* has one of the smallest nervous systems with just 231 neurons and is the second organism after *Caenorhabditis elegans* to have a fully reconstructed anatomical connectome at the level of individual neurons and synapses. These properties make this minimal nervous system a unique model to study principles of neuronal circuits and signal flow. In our analyses, we use network science to characterize statistical and topological properties of the chemical synapse network of *Ciona intestinalis* (L.). We calculate properties of the connectome such as degree distributions and clustering coefficients to identify the connectivity and density of network organization. We also identify neurons that are most central in information processing, and network motifs that indicate there is significant reciprocity in the nervous system of this organism. We characterize rich club and diverse club neurons, which therefore have highest efficiency of connection and diversity of connection, respectively,

and may indicate functional importance in information processing and global network integration. Further, our work demonstrates that *Ciona intestinalis*, *C. elegans*, and mammalian neocortex belong to the same superfamily of networks in terms of subgraph (network motif) distribution, thereby elucidating a possible evolutionary convergence in their wiring diagram. We further use comparative connectomics to explain how statistical properties of *Ciona intestinalis* are similar to those found in *C. elegans* and mammalian neocortex and biologically interpret results. Our work proposes mechanistic bases of behavior by generating predictions on experiments involving genetic perturbations, laser ablations or monitoring neuronal activity propagation in response to stimulation. The statistical results and biological interpretation help in understanding various behaviors displayed by *Ciona intestinalis,* and in identifying key neurons responsible for the multitude of behaviors the nervous system regulates.

Disclosures: C. Bhatia: None. **L. Varshney:** None.

Digital Abstract Session

P369. Data Analysis and Statistics: Neuronal Networks

Program #/Poster #: P369.02

Topic: I.07. Data Analysis and Statistics

Support: NIH RF1-MH117809-01

Title: Syconn v2: Scalable synaptic connectivity inference toolkit for volume EM

Authors: *P. J. SCHUBERT[1], S. M. DORKENWALD[2], J. KLIMESCH[1], M. S. FEE[3], W. DENK[1], J. KORNFELD[3];
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Abstract: The ability to acquire ever larger datasets of brain tissue using volume electron microscopy (VEM) leads to an increasing demand for reliable and fast automated analysis methods. We introduce SyConn v2, a cellular-resolution connectome analysis framework, which scales nearly linearly with the number of compute nodes and the processed volume. Importantly, it is compatible with classical HPC environments at universities or national labs and commercial cloud providers. Our framework combines synapse detection with the analysis of cell morphology and the identification of cellular organelles to extract a comprehensive connectivity matrix. Instead of VEM images, our models use a point cloud representation of the cell to perform supervised type classification, semantic segmentation of cellular compartments and unsupervised clusterings for cell type discovery. We show that point-based models are computationally efficient and competitive with previous approaches in classification accuracy (Li et al., 2020; Schubert et al., 2019). Finally, we test SyConn v2 on a > 25 million cubic µm VEM data set of brain tissue, suggesting that the architecture and design choices are sufficiently scalable for the massive data challenges that connectomics faces in the next decade.

Disclosures: **P.J. Schubert:** None. **S.M. Dorkenwald:** None. **J. Klimesch:** None. **M.S. Fee:** None. **W. Denk:** None. **J. Kornfeld:** None.

Digital Abstract Session

P369. Data Analysis and Statistics: Neuronal Networks

Program #/Poster #: P369.03

Topic: I.07. Data Analysis and Statistics

Title: Representation learning for cortical cell types based on local peptidergic networks

Authors: ***F. BAFTIZADEH**, Y. M. MARGHI, R. GALA, M. HAWRYLYCZ, S. J. SMITH, U. SÜMBÜL;
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Abstract: Analysis of a large body of single-cell RNA-seq data from mouse cortex [1] allowed Smith et al [2] to predict recently the existence of dozens of molecularly distinct neuropeptidergic modulatory networks interconnecting all cortical neurons. The predicted signaling pathways are cell type specific [2], suggesting a way to relate the very extensive diversity observed in single-cell transcriptomic studies to circuit function. In particular, predicted cell-type-specific paracrine signaling can be formalized as a network where nodes represent cell types and directed edges represent the expression of genes encoding a peptide precursor protein and its cognate G-protein coupled (GPCR) in source and target types, respectively. Joint analysis of the multitude of networks with directed, non-symmetric connections is, however, quite challenging. Here we develop a method based on graph neural networks to obtain low-dimensional embeddings of the underlying directed graph structures. We demonstrate that these embeddings can accurately predict peptidergic cell type-cell type interactions and shed light on network level differences between cell types defined by their transcriptomes.
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Disclosures: **F. Baftizadeh:** None. **Y. M. Marghi:** None. **R. Gala:** None. **M. Hawrylycz:** None. **S. J. Smith:** None. **U. Sümbül:** None.

Digital Abstract Session

P369. Data Analysis and Statistics: Neuronal Networks

Program #/Poster #: P369.04

Topic: I.07. Data Analysis and Statistics

Support: Allen Institute

Title: Joint identification of neuron types and type-specific activity-regulated genes

Authors: *Y. M. MARGHI, R. GALA, Z. YAO, B. TASIC, U. SÜMBÜL;
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Abstract: The striking diversity of neurons has been appreciated since the early days of modern neuroscience. Recent advances in single-cell transcriptomics uncovered an even greater diversity than previously appreciated [1,2]. While the previous studies confirmed the existence of easily-distinguishable broad neuron classes, they also pointed towards a complicated landscape where neuron types often appear to overlap or form gradients in gene expression [1,2,3]. Therefore, a crucial step toward elucidating the neuronal circuits is to jointly identify the discrete and the continuous factors of variability controlling the identity and function of the neurons that participate in those circuits. Although significant progress has been achieved in documenting the diversity of neurons, the discrete and continuous transitions between types pose unresolved challenges [1,2,4]. Moreover, from a computational perspective, such transitions are hindered by the unknown relative abundances of the underlying cell types. Here, we propose an unsupervised computational method that identifies discrete neuron types and the continuous variability within individual types. Taking advantage of deep generative models, we developed a generative framework using coupled artificial neural networks to disentangle the discrete and continuous aspects of neuronal diversity. At its core, we pursue the idea that different states of the same neuron should still share the same discrete category [2]. We demonstrate the application of our method to a stand-alone single cell RNA sequencing dataset, which defined over 100 transcriptomic cell types in the mouse cortex [3]. Our results illustrate that our framework can jointly identify discrete types as well as type-specific, activity-regulated genes whose expression contributes to the observed gradients and refine the existing classifications of neuronal identity.

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Disclosures: Y.M. Marghi: None. **R. Gala:** None. **Z. Yao:** None. **B. Tasic:** None. **U. Sümbül:** None.

Digital Abstract Session

P369. Data Analysis and Statistics: Neuronal Networks

Program #/Poster #: P369.05

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 1RF1MH123220-01
Allen Institute

Title: Consistent cross-modal identification of cortical interneurons with coupled autoencoders

Authors: *R. GALA, A. BUDZILLO, F. BAFTIZADEH, J. MILLER, N. GOUWENS, A. ARKHIPOV, G. MURPHY, B. TASIC, H. ZENG, M. HAWRYLYCZ, U. SÜMBÜL;
Allen Inst., Seattle, WA

Abstract: Consistent identification of neurons in different experimental modalities is a key problem in neuroscience. Parsing complex relationships across different modalities to uncover neuronal identity is a growing challenge. Here, we present an optimization framework to learn coordinated representations of multimodal data, and apply it to a large multimodal dataset profiling mouse cortical interneurons. Our approach reveals strong alignment between transcriptomic and electrophysiological characterizations, enables accurate cross-modal data prediction, and identifies cell types that are consistent across modalities.

Disclosures: R. Gala: None. **A. Budzillo:** None. **F. Baftizadeh:** None. **J. Miller:** None. **N. Gouwens:** None. **A. Arkhipov:** None. **G. Murphy:** None. **B. Tasic:** None. **H. Zeng:** None. **M. Hawrylycz:** None. **U. Sümbül:** None.

Digital Abstract Session

P369. Data Analysis and Statistics: Neuronal Networks

Program #/Poster #: P369.06

Topic: I.07. Data Analysis and Statistics

Title: Statistical analysis of the temporal-frontal and parieto-frontal networks in the macaque cortex

Authors: *F. GIARROCCO, B. AVERBECK;
NIH, Bethesda, MD

Abstract: The anatomical connections between architectonically defined areas of the frontal, temporal, and parietal lobes has been extensively mapped through tract tracing methods, providing a wide and complex dataset of connectivity. However, a comprehensive description of the statistical organization of the resulting networks, as well as a description of their functional architecture, has been marginally investigated. To tackle this issue, we carried out a maximum

likelihood hierarchical cluster analysis on 32 frontal, 20 temporal, and 17 parietal areas based on their inputs. This method clusters together areas that shared similar inputs, and allowed us to identify a well-defined hierarchical organization of frontal, temporal and parietal areas. Based on temporal inputs, frontal areas were divided into ten clusters, including multiple prefrontal, cingulate, premotor, and motor cortex domains. Based on frontal inputs, temporal and parietal areas were divided into six and four clusters, respectively. Temporal clusters were composed of inferotemporal, parahippocampal, hippocampal, entorhinal/perirhinal, insular, and amygdala domains. Parietal clusters were composed of ventral, lateral-medial, dorsal, and dorsal-medial parietal domains. As a general rule, areas grouped into the same cluster shared basic functional proprieties and, in most instances, were anatomically contiguous areas. Furthermore, each cluster showed a specific set of dominant inputs, which reflected its functional proprieties. Thus, we provided a new and simplified picture of the connectivity between frontal, temporal and parietal cortices, strengthening our understanding of these complex networks.

Disclosures: F. Giarrocco: None. **B. Averbeck:** None.

Digital Abstract Session

P369. Data Analysis and Statistics: Neuronal Networks

Program #/Poster #: P369.07

Topic: I.07. Data Analysis and Statistics

Support: JSPS KAKENHI: 15KK0010
JSPS KAKENHI: 16K00330
JST CREST: JPMJCR1914

Title: Online data-driven estimation and control of nonlinear model for neuronal dynamics

Authors: *S. FUKAMI, T. OMORI;
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Abstract: In recent years, experimental techniques in neuroscience regarding measurement and stimulus have been significantly developed. Along with this, there have been attempts to estimate the neural dynamics from measurement data. As a method for extracting the dynamics model of a neuron from data, the data-driven approach for estimating the mathematical model has been studied in the framework of Bayesian statistics. For example, statistical approaches for estimating non-linear dynamics for single neurons have been proposed.
In order to control neuronal state, a method to determine the feedback stimulus based on the estimated neuronal state has been proposed. In the previous study, the state of a neuron can be controlled more accurately by determining the control signal from the estimated value, whereas the state of neuron cannot be controlled accurately by noisy observable data. In the previous method, the state estimation is performed by assuming that the parameters of the model are known. In realistic situation, it is necessary to conduct online estimation of both parameters and state in order to determine the feedback control signal from the estimated state in the presence of

unknown parameters. However, in the usual estimation of dynamics by Bayesian statistics, estimation is performed using observed data in batch processing. In order to perform feedback control based on the estimated values, it is necessary to estimate parameters online. Moreover, the previous method is restricted to only single neurons.
In this study, we propose an online data-driven method for simultaneous realization of estimation of both parameters and states, and feedback control based on those estimated values. First, nonlinear state space model is derived for neural systems such as single neuron models and neuronal network models consisting of conductance neuron models. Next, sequential Monte Carlo methods, which is a state estimation method applicable to non-linear dynamics, is applied to the derived state space model with the feedback control. In addition, online parameter estimation is performed by using stochastic EM algorithm. A signal of feedback control is determined from the estimated hidden variables and parameters. Using the proposed method, we show that accurate feedback control can be performed by using the proposed method in the situation where the only observed value is noisy membrane potential. Furthermore, we find that the proposed method realizes accurate control of neural systems for not only single neuron but also neuronal network with multiple neurons.

Disclosures: **S. Fukami:** None. **T. Omori:** None.

Digital Abstract Session

P370. Gene, Protein, or Cell-Based Approaches

Program #/Poster #: P370.01

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH Grant 1R21NS101182
National Ataxia Foundation Grant YI-SCA
American Heart Association Grant 13SDG16150007

Title: Evaluation of positive allosteric modulators of SK2 channels using QPatch

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Abstract: Small-conductance Ca^{2+}-activated K^{+} (SK) channels mediate afterhyperpolarization in neurons and dampen the firing frequency of action potentials. Calmodulin is constitutively associated with the SK2 channels and serves as the Ca^{2+} sensor for the SK-calmodulin complex. The SK2 channel subtype plays a key role in the regulation of excitability of Purkinje cells in the cerebellum. Given their importance in Purkinje cells, SK2 channels are a promising drug target for ataxia, a movement disorder. Automated patch clamp (APC) machines, such as Qpatch, have been used in pharmaceutical industry to study drug interaction with varieties of ion channels. Over the past decade, the technology has contributed significantly to pharmaceutical research and drug discovery. Here, we report to use QPatch as a tool for testing and evaluating the

positive allosteric modulators of SK2 channels. A stable cell line of the rat SK2 channel tagged with GFP was established through transfection of HEK293 cells followed by puromycin selection and enrichment using repeated GFP fluorescence-activated cell sorting. Positive allosteric modulators were tested under whole-cell voltage clamp configuration with automated QPatch. The compounds that positively modulate the SK2 channels in the automated QPatch was further tested with the inside-out patch manual recordings. The results from automated whole-cell recordings and inside-out patch manual recordings were consistent.

Disclosures: **W. Yu:** A. Employment/Salary (full or part-time):; Sophion Bioscience Inc. **S.H. Park:** None. **M. Zhang:** None. **N. Salem:** A. Employment/Salary (full or part-time):; Chapman University. **Y. Nam:** A. Employment/Salary (full or part-time):; Chapman University. **K. Parang:** A. Employment/Salary (full or part-time):; Chapman University. **M. Zhang:** A. Employment/Salary (full or part-time):; Chapman University.

Digital Abstract Session

P370. Gene, Protein, or Cell-Based Approaches

Program #/Poster #: P370.02

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Research start-up fund from the College of Medicine, University of Saskatchewan.

Title: Whole tissue proteomics of in vitro and in vivo primed dorsal root ganglia

Authors: M. BAUTISTA, P. CHUMALA, S. DWIVEDI, G. KATSELIS, *A. KRISHNAN; Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Peripheral nerve injury is accompanied by immediate neurodegenerative events followed by active regeneration. In line with this, previous studies established that regenerative reprogramming of sensory neurons occurs as early as 2-3 days after injury and is associated with dynamic expression kinetics of growth regulatory molecules in the dorsal root ganglia (DRG). The early molecular changes in DRGs favoring nerve regeneration are collectively known as '*in vivo* priming' or injury-driven 'pre-conditioning' effect. The *in vivo* primed neurons outgrow extensively, and this model has been used for decades to understand the biology of nerve regeneration. A similar pre-conditioning effect was recently observed in a paradigm involving *in vitro* priming of DRGs. This new paradigm was designed independent of classical nerve injury, thus avoiding painful axotomy for animals. The objective of this study was to characterize the proteomics profile of *in vitro-primed* DRGs and compare it with that of uninjured and axotomized (*in vivo-primed)* DRGs from adult SD rats. We performed liquid chromatography-tandem mass spectrometry for the proteomics analysis and found several proteins differentially upregulated in *in vitro* primed DRGs. Of particular interest, several Ras-related proteins and the components of ubiquitin-proteasome machinery were differentially expressed after *in vitro priming*. The key transcription factor, signal transducer and activator of transcription 3 (STAT 3)

showed downregulation after *in vitro* priming, whereas it upregulated after *in vivo* priming. Similarly, mitogen-activated protein kinase (MAPK) proteins were preferentially upregulated in *in vivo* primed DRGs. Altogether, the proteomics profiling revealed differential molecular regenerative reprogramming of neurons after *in vitro* and *in vivo* priming. In conclusion, *in vitro* priming of DRGs is a valid model to explore additional molecular mediators of nerve regeneration. The proteomics database generated in this study will serve as a resource for future research aimed at developing nerve regeneration therapeutics.

Disclosures: **M. Bautista:** None. **P. Chumala:** None. **S. Dwivedi:** None. **G. Katselis:** None. **A. Krishnan:** None.

Digital Abstract Session

P370. Gene, Protein, or Cell-Based Approaches

Program #/Poster #: P370.03

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: CIRM DISC2-09032
Diversity Supplement 3R24OD021606-02ZF
NIGMS Pharmacology T32 GM099608
NIH NCATS UL1 TR001860-TR001861

Title: Cell-based delivery strategies for zinc finger proteins in preclinical animal models of Angelman Syndrome

Authors: *P. DENG[1], A. ADHIKARI[2], D. CAMERON[1], N. A. COPPING[2], J. J. WALDO[1], J. A. N. M. HALMAI[1], H. O'GEEN[4], J. A. NOLTA[3], J. L. SILVERMAN[2], D. J. SEGAL[4], K. D. FINK[1];
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Abstract: Therapies based on DNA-modifying proteins such as zinc finger, transcription activator-like effectors, and CRISPR/Cas9 to regulate gene expression are becoming viable strategies to treat genetically linked disorders through manipulation of endogenous gene regulation. The identification of an effective delivery systems for these proteins *in-vivo* remain a major translational hurdle. In this work, we evaluate a mesenchymal stem/stromal cell (MSC)-based delivery system as a putative cell-based strategy for the secretion of DNA modifying proteins. MSCs are advantageous as a delivery system due to their favorable ease of culturing, immunomodulatory properties, and favorable clinical safety profile. Presently, we report the first the use of a Zinc Finger secreting MSC (ZF-MSC) in transgenic Angelman Syndrome (AS) mouse models. In our *in-vivo* work we evaluate two routes of administration for ZF-MSC - direct intracranial injection or access into the cerebral spinal fluid space. Secreted ZF protein from mouse ZF-MSC is detectable in the murine hippocampus 1-week following either intracranial or cisterna magna injection. This secreted ZF is able to activate the imprinted paternal Ube3a gene

in a transgenic Ube3a^{Yfp} reporter mouse at 1 and 3-weeks following either intracranial or cisterna magna injection of ZF-MSC. We detect high co-localization of secreted ZF protein within the CA1 and CA3 regions of the hippocampus in the Ube3a^{Yfp} reporter mouse. ZF-MSC were detectable along cerebral spinal fluid rich regions such as the lateral ventricle, 3rd ventricle, and cerebellum following either route of administration. A significant increase in Yfp+ neurons are observable 1-week following intracranial ZF-MSC administration. A significant increase in Ube3a^{Yfp} protein expression is observable in the hippocampus, midbrain, and cerebellum 3-weeks following a cisterna magna injection of ZF-MSC. An amelioration of motor deficits in rotarod and forepaw propulsion is observed 3-4 weeks following intracranial injection of ZF-MSC in the Ube3a$^{mat-/pat+}$ AS mouse. Overall the results of these studies demonstrate that ZF-MSC secrete functionally active ZF protein to activate paternal Ube3a in AS mouse models. This approach may provide a less-invasive, non-surgical means to deliver gene modifying therapies into the CNS through access of the cerebral spinal fluid injections.

Disclosures: P. Deng: None. **A. Adhikari:** None. **D. Cameron:** None. **N.A. Copping:** None. **J.J. Waldo:** None. **J.A.N.M. Halmai:** None. **H. O'Geen:** None. **J.A. Nolta:** None. **J.L. Silverman:** None. **D.J. Segal:** None. **K.D. Fink:** None.

Digital Abstract Session

P370. Gene, Protein, or Cell-Based Approaches

Program #/Poster #: P370.04

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Rappaport Thematic Grant

Title: Novel viral vectors for infection of birds' brain cells

Authors: *S. ZOABI, Y. GUTFREUND, S. BERLIN;
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Abstract: Contemporary optical tools, such genetically-encoded calcium indicators and optogenetic actuators, provide users with exquisite means to trace, measure and manipulate cells *in vivo*, with unprecedented spatiotemporal resolution. Delivery of these *in vivo* can be efficiently obtained by use of viruses, commonly the adeno-associated virus (AAV). However, current AAVs are suitable to but a handful of species; mostly rodents. This gap has hindered the progression of studies in other animal species. We sought to bridge this gap by developing new means to introduce genetic tools to avian species, explicitly to the domestic Japanese quail (*Coturnix japonica)*. Our prime obstacle is that common viral vectors do not infect most avian models and, in the case of quails, remains untested. We first sought to develop a protocol for growing primary neuronal cultures from Quail embryos, as cultures are more suitable for high throughput screening of viral candidates. We succeeded and screened multiple viruses, *in vitro*. All viruses tested showed substantially reduced ability to infect Quail neurons. We examined the infection routes of AAVs, leading us to discover that the putative receptor for AAV1 and 2 in

Quails is different than the mammalian receptor. Educated mutagenesis was introduced to the viral capsid with the hope to improve receptor binding and infection in Quails. We produced a small library of new serotypes; of which one particular variant, denoted AAV1*, showed strikingly high infection efficiency, *in vitro*. Unexpectedly, AAV1* shows exclusive tropism to glial cells. We have established a robust culturing protocol from Quail embryos and engineered a highly efficient AAV for introducing genetic material to glia cells.

Disclosures: S. Zoabi: None. **Y. Gutfreund:** None. **S. Berlin:** None.

Digital Abstract Session

P370. Gene, Protein, or Cell-Based Approaches

Program #/Poster #: P370.05

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: BBRF NARSAD 27737
NIH 5UG3MH120102-02

Title: A viral vector engineered for improved spatially-specific noninvasive gene delivery to the brain.

Authors: *J. O. SZABLOWSKI[1], H. LI[2], J. HEATH[2], M. SHAPIRO[3];
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Abstract: Gene delivery to the brain with adeno-associated virus (AAV) has been a mainstay of in vivo neuroscience research and has significant therapeutic potential. However, gene delivery to the brain is typically done invasively, through surgeries that damage the studied tissues and prevent targeting of large brain regions e.g. in large animal models. Focused ultrasound (FUS) can be used to open the blood-brain barrier (BBB) to enable the delivery of AAVs into the brain from the blood. Recent studies optimized the ultrasound parameters and choice of serotypes for more efficient delivery. However, the delivery of large molecules, e.g. AAVs, remains challenging. AAV delivery requires FUS pressures close to the safety margins of the FUS BBB opening (FUS-BBBO); intravenous injection of AAVs exposes peripheral organs to the virus, leading to off-target peripheral tissue transduction. Here, we engineer a new AAV with improved brain transduction efficiency and tissue specificity after FUS-BBBO.To obtain a new AAV strain optimized for FUS-BBBO we modified a viral vector engineering method called Cre-recombination-based AAV targeted evolution (CREATE). In CREATE, a library of AAVs with mutated capsid proteins is injected intravenously into mice. When a particular AAV clone transduces a cell expressing Cre, its viral genome is modified and becomes detectable by a Cre-dependent PCR. We modified CREATE to engineer a FUS-BBBO-optimized AAV, which we called AAV.FUS. We first generated a library of viral vectors with a capsid modified by insertion of a randomized 7 amino-acid sequence between residues 588 and 589 of the AAV9 capsid. We chose AAV9 due to our preliminary studies and published work showing its efficient

gene delivery in mice. We then used FUS-BBBO to deliver the AAV library to one hemisphere of hSyn1-Cre mice that express Cre in neurons and after selection identified 5 clones most enriched in the FUS-targeted. Histological analysis revealed higher numbers of transduced cells in the brain for all candidates (20-130% improvement over AAV9) at safe ultrasound pressures. At the same time, each serotype transduced the liver less effectively (range: 13 - 643% reduction compared to AAV9). The liver is strongly transduced by many AAVs, e.g. AAV9. The top AAV.FUS candidate (AAV.FUS.3) showed a 12.1-fold improvement in overall brain-tissue specificity after FUS-BBBO. When tested the cell-type specificity of AAV.FUS candidates they have shown improved neuronal tropism when compared to AAV9.Overall, we conclude that FUS-BBBO gene delivery can be improved by engineering new AAV viral vector strain - AAV.FUS. JOS and HL contributed to this study equally.

Disclosures: J.O. Szablowski: None. **H. Li:** None. **J. Heath:** None. **M. Shapiro:** None.

Digital Abstract Session

P370. Gene, Protein, or Cell-Based Approaches

Program #/Poster #: P370.06

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NINDS Division of Translational Research

Title: The NINDS Ultra-Rare GENe-based Therapies network: Responding to an URGenT need

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Abstract: Gene-based therapies have begun to change the options and outcomes for patients with rare and ultra-rare genetic disorders. Availability of these therapies is limited by cost, risk of failure, challenges in manufacturing, and novel regulatory requirements. Furthermore, institutions differ widely in the resources, expertise, and risk tolerance they can apply to providing patients with such individualized therapies. NINDS seeks to create a mechanism that enables wider development and deployment of gene-based therapies. In April 2019, a workshop entitled “Advancing Gene-Targeted Therapies for Central Nervous System Disorders” was held by the National Academy of Medicine. In September 2019, a workshop entitled “Next Generation Strategies for Gene-Targeted Therapies of Central Nervous System Disorders” was held by NINDS to convene thought leaders and experts in diverse aspects of gene therapy, including target gene regulation of expression, target distribution, development of preclinical assays and models, choice of viral vector or delivery system, manufacture and scale-up, clinical trial challenges, collaborative network models, and regulatory requirements and standards. Finally, in December 2019, a meeting entitled “Facilitating Access to Gene Therapy for Rare Diseases: Opportunities for Collaboration” was held by the Foundation for NIH (FNIH) to bring together experts from the government, academia, industry, and nonprofit advocacy sectors to

prioritize challenges, such as preclinical scientific, technical, regulatory, and quality of life, for study and solution. FNIH has since launched an effort to create an atlas of adeno-associated viral vector platforms; NCATS has also initiated platform strategies with which to begin performance of gene therapy trials for systemic and neuromuscular junction disorders. The culmination of our efforts results in the ongoing formation of the Ultra-Rare Gene-based Therapy (URGenT) network - an NINDS late-stage therapy development program that aims to speed the delivery of state-of-the-art gene-based therapies to patients with ultra-rare diseases of the nervous system, standardize and harmonize best practices, and encourage innovation in clinical trials. URGenT was approved by the NINDS Council in February 2020. The network will provide, on a competitive basis, both grant funding and access to in-kind resources for planning and execution of therapeutic agent optimization, scale up and manufacture, IND-enabling studies, regulatory affairs support including IND preparation and submission, and clinical trial performance. The first requests for applications are anticipated to be issued in 2021.

Disclosures: **N.F. Schor:** None. **A. Tamiz:** None. **A. Broome:** None. **J. Morris:** None. **C. Boshoff:** None. **M.H. Skiadopoulos:** None. **J.L. Bachman:** None. **G. Lind:** None. **W.J. Koroshetz:** None.

Digital Abstract Session

P370. Gene, Protein, or Cell-Based Approaches

Program #/Poster #: P370.07

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Support for this study was provided by the Neuroscience program, Biochemistry, Cell and Molecular Biology program, College of Medicine, Department of Chemistry and Biochemistry, and the John G. Kulhavi Professorship in Neuroscience at CMU, and the Fi

Title: Use of curcumin degradation products and curcumin encapsulated polyamidoamine dendrimers as a potential treatment in a mouse model of glioblastoma

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Abstract: Glioblastoma (GB) is a highly aggressive and malignant glioma of the central nervous system. Despite significant improvements in surgical techniques and advancements in chemotherapy over the last few decades, the prognosis of GB has remained virtually the same. New developments in nanomedicine may fill a large void present in cancer therapeutics. Poly-

amidoamine (PAMAM) dendrimers are nanomolecules whose properties and ionic charge can be adjusted for GB treatment. While traditional PAMAM dendrimers have a toxic amine surface, we have developed a de novo method to synthesize G4 PAMAM dendrimers with a modified surface (G4-90/10) consisting of 90% OH and 10% NH2. Their small size (4 nm) and ability to solubilize hydrophobic molecules, facilitating cargo delivery, make them ideal candidates for nanomedicine. One such cargo under exploration for the treatment of GB is the polyphenol curcumin. It has many reported anticancer effects but has been largely unsuccessful in clinical trials due to its poor bioavailability, solubility, rapid degradation, and systemic elimination. Curcumin degradation products, however, are highly bioavailable, and isolation of these compounds have shown promise as anticancer agents. By encapsulating curcumin in a surface modified G4 PAMAM dendrimer (D-Cys-Cur), its solubility and release kinetics are greatly improved. UV Spectrometry shows that when encapsulated in a PAMAM dendrimer, curcumin is steadily released at increasing amounts over a 24-hour period compared to curcumin in water. The goal of this study is to use curcumin degradation products or curcumin by-products (CBP), and (D-Cys-Cur) to find a dose dependent treatment in vitro and in vivo in a GL261 Red-FLuc mouse model of GB. An MTT assay was run to examine the toxicity profiles of CBP and D-Cys-Cur against Gl261 mouse GB cells, using mouse primary cortical cultures (PCCs) as a healthy control. Results show that D-Cys-Cur significantly kills GL261 cells at 0.1-, 0.2-, and 0.6-mg/mL, while showing no significant loss to PCCs at their respective doses. Additionally, an MTT assay of CBPs against GL261 mouse GB cells show significant cell death at 0.04-, 0.08-, and 0.12-mg/mL, while showing no significant loss to PCCs at their respective doses. Fifteen animals with tumors were left untreated to establish a baseline length of survivability. When CBPs are administered intratumorally in GB mice, the animals live significantly longer than the control group. Future directions will examine if this is due to a reduction in tumor size after treatment.

Disclosures: **J.A. Gallien:** None. **J.W.E. Smith:** None. **A. Poudel:** None. **B. Srinageshwar:** None. **B.H. Somerville:** None. **B. McDonald:** None. **S. Koneru:** None. **K. Gallo:** None. **G. Holtgrefe:** None. **A. Sharma:** None. **G. Dunbar:** None. **J. Rossignol:** None.

Digital Abstract Session

P370. Gene, Protein, or Cell-Based Approaches

Program #/Poster #: P370.08

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH R21 (1R21EY030012)
Neuroscience program
College of Medicine
Department of Chemistry and Biochemistry
John G. Kulhavi Professorship in Neuroscience at CMU
Field Neurosciences Institute
Central Michigan University Office of Research and Graduate Studies

Title: Plasmid Expression in Neurons following Transfection using Different Sized PAMAM Dendrimers

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Abstract: Dendrimers are highly branched nano molecules that can be used to deliver drugs and biomolecules to neurons *in vitro* and *in vivo*. The delivery of therapeutic genes to the brain using dendrimers has great potential in the treatment of neurodegenerative diseases. Dendrimers are defined by three main components: a central core, an interior dendritic structure (generations G), and an exterior surface with functional surface groups. The generation G indicates the size of the dendrimers such as G1 dendrimer is 1 nm in diameter and so on. The widely used G4 100% amine surface dendrimers are found to be highly toxic to cells, due to their highly positive surface charge density. Therefore, we developed dendrimers with different surface compositions and less surface charge density (% hydroxyl/%amines ratio), such as G4-90/10, and G4-70/30. These dendrimers were found to be less toxic, due to their modified surface with less positive charge. We used five different dendrimer types with a cystamine core (such as G0 cys amine, G1 cys amine, G2 cys amine, G4 90/10, and G4 70/30) which were complexed with a pcDNA plasmid expressing a fluorescent reporter under control of a ubiquitous promoter at N/P ratios of 100:1 and 10:1. These complexes were then transfected into primary cortical neurons i*n vitro*. The first transfection included all 5 dendrimer types and was used to determine the type that expressed the plasmid with the highest efficiency. The G4-70/30 dendrimer yielded the highest transfection of plasmid into neurons when added at different concentrations at 48h following transfection. This is distinct from transductions with the adeno-associated virus (AAV) which begin to express around two weeks after transduction. We are continuing to optimize efficacy of dendrimers varying in N/P ratios.

Disclosures: N. Singh: None. **B. Srinageshwar:** None. **M. Tree:** None. **K. Riselay:** None. **A. Sharma:** None. **D. Swanson:** None. **G.L. Dunbar:** None. **U. Hochgeschwender:** None. **J. Rossignol:** None.

Digital Abstract Session

P370. Gene, Protein, or Cell-Based Approaches

Program #/Poster #: P370.09

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Department of Neuroscience

Department of Biochemistry, Cell and Molecular Biology
College of Medicine
Department of Chemistry and Biochemistry
John G. Kulhavi Professorship in Neuroscience at CMU
Field of Neurosciences Institute

Title: Knockout of 14-3-3β and its role in proliferation, migration and invasion in glioblastoma using the PAMAM dendrimer as a delivery vehicle

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Abstract: Glioblastomas (GB) are the most common and malignant brain tumor in adults, with an incidence rate of 5.26 per 100,000 people each year. Challenges in treating GB include resistance to the commonly used chemotherapy drug temozolomide and incomplete removal of glioblastoma stem cells following tumor resection. In previous studies, it has been demonstrated that 14-3-3β is an important protein involved in tumorigenesis and is commonly found to be upregulated throughout the initiation and progression of GB. Aiming to look further at 14-3-3β and its effects on GB, we used 14-3-3β small interfering RNA to silence the expression of this gene in U-87 MG human GB cell lines in *vitro*. Following confirmation of the knockdown using RT-PCR, we saw a decrease in proliferation occurring in the human GB cells over the span of 3 and 5 days confirming that a decrease in 14-3-3β expression may lead to a decrease in the proliferation of U-87 MG cells. To further investigate this, we are using CRISPR/Cas9 to completely knock out the expression of this protein to study its effects on proliferation, migration and invasion in U-87 MG cells. The issue of an efficient, non-immunogenic delivery system that can carry large cargo continues to be a problem in the delivery of biomolecules. To circumvent this issue, the poly-amidoamine (PAMAM) dendrimer has become increasingly popular over the years due to its water solubility, biocompatibility, and its ability to carry large cargo. However, due to their large number of cationic amine groups increasing with generation size (and cargo carrying capacity), they have been shown to have toxic effects on the lipid membrane bilayer. Specifically, the generation 4 (G4) NH_2 surface dendrimers have 64 amines that are toxic in a dosage-dependent manner. Recent modifications to traditional PAMAM NH_2 surface dendrimers to contain primarily neutral hydroxyl groups (70% -OH, 30% NH_2) instead of primary amine groups have shown to decrease previous toxicities. Additionally, we have confirmed successful transfection of the G4 70/30 dendrimers complexed with the 14-3-3β into U-87 MG cells. Following the knockdown of 14-3-3β, we plan to create a xenograft mouse model to study the effects of the knockout on survivability and tumor mass by delivering it intracranially using the generation 4 NH_2 surface dendrimer as our vehicle.

Disclosures: **K. Gallo:** None. **B. Srinageshwar:** None. **J. Gallien:** None. **B. Swanson:** None. **A. Sharma:** None. **G. Dunbar:** None. **J. Rossignol:** None. **J. Bakke:** None.

Digital Abstract Session

P370. Gene, Protein, or Cell-Based Approaches

Program #/Poster #: P370.10

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH R21 (1R21EY030012)
Neuroscience program
College of Medicine
Department of Chemistry and Biochemistry
John G. Kulhavi Professorship in Neuroscience at CMU
Field Neurosciences Institute

Title: Delivery of PAMAM dendrimer nanomolecules across the natural barriers (blood brain barrier and placental barrier) and analysis of large plasmid delivery-clearance from rodent brain

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Abstract: Dendrimers are 3-dimensional nanomolecules that have branches having various chemical and biomedicalapplications. They have been shown to be effective biomolecule carriers for the CNS as they can cross theblood-brain-barrier (BBB) following systemic injections. Previous research indicates that the G4dendrimers having 100% amine surface (G4-NH2) are highly toxic to cells *in vitro* and *in vivo*, due to theirpositively charged amine groups on the surface. Therefore, to reduce the toxicity of these dendrimers, wehave modified them to have more neutral functional groups, containing only 10% of the surface coveredwith NH2 and remaining 90% of the surface covered with hydroxyl groups (-OH; G4-90/10). Our workindicates that these surface-modified dendrimers are taken up by various cultured neurons, glia, and stemcells,) and by brain cells *in vivo*. The toxicity assay shows that these modified dendrimers are less toxiccompared to the pure 100% amine surface dendrimers. Moreover, the G4-90/10 dendrimers are capableof forming complex with plasmid DNA of various sizes (6kb and 10kb) and can deliver them to differentstem cells *in vitro* and to the glia cells *in vivo*. Continuing this line of research of using PAMAM dendrimers,we also focused on delivering them to the rodent fetal brain following i.p injections to analyze their abilityto cross the BBB and the placental barrier in pregnant mice. This can be applied for future purposes bydelivering therapeutic biomolecules using PAMAM dendrimers to treat fetal diseases affecting thenervous system such as infections of Zika virus, rubella virus, cytomegalovirus, and herpes frequentlyresult in numerous neurological deficits and malformations. Finally, we also injected

the PAMAMdendrimers and their complexes with large plasmids into the rodent brain and tracked the dendrimersand the complexes using in vivo imaging system (IVIS) to analyze the longevity of the dendrimers and thecomplex into the rodent brain. Our results show that the PAMAM dendrimers can successfully formcomplex with large plasmids and were able to deliver the plasmid into the cells *in vitro* and *in vivo*.Moreover, the PAMAM dendrimers were able to cross the maternal BBB and reach the brain, however,very negligible amounts of dendrimers were found in the fetal tissue, and majority of the dendrimers werefound in the placental tissue. In conclusion, we were able to track the PAMAM dendrimers and thecomplexes in the rodent brain using IVIS showing steady clearance of these nanomolecules over time.

Disclosures: B. Srinageshwar: None. **E.R. Kuhn:** None. **M. Florendo:** None. **S. Peruzzaro:** None. **M.M. Andrews:** None. **D. Swanson:** None. **A. Sharma:** None. **G.L. Dunbar:** None. **J. Rossignol:** None.

Digital Abstract Session

P371. Genomics, Proteomics, and Systems Biology

Program #/Poster #: P371.01

Topic: I.02. Systems Biology and Bioinformatics

Support: Department of Translational Neuroscience, College of Human Medicine internal fund, Michigan State University

Title: EFhd2 brain interactome reveals its association with different cellular and molecular processes

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Abstract: EFhd2 is a calcium-binding protein that is highly conserved from human to nematodes. EFhd2 has a very dynamic structure that leads to self-oligomerization and aggregation mediated by its coiled-coil domain. We showed that EFhd2 is widely expressed in the body with predominant levels in the central nervous system. In addition, we demonstrated that EFhd2 is associated with tauopathies (e.g. Alzheimer's disease). In particular, EFhd2 co-purified with insoluble tau in a mouse model of tauopathy and postmortem brains. Our findings instigated more research to decipher EFhd2 function in the brain. Despite the strides we and colleagues have made to unveil EFhd2 function in the brain, many questions about the molecular mechanisms thereof remain unanswered. Herein, we used a proteomics approach to investigate the physiological role of EFhd2 in the brain. EFhd2 was immunoprecipitated from cortical and subcortical brain regions of $EFhd2^{+/+}$ mice, and $EFhd2^{-/-}$ mice were used as control. EFhd2-associated proteins were identified by tandem mass spectrometry. EFhd2 interactome was generated and the associated proteins were categorized into their known biological functions.

The analysis showed that EFhd2 markedly associates with proteins regulating cytoskeleton, vesicle trafficking, mitochondrial metabolism, and stress response. These findings were corroborated by examining human EFhd2 interactome from postmortem temporal cortex and cerebellum of normal-aging individuals. In fact, the human and mouse EFhd2 interactome are comparable. Interestingly, fourteen proteins that copurified with EFhd2 in both temporal cortex and cerebellum are also linked to several neurological disorders like Alzheimer's disease, Parkinson's disease, and epilepsy. Among these proteins, we found MAPT (tau), which is the main pathological culprit in tauopathies and GLUL (glutamate synthase) whose function is dysregulated in several neurological diseases. Moreover, we employed label-free quantification (LFQ) to determine proteome changes induced by EFHD2 gene deletion ($EFhd2^{-/-}$). Gene ontology analysis revealed that the differentially abundant proteins in $EFhd2^{-/-}$ mice are associated with metabolism, stress response, transport, and protein regulation compared to $EFhd2^{+/+}$ in both cortical and subcortical brain regions. Our results open a gateway to understanding the impact of EFhd2 gain- and loss-of-function on different physiological and pathological pathways.

Disclosures: **A.S. Soliman:** None. **A. Umstead:** None. **A. Lee:** A. Employment/Salary (full or part-time):; NeuroInitiatives. **J. Ryan:** A. Employment/Salary (full or part-time):; NeuroInitiatives. **J. Lamp:** None. **I.E. Vega:** None.

Digital Abstract Session

P371. Genomics, Proteomics, and Systems Biology

Program #/Poster #: P371.02

Topic: I.02. Systems Biology and Bioinformatics

Support: NORTE-01-0145-FEDER-028623
SFRH/BD/136760/2018

Title: A microfluidic platform for tension driven axon elongation

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Abstract: During development, axonal elongation is mediated by growth cone progression. Once a synapse is established, body growth results in an increased distance between the neuronal somata and their targets. This distinct biophysical process is known as axon stretch growth (ASG). Players underlying ASG remain mostly unknown. Thus, to uncover the molecular mechanisms governing ASG, we designed a microfluidic platform (Fig.1A and B) to promote axon stretch in dorsal root ganglia (DRG) neuron explants from C57BL/6 animals grown in compartmentalized microchannels. Our microfluidic platform promotes a uniformly distributed

stretch, which is computer controlled by specifically developed firmware and software (Fig. 1C). All structural elements were 3D-printed in polylactic acid (PLA), a biocompatible polymer/plastic, whereas Arduino and C++/Qt were used for, respectively, the firmware and software controlling the stretcher stepper motor. DRG explant cultures in microfluidics have been optimized in a stretchable substrate - polydimethylsiloxane (PDMS). With this strategy we are able to mimic physiological conditions: with separated soma and neurites, which form synapses in co-culture with fibroblasts (Fig. 1D). Currently, our system is being optimized for live-cell imaging of cytoskeleton dynamics and transport, alongside with other techniques. Exploring molecular changes in response to physiological axon stretch, may provide insight into molecules that may find application in conditions where axon regeneration is needed.

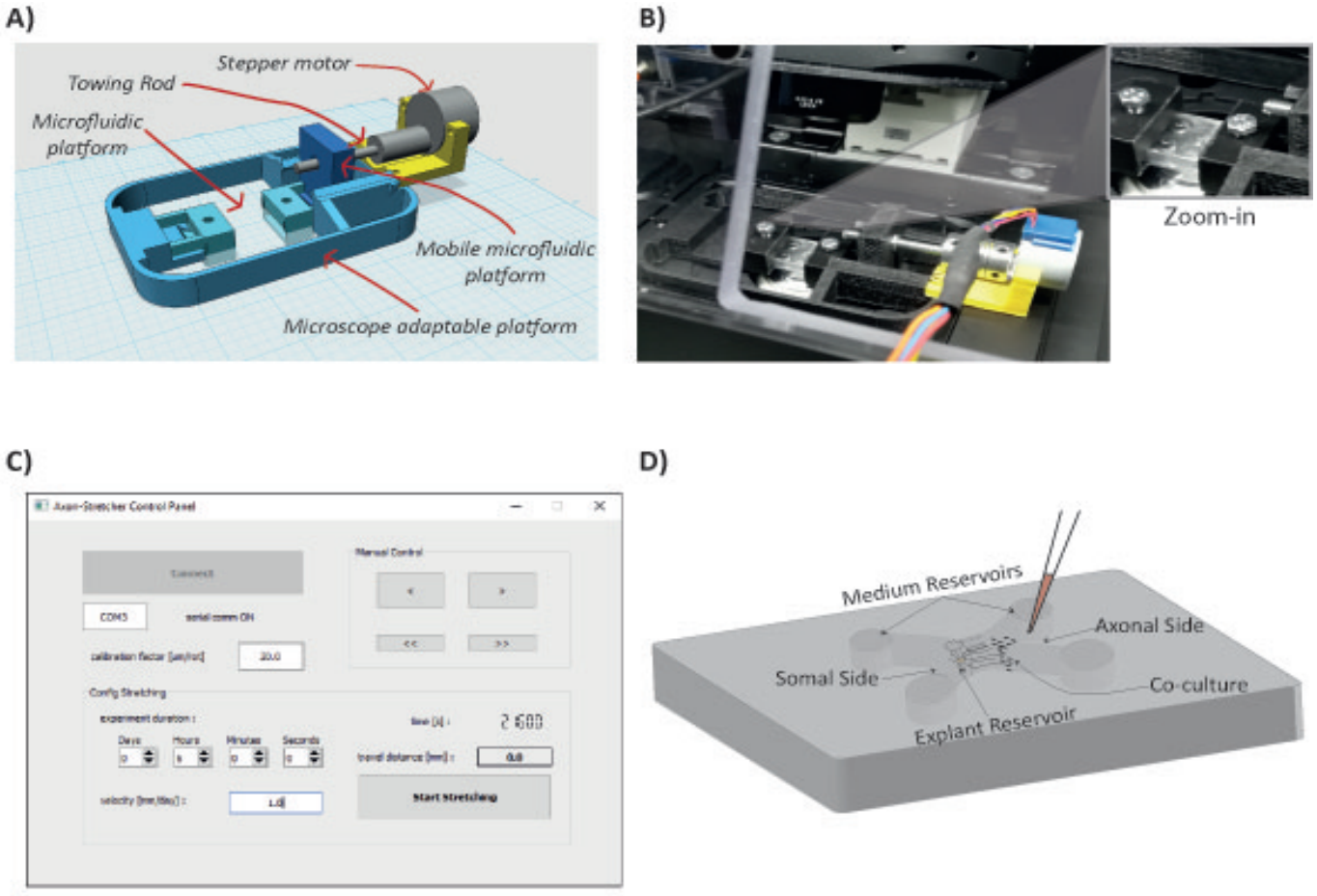

Figure 1 – Prototype of the microfluidic platform. A) 3D- schematic of microfluidic platform. B) Prototype of the microfluidic platform on the Leica SP8 microscope stand. The stretchable microfluidic platform is zoomed-in on the right. C) Control panel of the software currently being developed to control stretch rates. D) Schematic representation of the microfluidic co-culture system between DRG neuron explants and mouse embryonic fibroblasts. DRG explants will be plated in the explant reservoir (somal side) and fibroblasts plated in the axonal side to allow for a co-culture with the formation of synapses.

Disclosures: **S.C. Sousa:** None. **M. Aroso:** None. **T. Silva:** None. **P. Brites:** None. **C. Lopes:** None. **P. Aguiar:** None. **M. Sousa:** None.

Digital Abstract Session

P371. Genomics, Proteomics, and Systems Biology

Program #/Poster #: P371.03

Topic: I.08. Methods to Modulate Neural Activity

Title: Exploring laminar connectivity in the macaque brain

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Abstract: Over the past two decades, substantial progress has been made in neuroimaging of both global white matter connectivity (Sporns et al. 2005, Leemans et al. 2009, Setsompop et al. 2013), as well as cortical grey matter structure (Barazany and Assaf 2012, Lifshits et al. 2018, Shamir et al. 2019). Nevertheless, current techniques for exploring the brain's wiring diagram remain biased by the definition of the cortex as a single homogenous unit. To overcome this bias, we must expand the basic unit used in connectomics to a more descriptive representation of the heterogeneous laminar substructure of the cortex. In this study, we model and explore the macaque laminar connectome by integrating multi-modal ex-vivo magnetic resonance imaging via a simple model of laminar connectivity. The MRI acquisition includes a T1w sequence, an IR sequence performed using 3D FLASH and a DWI sequence. The model used for integration is based on a meta-analysis of histological findings concerning the interconnectivity of layers (Shamir and Assaf 2020). Our sample includes 7 macaque brains, all obtained from the Mammalian MRI (MaMI) database (Assaf et al. 2020). In order to validate our results, we revisit Felleman and Van Essen's seminal work regarding hierarchical processing in the primate cerebral cortex (Felleman and Van Essen 1991) and attempt to recreate it on the excised macaque brains. The resulting network of cortical laminar connectivity is compared in the visual cortex to Felleman and Van Essen's findings, resulting in a an accuracy level of 83%. By using an unbiased definition of the cortex that takes into account its laminar composition, we are able to use MRI neuroimaging to explore a new and more detailed laminar-level connectome.

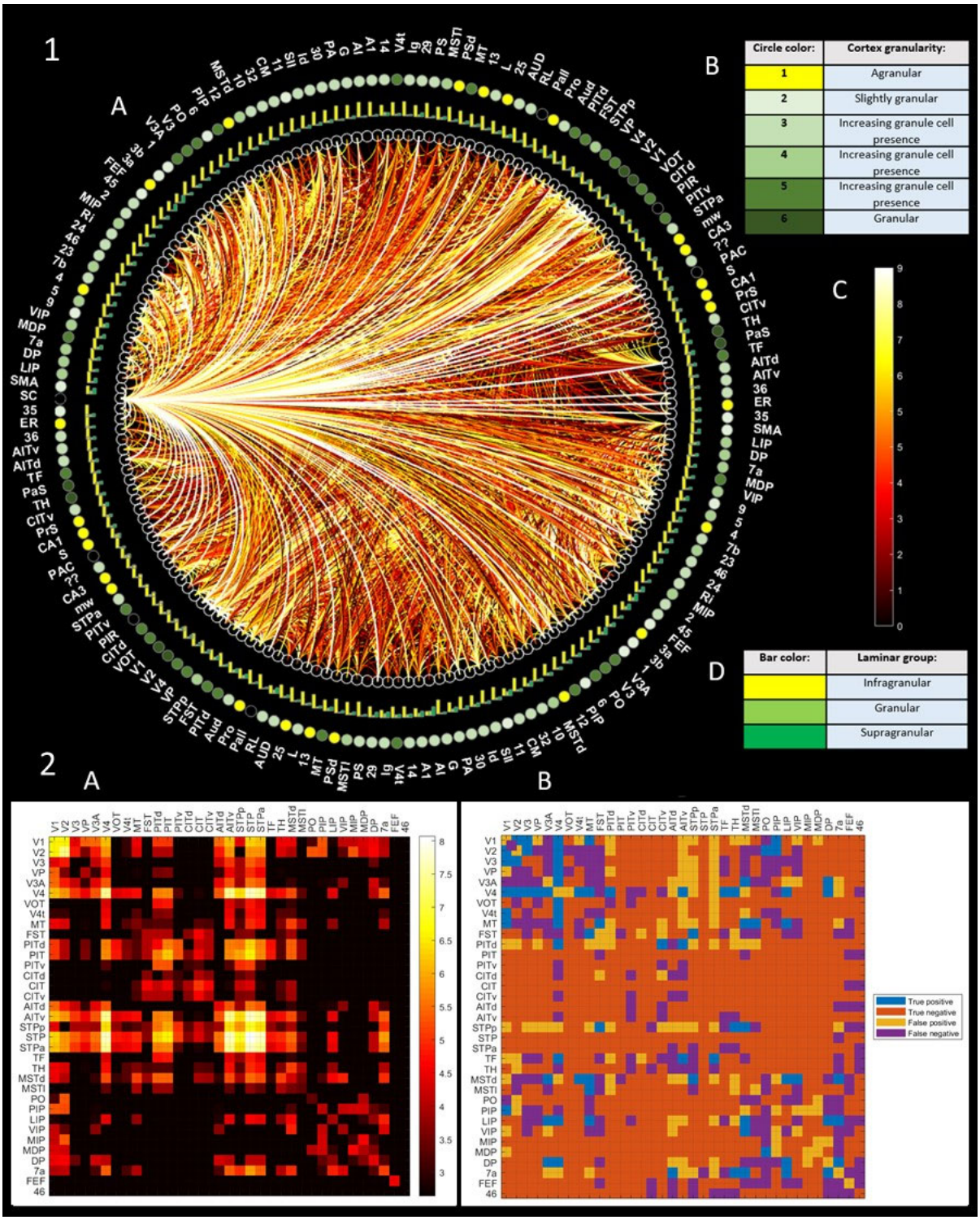

Fig. 1 Macaque cortical connectivity: **Part 1:** A circular network graph representing all model input datasets for the macaque brain; **Part 2:** Macaque visual cortex connectivity: A- Tractography-based connectivity matrix: log(number of tracts); B- Comparison of connectivity patterns: predicted values (tractography-based) to actual results (study findings)

Disclosures: **I. Shamir:** None. **Y. Assaf:** None.

Digital Abstract Session

P372. Bioinformatics

Program #/Poster #: P372.01

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH NIA Grant AG037868
NIH NIA Grant AG057461
NIH UL1 Grant TR001998

Title: Reduced RNA integrity indicates pathway-selective mRNA degradation in post-mortem human brain tissue

Authors: ***E. S. JOHNSON**, K. E. STENZEL, S. LEE, E. M. BLALOCK;
Dept. of Pharmacol. and Nutritional Sci., Univ. of Kentucky, Lexington, KY

Abstract: Objectives. RNA degradation can be influenced by many factors (e.g., post-mortem interval, sample preparation, storage duration), and the degree to which RNA is degraded prior to quantification can affect downstream measurements (e.g., situ hybridization, RT-PCR, transcriptional profiling). Agilent Technologies introduced the RNA integrity number (RIN; 1 being the worst to 10 being the best) to help quantify and standardize degradation across samples and labs. Recent studies have shown that RIN levels are associated with mRNA quantification, though relatively little work has been done to determine whether RNA is degraded across the genome or instead is focused in certain biological pathways. **Methods/ Results.** Using raw transcriptional data (accessed through the Gene Expression Omnibus) for multiple transcriptional profiling studies of post-mortem brain tissue from different individuals, we identified a robust and selective expression profile of degradation vulnerable mRNAs. Importantly, we report that many of the pathways supported by these degradation-sensitive genes are neuronal, particularly: vesicles, mRNA transport, synapses, and mitochondria. This suggests that neuronal synaptic mRNA is particularly vulnerable to degradation. Using a mean subtraction method, we determined the most common templates a gene's RNA degradation followed and plotted the gene expression values with the template. **Conclusion.** Our data found RNA degradation causes major drops in the signal of selective mRNA species around a RIN of 6.7-7 and that there is no appreciable correlation between gene expression and RIN if RIN is $\geq$ 8.3. Second, our data suggests this effect is pathway selective, possibly having important consequences for current bioinformatic RIN correcting procedures. Finally, this may have implications for neurodegeneration research like Alzheimer's disease, where the disease itself, rather than technical issues associated with harvesting and storage, may cause pathway selective mRNA degradation.

Disclosures: **E.S. Johnson:** None. **K.E. Stenzel:** None. **S. Lee:** None. **E.M. Blalock:** None.

Digital Abstract Session

P373. Single-Cell Genomic and Transcriptomic Techniques

Program #/Poster #: P373.01

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIMH U19MH114831

Title: Epigenomic Diversity of Cortical Projection Neurons in the Mouse Brain

Authors: *Z. ZHANG[1], J. ZHOU[1,8], P. TAN[1,11], Y. PANG[2], A. RIVKIN[1], M. A. KIRCHGESSNER[2], E. WILLIAMS[3], C.-T. LEE[4], H. LIU[1,9], A. D. FRANKLIN[2], P. A. MIYAZAKI[2], A. BARTLETT[1], A. ALDRIDGE[1], C. FITZPATRICK[5], J. R. NERY[1], R. CASTANON[1], L. BOGGEMAN[5], C. O'CONNOR[5], K.-F. LEE[4], X. JIN[3], E. A. MUKAMEL[10], M. BEHRENS[6], J. R. ECKER[1,7], E. M. CALLAWAY[2];
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Abstract: Neuronal cell types are classically defined by their molecular properties, anatomy, and functions. While recent advances in single-cell genomics have led to high-resolution molecular characterization of cell type diversity in the brain, neuronal cell types are often studied out of the context of their anatomical properties. To better understand the relationship between molecular and anatomical features defining cortical neurons, we combined retrograde labeling with single-nucleus DNA methylation sequencing to link neural epigenomic properties to projections. We examined 11,827 single neocortical neurons from 63 cortico-cortical (CC) and cortico-subcortical long-distance projections. Our results revealed unique epigenetic signatures of projection neurons that correspond to their laminar and regional location and projection patterns. Based on their epigenomes, intra-telencephalic (IT) cells projecting to different cortical targets could be further distinguished, and some layer 5 neurons projecting to extra-telencephalic targets (L5 ET) formed separate clusters that aligned with their axonal projections. Such separation varied between cortical areas, suggesting area-specific differences in L5 ET subtypes, which were further validated by anatomical studies. Interestingly, a population of CC projection neurons clustered with L5 ET rather than IT neurons, suggesting a population of L5 ET cortical neurons projecting to both targets (L5 ET+CC). We verified the existence of these neurons by dual retrograde labeling and by anterograde tracing of CC projection neurons, which revealed axon terminals in ET targets including thalamus, superior colliculus, and pons. These findings highlight the power of single-cell epigenomic approaches to connect the molecular properties of neurons with their anatomical and projection properties.

Disclosures: Z. Zhang: None. **J. Zhou:** None. **P. Tan:** None. **Y. Pang:** None. **A. Rivkin:** None. **M.A. Kirchgessner:** None. **E. Williams:** None. **C. Lee:** None. **H. Liu:** None. **A.D.**

Franklin: None. **P.A. Miyazaki:** None. **A. Bartlett:** None. **A. Aldridge:** None. **C. Fitzpatrick:** None. **J.R. Nery:** None. **R. Castanon:** None. **L. Boggeman:** None. **C. O'Connor:** None. **K. Lee:** None. **X. Jin:** None. **E.A. Mukamel:** None. **M. Behrens:** None. **J.R. Ecker:** None. **E.M. Callaway:** None.

Digital Abstract Session

P373. Single-Cell Genomic and Transcriptomic Techniques

Program #/Poster #: P373.02

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Rnascope™ Hiplex assay and hiplexup reagents in situ hybridization assay enables high throughput multiplexed spatial mapping of diverse gene signatures and cell type specific markers across mouse brain

Authors: J. SHELDON, **M. YU**, H. LU, H. ZONG, B. ZHANG, *J. V. PULLIAM, X.-J. MA; BioTechne, Newark, CA

Abstract: Transcriptomic research has ushered into an era of single cell technologies that are crucial for both classifying and characterizing known and novel cell populations of complex heterogenous tissues. However, such techniques are limited by the use of dissociated cells that result in the loss of spatial organization of these cell populations, thus requiring a highly multiplexed approach that can interrogate gene expression at a single cell resolution while retaining the morphological context. We sought to utilize the RNAscope HiPlex^{TM} assay with HiPlexUp reagents to resolve and spatially map diverse gene signatures identified by single cell RNA sequencing (scRNAseq) and known neuronal cell-type specific markers. With the previous HiPlex-12 reagent workflow, we spatially mapped the novel medium spiny neuronal (MSN) D1 and D2 subtypes identified by scRNAseq (Gokce *et al*, *Cell Rep*, 16(4):1126-1137, 2016). Our new HiPlexUp reagent workflow enables extension of the previous workflow for simultaneous detection of up to 48 targets on a single tissue section. This iterative target detection process allows for a highly sensitive and specific mRNA visualization without compromising the structural integrity of the tissue morphology. In addition to visualizing the previously confirmed major and minor D1 and D2 MSN subtypes (*Drd1, Htr7, Pcdh8, Th, Synpr, Crym, Wfs1, Calb1, Drd1, Cnr1*, and *Foxp1*) in the mouse striatum, we also visualized neuronal markers (*Fam84b, Lhx6, Crh, Vip, Tac1, Moxd1, Slc6a1, Sst, Chrna2, Gad2, Slc32a1, Gria1, Grin1, Cx3cr1, Chrm1, Chrm3, Oprd1, Chrnb2, Gabr2, Vglut1, Vglut2, Gad2, Calb2*, and *Pvalb*) and ubiquitously expressed genes that served as positive control (*Polr2a, Ppib, Ubc, Hprt, ActB, Tubb3, Bin1, Ldha, Gapdh, Pgk1, Bhlhe22*, and *Cplx2*) of the mouse brain. The markers were expressed across various region of interests such as the olfactory bulb, caudate putamen, hypothalamus, and cerebral cortex. These diverse expression patterns serve as an invaluable tool is understanding the region-specific functional significance of these diverse neuronal genes. Lastly, we demonstrated the utility of our HiPlex image registration software resolving this 48-plex data by zoning into our targets of interest. In conclusion, single-cell transcriptomics combined with spatial mapping by the RNAscope technology is well suited for resolving

heterogeneous tissues at cellular resolution and providing insights into cellular organization and function of diverse cell types in both healthy and disease states

Disclosures: **J. Sheldon:** None. **M. Yu:** None. **H. Lu:** None. **H. Zong:** None. **B. Zhang:** None. **J.V. Pulliam:** None. **X. Ma:** None.

Digital Abstract Session

P373. Single-Cell Genomic and Transcriptomic Techniques

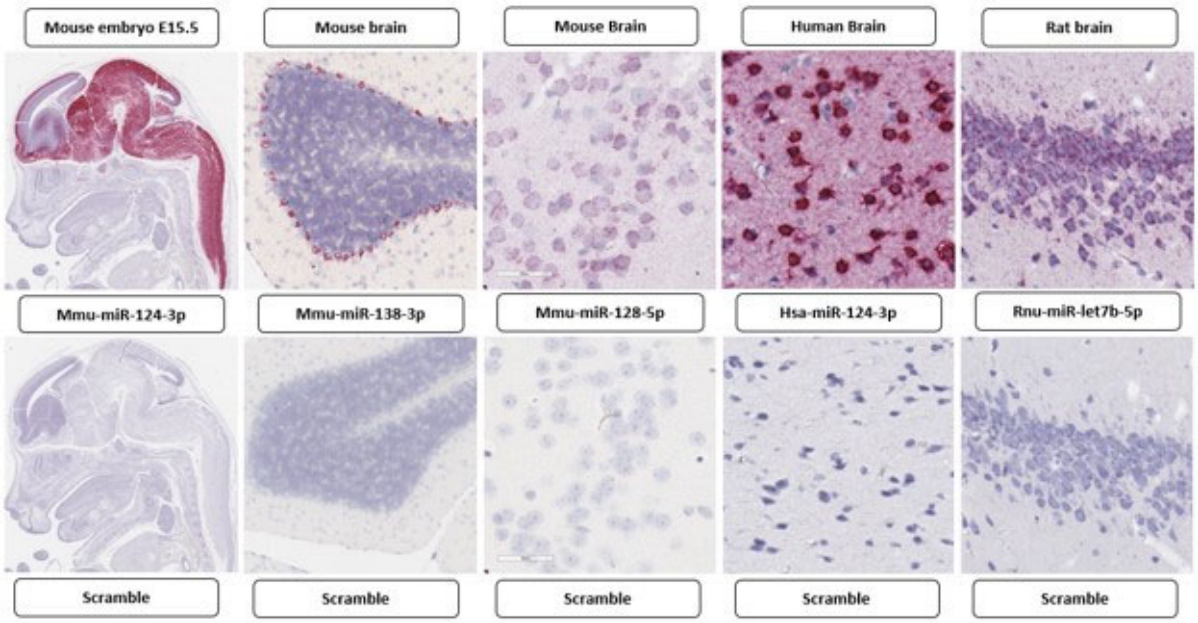
Program #/Poster #: P373.03

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Mirnascope a novel in situ hybridization technology for the detection of small regulatory rnas with spatial and morphological context

Authors: M. MATA[1], **A. SAHAJAN**[2], *L. WANG[2], B. ZHANG[1], X.-J. MA[1];
[1]Advanced Cell diagnostics, Newark, CA; [2]ACD a bio-techne brand, Newark, CA

Abstract: microRNAs (miRNAs) are 18 to 22 nucleotide small regulatory RNA molecules that are expressed in a tissue, cell-type, and cell-state specific manner. miRNAs regulate gene expression in developing neuronal cells and are implicated in pathogenesis of many neurological disorders, neuroinflammation and immune regulation. Attempts at studying miRNAs have relied on small RNA sequencing approaches, microarray, and qPCR. While these methods deliver bulk expression levels, they do not provide detailed spatial information for miRNA expression. Additionally, existing small RNA ISH technologies suffer from poor reproducibility and sensitivity, extensive optimization at the level of sample pretreatment, target probe hybridization, and post-hybridization. This emphasizes the need for a technology that can reliably detect cell-specific small RNA species in the brain and support biomarker discovery and small RNA therapeutics. To address this need ACD developed an RNA *in situ* hybridization (ISH) method known as miRNAscope™ for detection of miRNAs and small RNAs. It is a "Ready-to-Use" small RNA detection ISH assay supplied with universal tissue pretreatment reagents and needs zero target probe optimization. As proof of concept, miRNAscope probes specific for human Hsa-miR-124-3p, mouse Mmu-miR-124-3p-S1, Mmu-miR-138-3p, Mmu-miR-128-5p, rat Rnu-miR-let7b-5p were designed and its expression was evaluated in brain. A control scramble probe was used to determine background signal. The miRNAscope assay demonstrated high sensitivity and specificity for the cell-specific miRNA detection for the targets highlighted above, demonstrating the functionality of this assay. In summary, miRNAscope is an RNA ISH assay for the detection of small RNA species with unparalleled detection sensitivity and specificity, enabling morphological information with single cell resolution. The assay will improve our understanding of small RNA species in their native context and will elucidate their associated gene regulatory networks involved in health and disease.



Disclosures: **M. Mata:** None. **A. Sahajan:** None. **L. Wang:** None. **B. Zhang:** None. **X. Ma:** None.

Digital Abstract Session

P373. Single-Cell Genomic and Transcriptomic Techniques

Program #/Poster #: P373.04

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Dissecting neurodegeneration mouse models with an automated high-plex spatial platform

Authors: B. COOK, I. OH, R. YIN, **M. OTERO**;
Rebus Biosystems, Santa Clara, CA

Abstract: Single-cell RNA sequencing (scRNA-seq) has fundamentally expanded our understanding of tissue composition and heterogeneity. While this methodology enables unbiased and comprehensive identification of cell types based on their transcriptomic profiles, it does not provide positional information in the tissue architecture context. Rebus Biosystems has developed a highly automated platform that combines Synthetic Aperture Optics (SAO), fluidics engineering and data processing to enable multiplex analysis of gene targets across large tissue sections with minimal user intervention. Here, we used the Rebus Biosystems platform to quantify expression levels of 31 target genes across three experimental conditions. First, we obtained fresh frozen mouse brain sections from two transgenic lines modeling a neurodegenerative disease and a third wild-type line serving as a control. We mounted one section from each of the three conditions (genotypes) on the same glass coverslip. Sections mounted together corresponded to the same antero-posterior levels of the hippocampus and adjacent somatosensory cortex. We ran nine of these three-section arrays to acquire single

mRNA molecule quantitative data for 31 genes using on-system cyclic single-molecule RNA fluorescence in situ hybridization (smFISH) chemistry. Detected mRNA spots were automatically assigned to individual DAPI-stained nuclei to render a single-cell gene-expression matrix. Like with scRNA-seq approaches, cell-type associated genes were used for unbiased classification of single cells into several distinct neuronal and glial cell types. Then, the spatial information was used to further dissect each cell type into distinct anatomical compartments and explore spatial relationships between cells. The expression levels of disease-associated genes were compared across experimental conditions, for each cell type and anatomical structure individually. The combination of single cell gene expression and spatial data allows for fine dissection of tissue heterogenicity, while the balanced tissue-array design minimizes technical variability and enables powerful statistical comparisons.

Disclosures: **B. Cook:** A. Employment/Salary (full or part-time):; Rebus Biosystems. **I. Oh:** A. Employment/Salary (full or part-time):; Rebus Biosystems. **R. Yin:** A. Employment/Salary (full or part-time):; Rebus Biosystems. **M. Otero:** A. Employment/Salary (full or part-time):; Rebus Biosystems.

Digital Abstract Session

P374. Software Tools

Program #/Poster #: P374.02

Topic: I.07. Data Analysis and Statistics

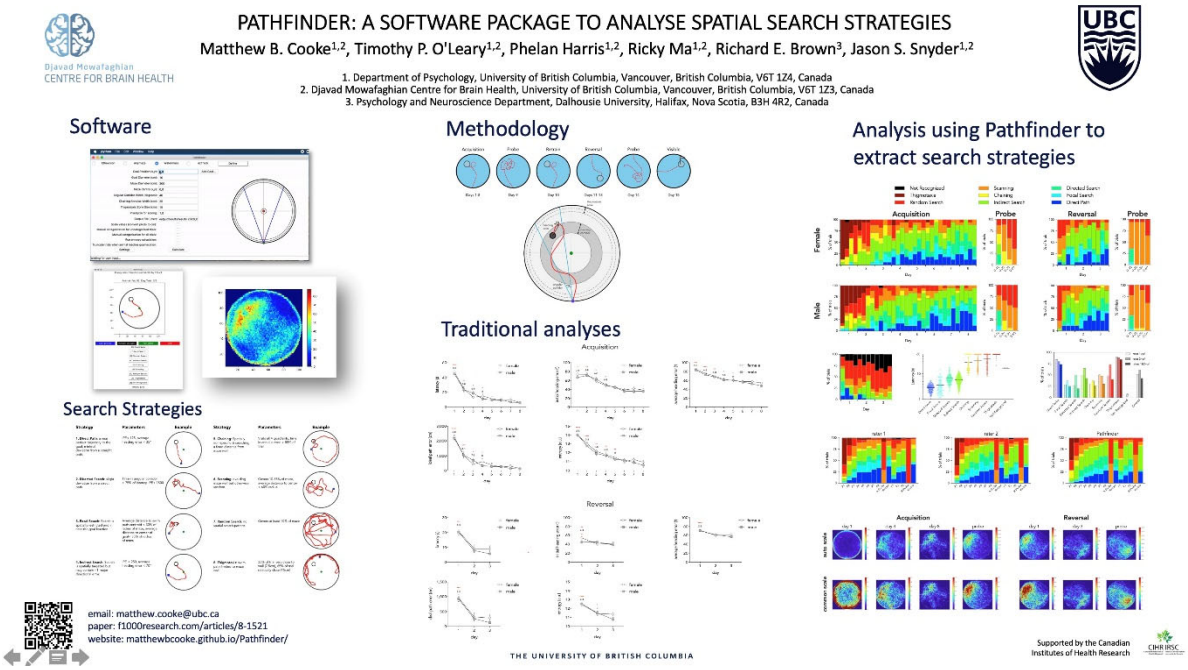
Support: CIHR Grant

Title: Pathfinder, open source software for analyzing spatial navigation search strategies

Authors: ***M. B. COOKE**[1], T. O'LEARY[2], P. HARRIS[2], R. MA[2], R. E. BROWN[4], J. S. SNYDER[3];
[1]Dept. of Neurosci., [3]Dept. of Psychology, [2]Univ. of British Columbia, Vancouver, BC, Canada; [4]Psychology & Neurosci., Dept. of Psychology and Neurosci., Halifax, NS, Canada

Abstract: Spatial navigation is a universal behavior that varies depending on goals, experience and available sensory stimuli. Spatial navigational tasks are routinely used to study learning, memory and goal-directed behavior, in both animals and humans. One popular paradigm for testing spatial memory is the Morris water maze. Researchers typically express learning as a function of the latency to escape, though this reveals little about the underlying navigational strategies. Recently, a number of studies have begun to classify water maze search strategies in order to clarify the precise spatial and mnemonic functions of different brain regions. However, despite their usefulness, strategy analyses have not been widely adopted due to the lack of software to automate analyses. We developed Pathfinder, an open source application for analyzing spatial navigation behaviour. Here we show Pathfinder's performance on a sample dataset, where it effectively characterized highly-specific spatial search strategies used during the trials. Our software provides support for inputs from commonly-used, commercially-available

and open-source software packages, is optimized for classifying search strategies, and can also be expanded easily to work with other species and spatial navigation tasks. Pathfinder has the ability to automatically determine the platform location as well as the size of the pool and related pool parameters. It can generate heatmaps of trials, analyze navigation with respect to multiple goal locations, and can be easily updated to accommodate future developments in spatial navigation behaviours.



Disclosures: **M.B. Cooke:** None. **T. O'Leary:** None. **P. Harris:** None. **R. Ma:** None. **J.S. Snyder:** None. **R.E. Brown:** None.

Digital Abstract Session

P374. Software Tools

Program #/Poster #: P374.03

Topic: I.07. Data Analysis and Statistics

Support: OT2OD030541

Title: Apinatomy infrastructure for multiscale connectivity

Authors: ***T. H. GILLESPIE**[1], N. KOKASH[3], M. C. SURLES-ZEIGLER[1], M. E. MARTONE[2], B. DE BONO[4];
[2]Neurosci., [1]UCSD, La Jolla, CA; [3]RUDN Univ., Moscow, Russian Federation; [4]Auckland Bioengineering Inst., Univerity of Auckland, Auckland, New Zealand

Abstract: A key challenge facing many large neuroscience projects is how to integrate data across a wide diversity of species and spatial and temporal scales. We face this in the NIH

SPARC project as we build systems for researchers to discover data about the peripheral nervous system and other organ systems on the basis of its potential physiological relevance to the organ systems they research. A central element of our approach is the use of the ApiNATOMY toolkit that supports data integration over a multiscale connectivity model of cells, tissues, and organs. ApiNATOMY consists of a knowledge representation and knowledge management tools that enable topological and semantic modeling of process routes and associated anatomical compartments in multiscale physiology. Domain experts provide model specifications in a semi-structured format based on templates for common topological structures (such as neural arborizations, etc.). ApiNATOMY tools then expand the specifications into a graph that can be visually manipulated, serialized, and integrated with other knowledge bases such as the NIF-Ontology and the SPARC Knowledge Graph.
ApiNATOMY models are annotated using the same vocabularies as datasets, allowing the connectivity knowledge to be leveraged to enhance navigation, classification, and search for data. For instance, if a researcher is designing a procedure on the Middle Cervical Ganglion (MCG), ApiNATOMY connectivity provides the means to discover existing data along the route of neurons passing through the MCG that could help plan the experiment.
Here we present our work on infrastructure for ApiNATOMY to make its connectivity models more FAIR (Findable, Accessible, Interoperable, and Reusable). This includes an interactive graphical web application, services to support identifier resolution, and tools to query linked metadata, discover datasets, track provenance, etc. We use a generalizable approach to make application specific JSON data structures FAIR by using JSON-LD to convert them to RDF. This approach has greatly reduced the complexity of the system and has made it easier to maintain.

Disclosures: **T.H. Gillespie:** None. **N. Kokash:** None. **M.C. Surles-Zeigler:** None. **M.E. Martone:** None. **B. de Bono:** None.

Digital Abstract Session

P374. Software Tools

Program #/Poster #: P374.04

Topic: I.07. Data Analysis and Statistics

Title: Analysim - a web platform for collaborative data sharing and analysis

Authors: H. DINH, D. THOMAS, A. DOLOC-MIHU, ***C. GUNAY**;
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Abstract: AnalySim is a website developed from scratch to help people create and share new projects that can include different types of datasets and analysis methods already implemented in the system. AnalySim aims to help with data sharing, data hosting for publications, interactive visualization, collaborative research, and crowdsourced analysis. It is especially designed for specialized for datasets with many changing parameters and recorded measurements, such those in computational neuroscience. Analysim provides tools that are specific for parameter search,

but at the same time allows to run custom code. Offering these features on an interactive web platform improves visibility of one's research and helps the reviewing process by allowing to reproduce others' analyses. In addition, it fosters collaborative research by providing access to others' public datasets and analysis, creating opportunities to ask novel questions, guide one's research, and start new collaborations or join existing teams. Analysim can be said to provide a "social scientific environment", which include features such as forking or cloning existing projects to customize them and tagging or following researchers and projects. In addition, one can filter datasets, duplicate analyses and improve them, and publish findings via interactive visualizations. In summary, Analysim is a Github-like tool specialized for scientific problems - especially when they are large and complex as in parameter search.

Disclosures: **C. Gunay:** None. **A. Doloc-Mihu:** None. **D. Thomas:** None. **H. Dinh:** None.

Digital Abstract Session

P374. Software Tools

Program #/Poster #: P374.05

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant U19MH114830
NIH Grant U01MH114812

Title: Common cell type nomenclature for the mammalian brain: a systematic, extensible convention

Authors: ***J. A. MILLER**, N. W. GOUWENS, B. TASIC, F. COLLMAN, C. T. J. VAN VELTHOVEN, T. E. BAKKEN, M. J. HAWRYLYCZ, H. ZENG, E. S. LEIN, A. BERNARD; Allen Inst. for Brain Sci., Seattle, WA

Abstract: The advancement of single cell RNA-sequencing technologies has led to an explosion of cell type definitions across multiple organ systems. Consortia like the BRAIN Initiative Cell Census Network (BICCN) and the Human Cell Atlas (HCA) have begun to standardize and centralize the intake of data and associated metadata from these projects; however, organization of cell types has largely been left to individual investigators, resulting in widely varying nomenclature and limited alignment between taxonomies. To facilitate cross-dataset comparison, the Allen Institute created the Common Cell type Nomenclature (CCN) for matching and tracking cell types across studies, allowing comparison of matched cell types between any two taxonomies, including a reference taxonomy. This concept is qualitatively similar to gene transcript management across different genome builds. The CCN can be readily applied to new or established taxonomies without changing cell type names previously assigned in an analysis or publication. Finally, the CCN proposes conventions for assigning accurate yet flexible cell types names in the mammalian cortex as a step towards community-wide effort to organize multi-source, data-driven information related to cell types taxonomies from any organ system or organism. To demonstrate its utility, the CCN was applied here to diverse cell type datasets

derived from multiple quantifiable modalities and several species. User-friendly executable code for applying the CCN is available on GitHub (https://github.com/AllenInstitute/nomenclature).

Disclosures: **J.A. Miller:** None. **N.W. Gouwens:** None. **B. Tasic:** None. **F. Collman:** None. **C.T.J. van Velthoven:** None. **T.E. Bakken:** None. **M.J. Hawrylycz:** None. **H. Zeng:** None. **E.S. Lein:** None. **A. Bernard:** None.

Digital Abstract Session

P374. Software Tools

Program #/Poster #: P374.06

Topic: I.07. Data Analysis and Statistics

Support: OT3OD025349

Title: Stimulating Peripheral Activity to Relieve Conditions (SPARC) Anatomy Working Group: Enriching ontology development

Authors: ***M. C. SURLES-ZEIGLER**, T. H. GILLESPIE, T. SINCOMB, M. E. MARTONE; Neurosci., UCSD, La Jolla, CA

Abstract: The Stimulating Peripheral Activity to Relieve Conditions (SPARC) program is a collaborative effort funded by the US National Institutes of Health to improve our understanding of the circuitry responsible for visceral control to develop the next generation of neuromodulation devices to treat conditions. SPARC comprises a consortium of researchers obtaining data on autonomic nervous system (ANS) connectivity between end organs and the central nervous system and the SPARC Data and Resource Center (DRC), which is fielding an infrastructure and tools for making these data available for use in models and simulations. As part of the DRC, we are developing FAIR vocabularies for annotating SPARC data, circuitry maps, and models. As anatomy forms the basis for this mapping effort, we have implemented an anatomical term pipeline that facilitates the use of anatomical terms from community ontologies like UBERON and FMA while augmenting these vocabularies with new terms as needed. New terms are needed when missing from community ontologies due to relatively poor coverage of the ANS.

Additionally, SPARC annotation spans several spatial scales, requiring even more precise anatomical annotations. In order to ensure that new anatomical terms are accurate and used consistently across these contexts, the SPARC Anatomy Working Group (SAWG) was formed comprising anatomical experts who provide an independent review and arbitration of SPARC's anatomical terminology. As part of this process, the SAWG developed a term request pipeline that enables SPARC investigators to regularly submit anatomical terms to the SPARC vocabularies housed within the InterLex database, an online database for viewing and building ontologies (https://scicrunch.org/sawg/). After review, appropriate terms will be contributed back to the community ontologies, thus improving their coverage of the PNS. Therefore, this project describes the novel process of triaging and standardizing anatomical terms and illustrates the

urgent need to contribute to community ontologies to enhance peripheral nervous system coverage.

Disclosures: M.C. Surles-Zeigler: None. **T.H. Gillespie:** None. **T. Sincomb:** None. **M.E. Martone:** None.

Digital Abstract Session

P375. Sample Preparation and Novel Probes

Program #/Poster #: P375.01

Topic: I.03. Anatomical Methods

Support: NIH Grant R37HD059288 (to ASC)

Title: Blocking cross-species secondary binding when performing double immunostaining with mouse and rat primary antibodies

Authors: *G. XIONG[1], S. MAO[2], B. N. JOHNSON[1], N. A. COHEN[3,4], A. S. COHEN[1,5]; [1]Anesthesiol. and Critical Care Med., Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Neurol., Renmin Hospital, Wuhan Univ., Wuhan, China; [3]Philadelphia Veterans Affairs Med. Ctr., Philadelphia, PA; [4]Otorhinolaryngology—Head and Neck Surgery, [5]Anesthesiol. and Critical Care Med., Perelman Sch. of Medicine, Univ. of Pennslyvania, Philadelphia, PA

Abstract: Immunostaining is a powerful technique and widely used to identify molecules in tissues and cells, although critical steps are necessary to block cross-reactivity. Here we focused at an overlooked cross immunoreactivity issue where the secondary antibody (secondary) cross-reacts with the primary antibody (primary) from a different species. We first confirmed the previously reported cross-species binding of goat anti-mouse secondary to rat primary. This was accomplished by staining with a rat primary against glial fibrillary acidic protein (GFAP) and visualizing with goat (or donkey) anti-mouse secondary. We then further revealed the converse cross-species binding by staining with a mouse primary against neuronal nuclear protein (NeuN) and visualizing with anti-rat secondaries. We speculate that mouse and rat primaries share antigenicity, enabling either secondary to recognize either primary. To block this cross-species binding in double staining experiments, we compared 3 protocols using mouse anti-NeuN and rat anti-GFAP, two primaries whose antigens have non-overlapping distributions in brain tissue. Simultaneous staining resulted in cross-species astrocytic staining (anti-mouse secondary to rat anti-GFAP primary) but no cross-species neuronal staining (anti-rat secondary to mouse anti-NeuN primary). Cross-species astrocytic staining was missing after sequential same-species staining with mouse anti-NeuN primary, followed by rat anti-GFAP. However, cross-species astrocytic staining could not be diminished after sequential same-species staining with rat anti-GFAP primary, followed by mouse anti-NeuN. We thus hypothesize that a competition exists between anti-mouse and anti-rat secondaries in their binding to both primaries. Single staining for NeuN or GFAP visualized with dual secondaries at different dilution ratio supported this hypothesis.

Disclosures: **G. Xiong:** None. **S. Mao:** None. **B.N. Johnson:** None. **N.A. Cohen:** None. **A.S. Cohen:** None.

Digital Abstract Session

P375. Sample Preparation and Novel Probes

Program #/Poster #: P375.02

Topic: I.03. Anatomical Methods

Title: An implantable kinematic headplate for repeatable submicron skull positioning in mice

Authors: ***S. J. KIM**, B. B. SCOTT;
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Abstract: Head immobilization is widely used in neuroscience studies to facilitate sensory stimulus control, behavioral monitoring, and neurophysiological recordings. Fixation strategies that provide mechanical stability and repeatable positioning over time or across instruments are particularly important for cellular resolution imaging or manipulation experiments. Headplates inspired by kinematic mounts enable precise micron-scale stability and position repeatability in rats (Rich et al. 2018). Here we sought to develop a head-restraint system capable of submicron registration accuracy that is small enough to be worn by mice. To achieve these goals, we designed a headplate and clamp based on the Maxwell kinematic coupling system (Slocum 2010). The clamp consisted of an aluminum mount with three vee grooves, oriented at 120 degrees relative to each other. The headplate was a stainless steel triangle, weighing 2.2 grams, with three half spheres at the triangular vertices, designed to mate with the vee grooves. Neodymium magnets attached to the clamp were used to provide the clamp force. We assessed the registration accuracy of the system using super resolution two-photon (2P) imaging of fluorescent microspheres fixed to the headplate following repeated attachments of the headplate., Registration accuracy (root mean square [RMS] displacement) for the solid stainless steel headplate was 1.35μm in x, 1.42μm in y, and 0.28μm in z. Scanning electron microscopy of the half spheres revealed mesoscale ridges and grooves in the metal surface, which could affect registration accuracy. Replacement of the stainless steel half spheres with ruby spheres improved repositioning accuracy to 0.17μm in x, 0.32μm in y, and 0.21μm in z. Mechanical stability of the headplate was assessed by 2P imaging of hippocampal pyramidal neurons of implanted, head fixed mice on a running wheel. RMS displacement was less than 4μm microns along horizontal (x) and vertical (y) axes of the imaging plane, and was small enough to be corrected by standard image registration algorithms. These results suggest that the mechanical stability of the headplate and clamp are sufficient for cellular resolution imaging in awake, behaving mice. We anticipate that the headplate clamp system reported here may facilitate experiments in mice where high precision and stability are required, such as imaging experiments involving voluntary head restraint (Kampf et al. 2010), time-lapse developmental studies, or registration of the same subject across multiple instruments.

Disclosures: **S.J. Kim:** None. **B.B. Scott:** None.

Digital Abstract Session

P375. Sample Preparation and Novel Probes

Program #/Poster #: P375.03

Topic: I.03. Anatomical Methods

Support: PAPIIT 306918

Title: Neuroanatomical Differences Between Brain Lobes Of Adults and Hatchlings Of Octopus maya

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Abstract: Octopuses are proposed as a good animal model for studying cognition and behavior because of their wide behavioral repertoire and complex neuroanatomy, however, the Octopus Maya, an endemic specie from Mexico, is not fully studied yet. Even though their lifespan is relatively short, previous findings state that hatchlings are born with a fully developed brain and exhibit similar behavior to adults, hence they can be used on experimental procedures since early life stages. Yet, some behavioral differences between young and adult specimens have been observed in octopuses breed in captivity, and more information is needed to elucidate the underlying causes. Brain organization, neuronal growth and overall system maturation have been seen as a crucial factor on behavioral refinement in other animal groups, therefore, in this study we compared neuroanatomical characteristics between brain lobes of adults and hatchlings of octopus maya, including quantity, type and distribution of neurons through light microscopy. We found that lobes of 1 month post-hantching specimens only have the equivalent of 36% of the amount of neurons present in adults. In addition, most neurons in young specimens are small cells arranged in 1 cellular layer, while adults exhibit 3-4 cellular layers comprised of small and large cells. These findings show the potential relevance of studying the developmental characteristics of octopus neurobiology, to improve the standardization of this emerging model of complex behavior and cognition.

Disclosures: H. Sanchez-Castillo: None. **P. García-Andaluz:** None. **A. González-Navarrete:** None. **D.B. Paz-Trejo:** None. **F. Vergara Ovalle:** None.

Digital Abstract Session

P375. Sample Preparation and Novel Probes

Program #/Poster #: P375.04

Topic: I.03. Anatomical Methods

Support: NIH Grant R01-DA-042057
NIH Grant F30MH122093
Wayne State University Graduate Research Award

Title: Quantifying Fos neuroactivity using high-resolution photoacoustic imaging in vivo and ex vivo after various routes of exogenous contrast agent administration

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Abstract: Current functional neuroimaging techniques, such as functional magnetic resonance imaging, rely on indirect consequences of neural activity (the hemodynamic response). With the advances in transgenic rodent technology and photoacoustic (PA) imaging, we propose a more direct method to map and quantify activated neurons using a Fos-LacZ transgene reporter system in rats. Fusion of Fos with the lacZ gene gives active (Fos+) cells the ability to cleave pro-chromogenic substrates, such as X-gal, into PA-detectable products. In this study, we quantified the neuroactivity-based PA signal in Fos-LacZ transgenic rats following cued fear conditioning using various routes of administration of X-gal to determine optimal *in vivo* PA imaging parameters and facilitate longitudinal analyses.Homozygous Fos-LacZ Wistar female and male rats underwent acquisition of cued fear conditioning immediately followed by X-gal administration through intrathecal, intravenous, or intraperitoneal routes 60 or 90 minutes (route dependent) after fear conditioning. Twenty-four hours later, rats were anesthetized and a cranial window was created in the skull. PA imaging of Fos-dependent neuroactivity in the medial prefrontal cortex (mPFC) *in vivo* was immediately conducted followed by transcardial perfusion to allow for subsequent validation with PA imaging of brains *ex vivo*. All PA imaging used an 18.5 MHz, 128 element L22-14v linear array ultrasound transducer to record PA signal produced by pulsed laser illumination directed with custom fiber optic cables. PA intensity within the mPFC *in vivo* and *ex vivo* of acquired images were then quantified using Matlab.We report quantified *in vivo* and *ex vivo* PA images acquired after multiple routes of X-gal administration demonstrating PA intensity differences between fear conditioned animals and vehicle controls.Using this novel approach, we propose a method of longitudinally monitoring activated (Fos+) neurons *in vivo* with high resolution and specificity. Our studies report the feasibility of all tested routes of X-gal administration with the best results reported from intrathecal administration. We then continued our exploration of this route to report preliminary *in vivo* imaging which will drive our future studies.

Disclosures: J.I. Matchynski: None. **R. Manwar:** None. **N. Sadik:** None. **S. Kallakuri:** None. **K. Makki:** None. **B.T. Hope:** None. **A.C. Conti:** None. **K. Avanaki:** None. **S.A. Perrine:** None.

Digital Abstract Session

P376. Connectomics and Circuit Tracing

Program #/Poster #: P376.01

Topic: I.03. Anatomical Methods

Support: Israel Science Foundation

Title: The AxCaliber connectome - investigating the axon-diameter weighted human brain network using MRI

Authors: *H. GAST, Y. ASSAF;
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Abstract: Considered to have a major role in the ability to define brain's function, the history of neuroscience is replete with attempts to understand the wiring pattern of the brain. Yet, one of the major drawbacks in studying the connectome, is how to weight its connections. In this study, we introduce AxCaliber3D method, under developing in our lab, to assess axon diameter distribution in the brain. Being related to the conduction velocity in axons (Ritchie, 1982), we intend to define the conduction-velocity based connectome of the human brain. We believe that this newly defined connectome, might provide a better ground for connectome properties estimation. 50 participants were scanned for a high resolution T1 MPRAGE, a high angular resolution diffusion imaging (HARDI) and a multi b-shell AxCaliber. We analyzed scans using AxCaliber3D, an under-development method for assessing axon-diameter distribution. In AxCaliber3D, we divide the analysis into two parts: Estimating hindered diffusion and fiber orientations (from the HARDI acquisition); and estimating the relative contribution of each of axonal components to the obtained signal, for 160 different axon components (0-20 μm). The diffusion within the axon are modeled based on the work of Vangelderen et al. (1994). We then performed Deterministic Maximum Direction Getter and CSD tractography, using Dipy library. Based on Schaefer et al.'s (2018) atlas, we labeled brain areas as nodes and weighted edges by the average number of tracts, FA values or axon-diameter between each pair of nodes, to compute connectivity matrices. We used Pearson correlation to compared the FA weighted and axon-diameter weighted connectomes, we were able to show that for some cases the two weighting methods demonstrate a positive correlation, in others a negative correlation and some nodes' pairs connections are not correlated at all. The different patterns between the two diffusion-based values, asses that the AxCaliber3D method measures a unique property of the brain's tracts, thus revealing a new tier in the human brain connectome. Moreover, while weighting edges using the number of tracts or the axon-diameter, connectivity matrices showed two different networks, as well as distinct node's properties distribution. Our results show different distributions of node's centrality measures (i.e. the node's degree and clustering coefficient) for the two connectomes. Being notably different from the so far explores weighted anatomical connectomes, the axon-diameter based connectome could expand our knowledge regarding the human brain and the complex network that upholds it.

Disclosures: **H. Gast:** None. **Y. Assaf:** None.

Digital Abstract Session

P376. Connectomics and Circuit Tracing

Program #/Poster #: P376.02

Topic: I.03. Anatomical Methods

Support: AMED JP20dm0207001

Title: The connectomic mapping of the marmoset prefrontal cortex

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Abstract: The prefrontal cortex (PFC) evolved greatly in primate lineage to enable highly complex cognitive behaviors, laying the basis for humanity. At the core of its function lies the intricate connectional network of cortical and subcortical brain regions. To gain insight into the architecture of such network, we performed a comprehensive mapping of PFC projections using marmosets as the model animal. We have now obtained 44 high-quality datasets with anterograde tracer injections into major PFC subdivisions, including dorsolateral and orbitomedial PFCs, putative frontal eye field as well as a few premotor areas. Each dataset covers the whole brain with >650 images of axon-level resolution (xy resolution; 1.3μm/pixel, z resolution; 50 μm/section). It offers both parcellation-based and parcellation-free measurements of tracer signals in a standardized 3d space for comparison across samples. Using this resource, we were able to model topographic projections of corticothalamic and corticostriatal terminals at high accuracy. Furthermore, by modeling the cortical surface by a stack of layer-specific surface map, we were able to quantitatively characterize the columnar convergence of axon terminals, which have been known for decades as a characteristic feature of primate PFC.

Disclosures: **A. Watakabe:** None. **H. Skibbe:** None. **K. Nakae:** None. **J. Wang:** None. **M. Takaji:** None. **H. Mizukami:** None. **A. Woodward:** None. **R. Gong:** None. **J. Hata:** None. **H. Okano:** None. **S. Ishii:** None. **T. Yamamori:** None.

Digital Abstract Session

P376. Connectomics and Circuit Tracing

Program #/Poster #: P376.03

Topic: I.03. Anatomical Methods

Title: Inter- and Inner- Connectivity Balance in The Human Brain

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Abstract: The connectome has a great impact on the brain's function, as its structure affects the speed and efficiency of information transfer. As such, brain networks are expected to have an organization that minimizes wiring costs while maintaining conductivity speed. A recent study spanning a variety of mammals showed consistent connectivity across species, explained by the fact that species with highly connected hemispheres appear to have weaker inter-hemisphere connections. While this phenomenon is harder to pinpoint in humans due to a smaller variability, we hypothesized that a similar phenomenon will be found across humans. Analyzing this phenomenon in the human brain allows us to compare network structures more easily. Thus, we can find anatomical and functional origins of this phenomena, an exploration that could not be done between species. This research was conducted on the HCP young adult dataset (N=970), including structural and diffusion MRI scans of pairs of monozygotic and dizygotic twins, non-twin siblings and other subjects. We performed multi-shell multi-tissue CSD based tractography on all subjects and computed structural connectivity matrices for each. Hemispheric connectivity was assessed by the network connectivity, and inter-hemispheric connectivity by the ratio of commissural tracts of the overall brain. To further examine the biological origins of this phenomenon, local connectivity measures were computed in order to examine their effect on the phenomenon. We observed a correlation (r^2 =0.25, $p<0.001$) between hemispheric efficiency and the commissural ratio. In node analysis, a strong connection was found between each node's centrality in the network and its effect on the balance. Rich club nodes, such as the precuneus, superior frontal and temporal cortices and the hippocampus, contribute highly to the phenomenon. Comparing the connectomes of monozygotic and heterozygotes siblings revealed higher hereditary values for commissural ratio (h^2=90%) than hemispheric efficiency (h^2=83%) and significant cross-twin-cross-correlation. Our results point to a trade off between inter intra hemispheric connectivity. In contrast to previous studies in mammals, here we explore the regional and hereditary basis of this phenomenon. These findings emphasize studies showing increased intra-hemispheric connections in subjects with agenesis of the corpus callosum, suggesting a reorganization of connections to balance the brain connectivity. This variability in hemispheric connectivity may underlie the specific abilities of an individual. Thus, further focus on the cognitive and behavioral implications of these findings is needed.

Disclosures: R. Krupnik: None. **Y. Yovel:** None. **Y. Assaf:** None.

Digital Abstract Session

P376. Connectomics and Circuit Tracing

Program #/Poster #: P376.04

Topic: I.03. Anatomical Methods

Support: NIH R01NS096720

Title: A multimodal connectomic atlas of the mouse brain

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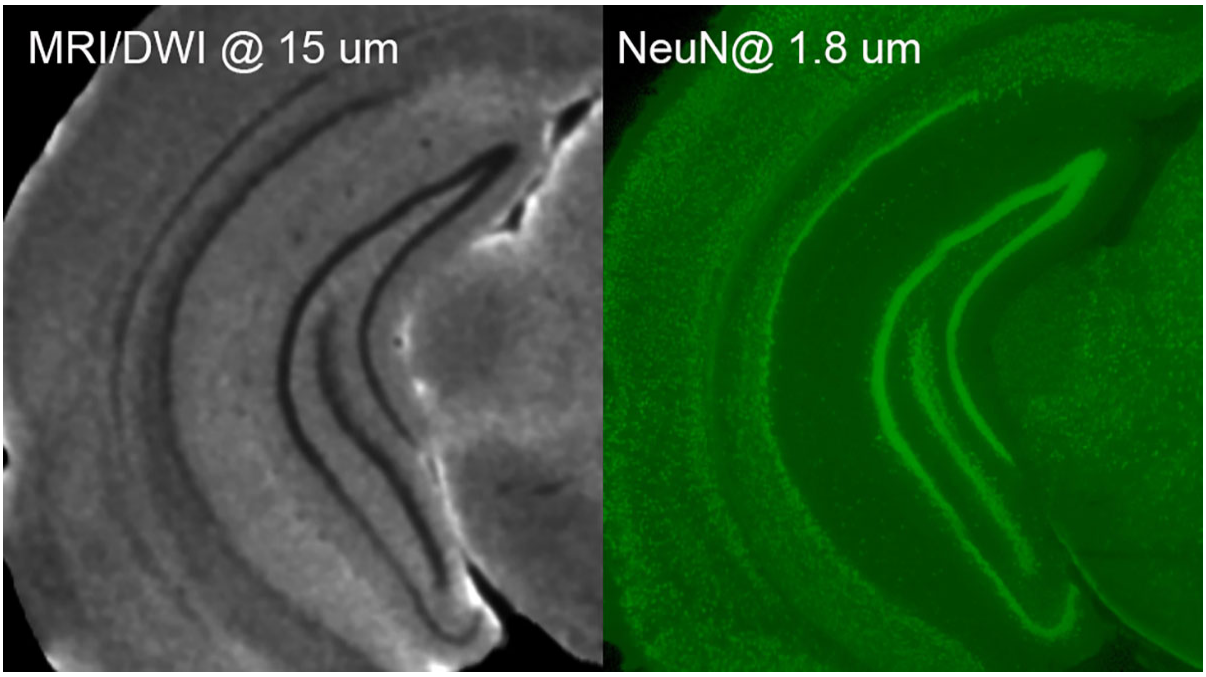
Abstract: Introduction: We present a multimodal connectomic atlas of the mouse brain that merges diffusion tensor images (DTI) magnetic resonance histology (MRH) at 15 μm isotropic resolution and light sheet microscopy (LSM) of the same brain registered into a common space (CCFv3).Methods: MRH was performed at 9.4 T using a novel compressed sensing pipeline. Brains were cleared/stained using LSM. LSM data were acquired with stains for Syto16 NeuN and MPB. LSM data were registered to MRH at full resolution. The CCFv3 atlas was registered to the MRH using diffusion-weighted (DWI) and fractional anisotropy (FA) images.Results: The MR/DTI datasets are ~ 600,000-times higher resolution than the human connectome and 250-fold higher when adjusting for total brain volume between human and mouse. We were successful in co-registering the CCFv3 atlas and LSM data to the MRH. Since the MRH data are acquired with the brain in the skull, there is almost no distortion from perfusion-fixation and no artifacts from cranial dissection, tissue sectioning and histological processing. The availability of multiple MRH contrasts—axial diffusivity (AD), radial diffusivity (RD), DWI, FA—all of which are inherently registered to the same space, provides anatomical and cytoarchitectural landmarks that are consistent with the CCFv3 atlas. The resolution index of the connectome data, that is, the product of angular and spatial resolution ($3.7x10^7$) is five orders of magnitude higher than clinical scans allowing quantitative comparison between DTI and ABA mouse brain connectomes. The combination of MRH and LSM provides the most accurate geometric correction possible with a comprehensive anatomical connectome for the same specimen. The atlas is providing a powerful, multidimensional platform for global surveys of structural, functional, behavioral, and connectomic variation, including a comprehensive evaluation of the BDX panel of transgenic mouse strains.



Disclosures: **G.A. Johnson:** None. **M. Chen:** None. **J. Cook:** None. **G. Cofer:** None. **J. Gee:** None. **R. Laoprasert:** None. **Y. Qi:** None. **L. White:** None. **N. Wang:** None. **P. Starewics:** None. **R. Williams:** None.

Digital Abstract Session

P376. Connectomics and Circuit Tracing

Program #/Poster #: P376.05

Topic: I.03. Anatomical Methods

Title: Exploring the spatial efficiency of the brain utilizing the mouse connectome

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Abstract: The mammalian brain is a complex set of highly interconnected, yet anatomically and functionally distinct, regions. Together, these brain regions are organized into a functional neural network, but it remains unclear what factors drive this organization. One possible factor driving the spatial orientation of discrete brain regions is the structural connectivity of the network which promotes efficient function, where a network is efficient if regions that are well-connected are placed closer together. However, a limited understanding of structural connectivity and efficiency of brain networks in mammalian systems confounds our understanding of the brain's organization. The Allen Mouse Brain Connectivity Atlas provides a high-resolution structural connectivity map of mouse brain regions derived from a series of viral tracing experiments. With these data, we explored our hypothesis that regions which are highly interconnected are located

proximal to each other resulting in an efficient spatial organization that is related to the network connectivity. To test this hypothesis, we composed a model of the mouse brain by first simplifying complex brain region geometry to spheres while maintaining original structure volume. Then, using accompanying axonal projection volume data from the Atlas, we defined the spatial-connective efficiency for the placement of brain regions in 3D space as the volume of axonal projections between each region divided by the distance between regions. With this parameter defined, we utilized the SciPy optimization library to derive a solution for the maximal spatial-connective efficiency, and therefore the optimal theoretical placement, of 157 wild-type mouse brain regions with connectivity data from 499 viral tracing experiments and compared it to the actual mouse brain. Through this comparison, we found that the mouse brain's connectivity correlates to the physical localization of brain regions, suggesting that the brain's spatial organization is partly driven by the efficiency of the network connectivity.

Disclosures: **N.R. Smith:** None. **A. Borzou:** None. **S. Miller:** None. **J. Kasper:** None. **J. Hommel:** None.

Digital Abstract Session

P376. Connectomics and Circuit Tracing

Program #/Poster #: P376.06

Topic: I.03. Anatomical Methods

Support: Unversity of Leeds International Research Scholarship
NIH OD 010943
EPSRC EP/J004057/1

Title: A multiscale brain map derived from whole-brain volumetric connectome reconstructions

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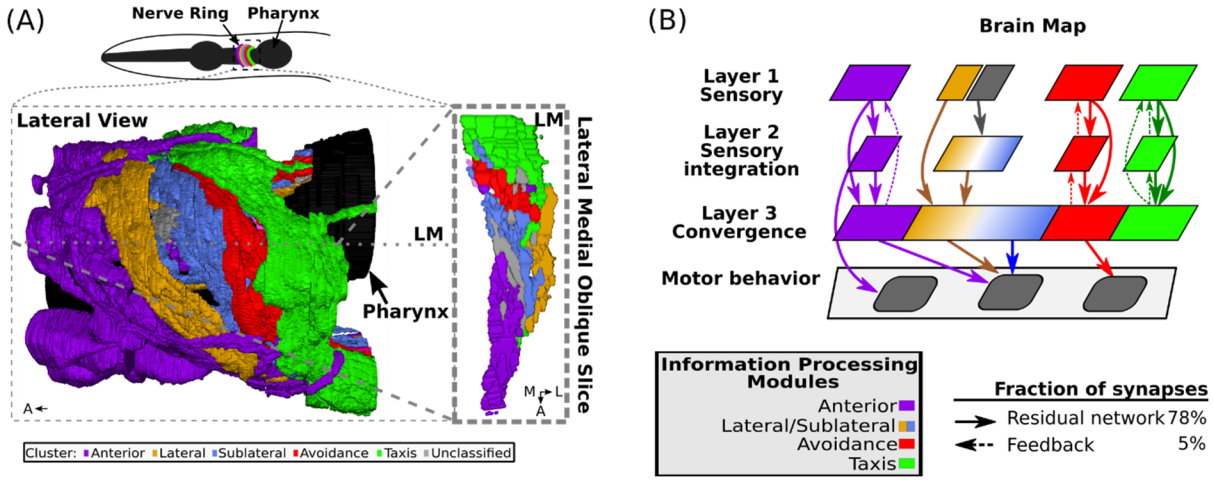
Abstract: Understanding how brain structure and function combine to generate behavior is a major goal of systems neuroscience. We present a multi-scale connectome and complete brain map of the *C. elegans* main neuropil, the nerve ring. We integrate our volumetric reconstruction of serial-sectioned EMs from 1 L4 larva and 1 young adult brain with corresponding synaptic and gap junctional connectomes. Exploiting bilateral symmetry of the neuropil, we generate 4 lateral datasets and assess the reproducibility of membrane contacts across datasets (M^{δ}), where δ=[1,4] labels the reproducibility degree. We find M^{δ} bimodally distributed, with high and low reproducibility peaks, and predict distinct populations of precisely-targeted versus variable contacts. Synaptic contacts (C^{δ}) on reproducible membrane contacts are also bimodal, pointing to significant variability in synaptic connectivity. We model membrane and synaptic contact distributions with 3 parameters: the target contact fraction, f<50% , precision, p>90%, and specificity (contact avoidance), s>20%, showing that <50% of synaptic contacts are precisely

targeted. Applying cluster analysis to reproducible M^4 (as proxies for core) contacts, we identify 5 spatial clusters of neurites (Figure A) that support similarly modular synaptic circuitry. Most neurons form predominantly intra-module contacts but 33 neurons are cross-modular and often exhibit reproducible microscale features linked to synaptic specialization. We posit that parallel information processing modules integrate over parallel sensory pathways before converging onto a recurrent distributed subcircuit, and construct a cellular resolution 3-layer modular brain map that accounts for 83% of core (C^4) contacts (Figure B). The *C. elegans* brain map is reminiscent of the layered cortical connectivity and its bio-inspired analogue - Residual Networks. The nested architecture supports both core, conserved, and variable, individual structures. Conserved features point to robust principles of brain organization that likely generalize across nervous systems.



Disclosures: **C.A. Brittin:** None. **S.J. Cook:** None. **D.H. Hall:** None. **S.W. Emmons:** None. **N. Cohen:** None.

Digital Abstract Session

P376. Connectomics and Circuit Tracing

Program #/Poster #: P376.07

Topic: I.03. Anatomical Methods

Support: Foundation of Westlake University to K.D.P.

Title: Optical connectome at single synapse resolution

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Abstract: How the brain works depends on its neuronal network wiring. A comprehensive understanding of the brain is not possible without revealing its wiring diagram at a single synapse level. Mapping neuronal circuits in mammalian brains requires reconstruction of large

brain samples at spatial resolution corresponding to the size of synapses, e.i. ~20-40 nm. Optical microscopy has been the most powerful technique in the neuroscience arsenal due to its low cost, wide usage in biological sciences and ability to read out information-rich molecular signals. However, conventional optical microscopes have the spatial resolution of optics limited by diffraction of light. To address this issue, we developed an integrated imaging method for optical connectome microscopy with unprecedented resolution and scales. The method based on a modified ExM protocol that enables physical magnification of large brain tissue samples by 8 times in linear dimension. The high expansion factor allows us to achieve ~40 nm resolution using conventional microscopy techniques. The method is compatible with standard fluorescent proteins and antibody staining enabling multiplex imaging of neuronal morphology and pre- and post-synaptic densities in mouse brain. Using a versatile tiling light-sheet microscope we imaged the entire primary visual cortex region of a mouse at 100 nm resolution. In addition, the developed technique is compatible with whole mouse brain preparation as well as intact C.elegans worms. We believe that our method will enable fast and cost-efficient acquisition of whole-brain optical connectome at a single synapse resolution for the model organisms. The successful delivery of the method would significantly facilitate the study of how the brain works by creating complete connectome maps.

Disclosures: **B. Liu:** None. **C. Sun:** None. **Y. Chen:** None. **L. Gao:** None. **K.D. Piatkevich:** None.

Digital Abstract Session

P376. Connectomics and Circuit Tracing

Program #/Poster #: P376.08

Topic: I.03. Anatomical Methods

Support: Smith Family Award Program for Excellence in Biomedical Research
NIH Grant R01AA024434
NIH Grant 5P20GM103645
NIH Grant R01DC017146
NIH Grant R01MH105368

Title: Transsynaptic mapping of Drosophila mushroom body output neurons

Authors: *K. M. SCAPLEN[1,2,3], M. TALAY[4,3], J. D. FISHER[3], R. M. COHN[5,6], A. SORKAC[3], Y. ASO[7], G. BARNEA[3], K. R. KAUN[3];
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Abstract: The Mushroom Body (MB) is a well-characterized associative memory structure within the *Drosophila* brain. Although previous studies have analyzed MB connectivity and provided a map of inputs and outputs, a detailed map of the downstream targets is missing. Using the genetic anterograde transsynaptic tracing tool, *trans*-Tango, we identified divergent projections across the brain and convergent downstream targets of the MB output neurons (MBONs). Our analysis revealed at least three separate targets that receive convergent input from MBONs: other MBONs, the fan shaped body (FSB), and the lateral accessory lobe (LAL). We describe, both anatomically and functionally, a multilayer circuit in which inhibitory and excitatory MBONs converge on the same genetic subset of FSB and LAL neurons. This circuit architecture provides an opportunity for the brain to update information and integrate it with previous experience before executing appropriate behavioral responses.

Disclosures: K.M. Scaplen: None. **K.R. Kaun:** None. **G. Barnea:** None. **J.D. Fisher:** None. **A. Sorkac:** None. **Y. Aso:** None. **M. Talay:** None. **R.M. Cohn:** None.

Digital Abstract Session

P376. Connectomics and Circuit Tracing

Program #/Poster #: P376.09

Topic: I.03. Anatomical Methods

Support: Australia Research Council DP180101395

Title: Mapping spinal cord and brain targets of colonic extrinsic nerves in the mouse.

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Abstract: Aim: Identify the central circuits into which extrinsic nerves from the distal colon convey nociceptive signalling in the mouse. **Methods:** Male and female adult mice underwent retrograde tracing from the distal colon using cholera toxin subunit b (CTb). CTb labelled sensory neurons were quantified within CLARITY cleared dorsal root ganglia (DRG, T9-S2) and nodose ganglia (N=6) after four days. CTb labelled projections were identified in spinal and medulla sections (N=5) after seven days. Anterograde trans-neuronal tracing with herpes simplex virus 1 expressing EGFP (HSV-H129-EGFP) was performed from the distal colon wall. Neuronal expression of EGFP was identified within spinal and brain sections after 72-120 hours (N=4/time). To identify brain regions engaged by colonic nociceptive stimuli the number of neurons activated by noxious *in vivo* colorectal distension (N=5) was compared to that evoked by innocuous (N=5) and no distension (N=5). Immunolabelling for phosphorylated MAP kinase (pERK) was used to identify activated neurons. **Results:** Colonic CTb-labelled neurons were present in the DRG, peaking in L1 and S1, and nodose ganglia. CTb-labelled projections were observed in the spinal cord dorsal horn, spanning T11-L1 (in lamina I/ V and in lateral tracts of

Lissauer) and L6-S2 (in lamina I, dorsal grey commissure and lateral tracts into the sacral parasympathetic nuclei (SPN)), and within the medulla (nucleus tractus solitarius (NTS) and area postrema). CTb-labelled cell bodies were present in the intermediolateral nuclei (IML), SPN and dorsal motor vagal nuclei (DMV). 72-96 hours after colonic injection, EGFP-expressing neurons were observed in the spinal cord dorsal horn spanning T11-L1 (lamina I, IML and around the central canal) and L6-S2 (laminae I-V, dorsal grey commissure and SPN) and within the caudal and rostral ventrolateral medulla (VLM) and the rostral ventromedial medulla (RVM). After 120 hours, EGFP-labelling was present in the DMV, NTS, hindbrain locus coeruleus (LC), sub-coeruleus, Barrington's nucleus, lateral parabrachial nucleus (lPbN) and pontine reticular A7. Labelling was also evident in the hypothalamic paraventricular nucleus, periaqueductal gray (PAG), thalamic nuclei and somatosensory motor cortex. Of the brain regions in which EGFP-neurons were observed, noxious CRD evoked a significant increase in neuronal activation relative to innocuous distension in the VLM, RVM, LC, lPbN, PAG and medial thalamic nuclei. **Conclusion:** These data localised colonic input within the spinal cord dorsal horn and medulla dorsal motor vagal complex and the subsequent brain circuits into which colonic nociceptive stimuli is conveyed.

Disclosures: A.M. Harrington: None. **V. Wang:** None. **J. Harris-Janson:** None. **A. McGovern:** None. **S.B. Mazzone:** None. **S. Brierley:** None.

Digital Abstract Session

P376. Connectomics and Circuit Tracing

Program #/Poster #: P376.10

Topic: I.03. Anatomical Methods

Support: NIH Grant MH108053-01

Title: Mapping neuroanatomical structures labeled in histologic sections to a whole brain standardized atlas coordinate system

Authors: *N. J. O'CONNOR[1], B. S. EASTWOOD[1], P. J. ANGSTMAN[1], A. D. LEDUC[1], C. R. GERFEN[2], J. R. GLASER[1];
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Abstract: Advances in molecular neuroanatomical tools have expanded the ability to map specific neuron subtype populations and their connections in the context of behavioral or pathological patterns of neuronal activity. Analysis of connectivity and neuronal activity across whole mouse brains that have been registered to a reference atlas reveals the functional organization of brain circuits related to behavior and pathologies that can be compared across animals, experiments, and laboratories. Here we present advances to our previous technique[1] for reconstructing images of mouse brain sections labeled with standard histochemical techniques into whole-brain volumes and registering those brain volumes to the Allen Mouse Brain Common Coordinate Framework (CCF) to enable automatic delineation of brain

regions.Specifically, the process for accurate registration to the CCF accounts for artifacts due to histological cutting, mounting, and fixation. A transform is estimated for a section that contains clear landmarks, and this transform is then propagated to other sections with linear and nonlinear refinements that preserve the histologic sectioning model. We present a quantitative comparison between our updated algorithms and a single 3D transformation of aligned serial section volumes. Manually placed fiducial markers in the experimental brain sections and in corresponding anatomies in the CCF are used to assess matching accuracy. Examples are provided of whole-brain analysis of connectivity using trans-synaptic rabies labeling of neurons providing inputs to Cre-expressing neuron subtypes and of analysis of behaviorally relevant brain circuits using fos labeling of activity in neurons correlated with specific behaviors. For analysis, coronal brain sections are reconstructed into a 3D volume, labeled neurons are automatically marked using a multiple scale feature detector assisted by a deep neural network, and brain sections and marked neurons are registered to the CCF. The number of detected neurons in each of the 2500 brain structures in the CCF are tallied, allowing for quantitative analysis between mice.

1. Eastwood B. S., Hooks B. M., Paletzki R.F., O'Connor N.J., Glaser J.R., and Gerfen C.R. "Whole Mouse Brain Reconstruction and Registration to a Reference Atlas with Standard Histochemical Processing of Coronal Sections." Journal of Comparative Neurology, December 14, 2018.

Disclosures: **N.J. O'Connor:** A. Employment/Salary (full or part-time):; MBF Bioscience. **C.R. Gerfen:** None. **J.R. Glaser:** A. Employment/Salary (full or part-time):; MBF Bioscience. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MBF Bioscience. **P.J. Angstman:** A. Employment/Salary (full or part-time):; MBF Bioscience. **B.S. Eastwood:** A. Employment/Salary (full or part-time):; MBF Bioscience. **A.D. LeDuc:** A. Employment/Salary (full or part-time):; MBF Bioscience.

Digital Abstract Session

P377. New Optical Sensors and Imaging Technologies

Program #/Poster #: P377.01

Topic: I.04. Physiological Methods

Support: Howard Hughes Medical Institute

Title: Estimation of protein turnover in mouse brains over multiple spatial scales with cell type specificity

Authors: *B. MOHAR, N. P. SPRUSTON, K. SVOBODA;
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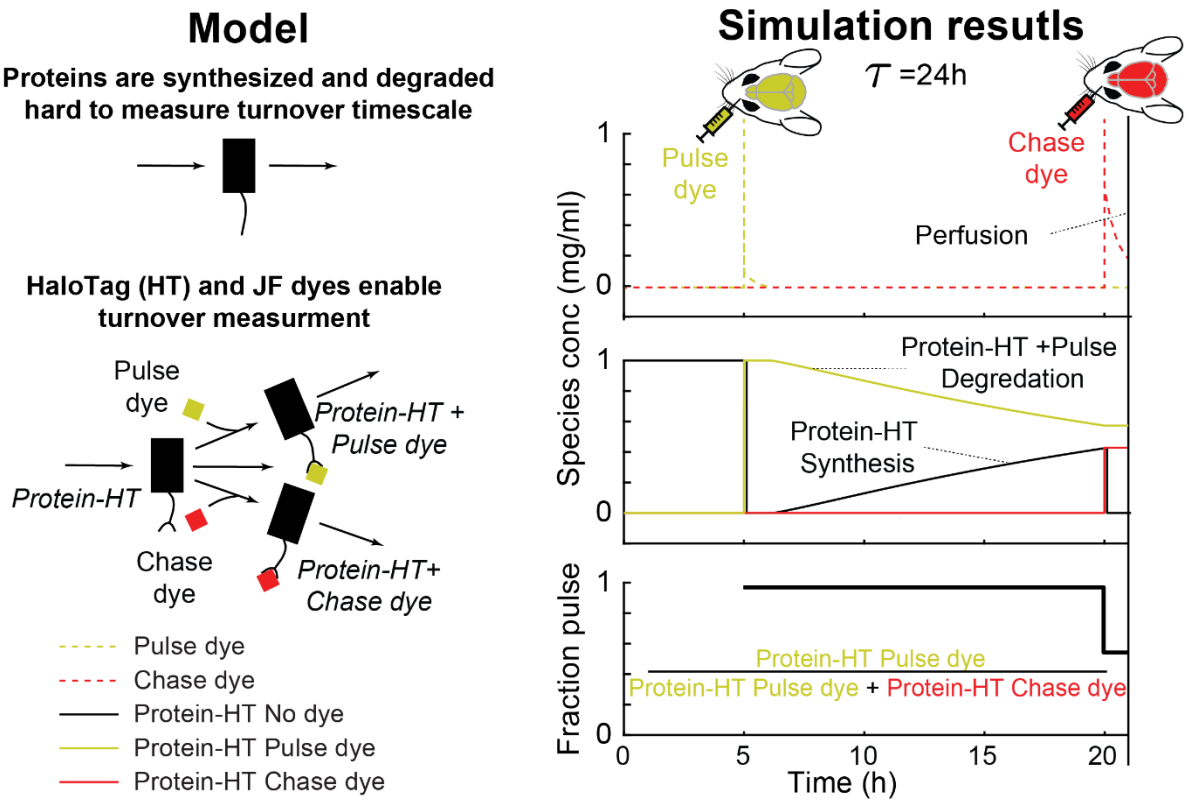
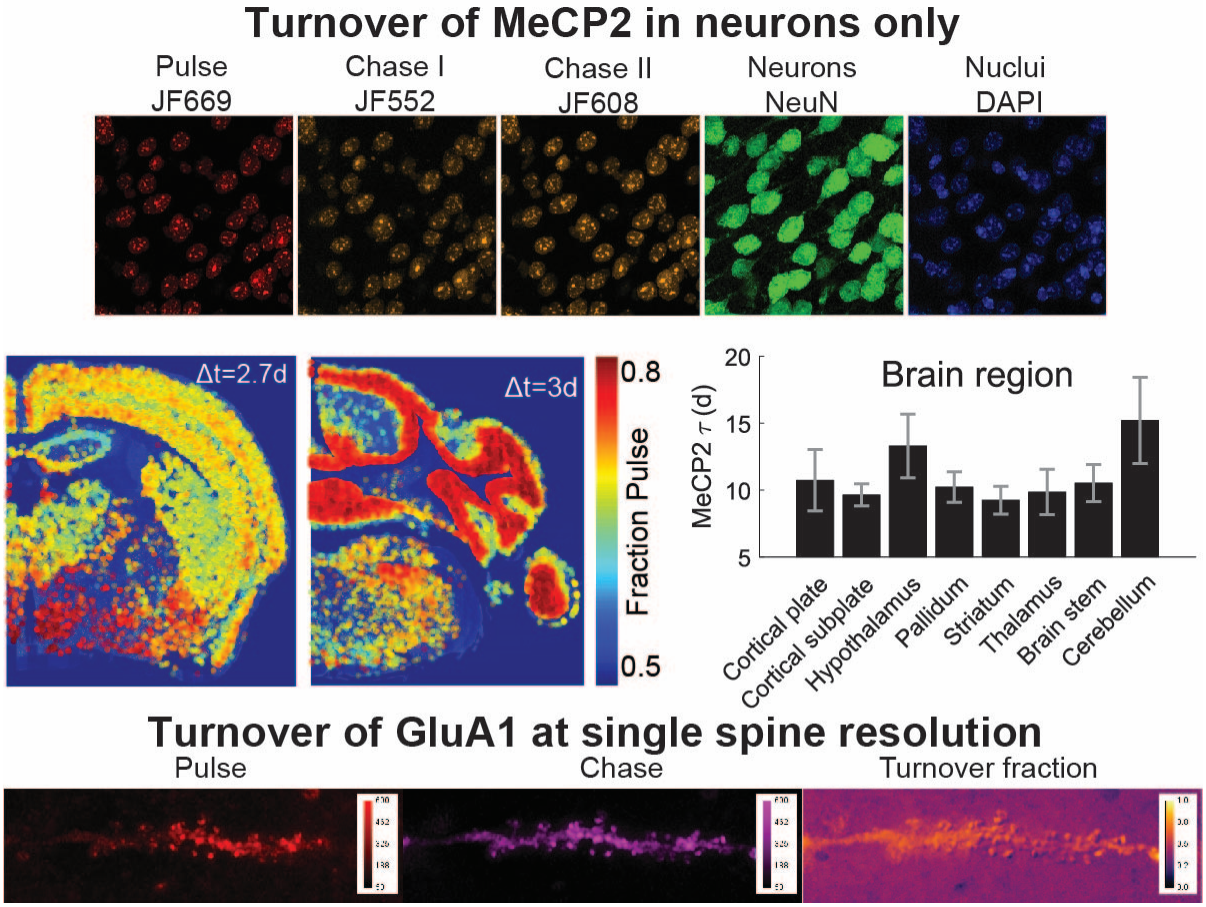
Abstract: Cells continuously synthesize and degrade their proteins (turnover), supporting cellular functions over a wide range of temporal and spatial scales. The brain presents special

challenges for studying turnover, due to its morphologically expansive neurons and the need to maintain synaptic weights used to store memories that may last as long as the lifetime of the animal. Thus, the dynamics of protein turnover may vary across brain regions, cell types, and subcellular compartments. Studying protein turnover in the nervous system requires a method that overcomes the brain's pharmacologically privileged status, and assesses protein turnover at very high spatial resolution and over large spatial and temporal scales. We tackled these challenges by tagging proteins with a self-labeling enzyme that covalently attaches a fluorescent ligand. We then measured turnover using a pulse-chase strategy with orthogonal fluorescent dyes. We screened dyes to maximize brain bioavailability, and characterized their temporal profile with in-vivo measurements and modeling. By combining this method with post-hoc immunofluorescence, we quantified cell-type and brain-region specific turnover of the nuclear protein MeCP2, as well as subcellular compartment-specific turnover of a glutamate receptor subunit, which turned over faster in the soma than in dendrites and spines. This new method enables high-resolution, brain-wide measurements of protein turnover in-vivo.





Disclosures: **B. Mohar:** None. **K. Svoboda:** None. **N.P. Spruston:** None.

Digital Abstract Session

P377. New Optical Sensors and Imaging Technologies

Program #/Poster #: P377.02

Topic: I.04. Physiological Methods

Support: JSPS KAKENHI 16H06532
JSPS KAKENHI 16H06524
JSPS KAKENHI 16K21734
JSPS KAKENHI 19K12190
JSPS KAKENHI 19K22990
JSPS KAKENHI 19H01142
JSPS KAKENHI 20H04341

Title: Optical membrane potential recordings with voltage-sensitive dye (VSD) enabling recordings of rare and unique neuronal activity patterns

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Abstract: Optical recording with voltage-sensitive dye (VSD) was developed in the early 1970s. Developments of imagers for VSD recording in the 1980s-90s have enabled us to record the dynamics of neural activities in the brain. Due to small fractional changes in VSD fluorescence upon membrane potential change in brain slice preparation, one must do averaging or accumulating of the signal over several trials to get clear signals, or in most cases to avoid photon shot-noise. Our improvements in optics and slice preparation handlings have improved this situation — they have allowed us to record much more data. We successfully recorded optical response every 200 ms, recording every 60 seconds at 0.1 ms/frame (10 kHz) for over 12 hours, without photo-damaging or photo-bleaching in area CA1 of mouse hippocampal slice upon Shaffer collateral stimulation, enabling us to record late-phase LTP. Each single-shot imaging trial has enough S/N without averaging.
The successful single-shot repetitive recording allows us to find "rare" network activity among many trials. This network activity occurred only rarely but was imaged clearly. One was the activity spread from the perirhinal cortex (PC) to area CA2 of the hippocampus. In the series of consecutive recordings, most of the recording only shows the spread between the PC and the entorhinal cortex (EC). On only rare occasions, the PC activity was followed by an activation in area CA2 of the hippocampus and then spread to the CA1 region in parallel with the spread to EC. The neural excitation transmission between two regions has been predicted from the anatomical study but was difficult to record with electrophysiological recording. This kind of probabilistic or quantal communication between regions in the brain could be substantial. Because of the evident propagation shown in the imaging system, even though it was rare, the spread is not just noise or fluctuation. The single-shot recording was also useful to see oscillations in the neural circuit. We present oscillatory activity in the hippocampus from CA2 to CA1 and PC-EC connection and long-range activity in the cortex. Because we could record those events with the wide-field optics, we argue that wide-field imaging with VSD is a handy tool in seeking unique network activity in the brain slice preparation.

Disclosures: T. Tominaga: None. **Y. Tominaga:** None. **R. Kajiwara:** None.

Digital Abstract Session

P377. New Optical Sensors and Imaging Technologies

Program #/Poster #: P377.04

Topic: I.04. Physiological Methods

Support: NSF Grant DBI-1707312

Title: Quantitative analysis of 1700-nm three photon red-shifted calcium imaging in the mouse brain

Authors: *C. WU, A. MOK, T. WANG, D. OUZOUNOV, W. GU, M. WARDEN, C. XU; Cornell Univ., Ithaca, NY

Abstract: Three-photon calcium imaging with combination of genetically encoded calcium indicators (GECIs) have allowed measurements of activity in large populations in deep brain regions. To achieve deeper depth and parallel applications, red-shifted GECIs were emerging as a useful tool. However, current red GECIs are not well quantified in large-scale neural activity imaging which entails a careful balance between recording fidelity and tissue heating.
We calculated and experimentally verified the excitation pulse energy to achieve the minimum photon count required for the detection of calcium transients in jRGECO1a-expressing neurons for 1064-nm two-photon and 1650-nm three-photon excitation, respectively. Brain tissue heating by continuous three-photon imaging was simulated with Monte Carlo method and experimentally validated with immunohistochemistry. We observed increased immunoreactivity with 75 mW excitation power at 0.8-, 1.0- and 1.2-mm imaging depths. Our analysis presents a translatable model for the optimization of three-photon red protein calcium imaging based on experimentally tractable parameters.

Disclosures: C. Wu: None. **A. Mok:** None. **T. Wang:** None. **D. Ouzounov:** None. **W. Gu:** None. **M. Warden:** None. **C. Xu:** None.

Digital Abstract Session

P377. New Optical Sensors and Imaging Technologies

Program #/Poster #: P377.05

Topic: I.04. Physiological Methods

Support: NSF grant 2027113
Perkin Elmer Postdoctoal fellowship

Title: Bioluminescent genetically encoded indicators of neuronal activity

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Abstract: Genetically encoded optical sensors and advancements in microscopy have revolutionized the scientific toolbox used for answering complex biological questions especially in terms of studying brain activity. However, there remains a need to develop new tools for reporting neuronal activity *in vivo* within deeper structures. We expect this can be achieved by replacing the fluorescent elements of the existing biosensors with bioluminescent producing elements. This will bypass the need of external light sources to illuminate the sensor and overcome limitations such as limited light penetration depth, light diffraction and tissue heating that are all caused by the excitation light used for fluorescent imaging. We are building on recent advancements in two fields: genetically encoded optical sensors for real-time sensing changes in neurotransmitters levels or voltage; and genetic evolution of new enzymes that produce

biological luminescence at unprecedented intensities. We have engineered a variety of bioluminescent voltage indicators (bGEVIs) and bioluminescent neurotransmitter indicators (bGENIs) by adapting sensing domains used in fluorescent indicators and incorporating bioluminescent producing luciferases. We have currently created bioluminescent indicators that report membrane depolarization, glutamate and dopamine with increases in luminescence. Our voltage indicator has a 60% increase in luminesce when mammalian cells are depolarized. Our glutamate indicator has a 255% increase and our dopamine indicator currently has a 20% increase in response to neurotransmitter presentation when expressed in mammalian cells (HEK cells). We expect these indicators to perform well for imaging neuronal activity in deep brain structures with imaging equipment that is readily accessible to researchers.

Disclosures: E.D. Petersen: None. **G. Santana:** None. **F. St-Pierre:** None. **N.C. Shaner:** None. **A. Gilad:** None.

Digital Abstract Session

P377. New Optical Sensors and Imaging Technologies

Program #/Poster #: P377.06

Topic: I.04. Physiological Methods

Support: Beijing Municipal Science & Technology Commission Grant Z181100001318002
Beijing Municipal Science & Technology Commission Grant Z181100001518004
National Natural Science Foundation of China Grant 31925017
NIH Grant NS103558
Shenzhen-Hong Kong Institute of Brain Science Grant NYKFKT2019013
Peking-Tsinghua Center for Life Sciences
State Key Laboratory of Membrane Biology at Peking University School of Life Sciences

Title: A fluorescent sensor for spatiotemporally resolved endocannabinoid dynamics in vitro and in vivo

Authors: *A. DONG[1,2,3], K. HE[1,2], B. DUDOK[4], J. S. FARRELL[4], W. GUAN[7], D. J. LIPUT[8,9], H. L. PUHL[9], R. CAI[1,2], J. DUAN[1,2], E. ALBARRAN[5], J. DING[4,6], D. M. LOVINGER[8], B. LI[7], I. SOLTESZ[4], Y. LI[1,2,3,10];
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Abstract: Endocannabinoids (eCBs) are retrograde neuromodulators that play an important role in a wide range of physiological processes; however, the release and in vivo dynamics of eCBs

remain largely unknown, due in part to a lack of suitable probes capable of detecting eCBs with sufficient spatiotemporal resolution. Here, we developed a new eCB sensor called $GRAB_{eCB2.0}$. This genetically encoded sensor consists of the human CB1 cannabinoid receptor fused to circular-permutated EGFP, providing cell membrane trafficking, second-resolution kinetics, high specificity for eCBs, and a robust fluorescence response at physiological eCB concentrations. Using the $GRAB_{eCB2.0}$ sensor, we monitored evoked changes in eCB dynamics in both cultured neurons and acute brain slices. Interestingly, in cultured neurons we also observed spontaneous compartmental eCB transients that spanned a distance of approximately 11 μm, suggesting constrained, localized eCB signaling. Moreover, by expressing $GRAB_{eCB2.0}$ in the mouse brain, we readily observed foot shock-elicited and running-triggered eCB transients in the basolateral amygdala and hippocampus, respectively. Lastly, we used $GRAB_{eCB2.0}$ in a mouse seizure model and observed a spreading wave of eCB release that followed a Ca^{2+} wave through the hippocampus. Thus, $GRAB_{eCB2.0}$ is a robust new probe for measuring the dynamics of eCB release under both physiological and pathological conditions.

Disclosures: **A. Dong:** None. **K. He:** None. **B. Dudok:** None. **J.S. Farrell:** None. **W. Guan:** None. **D.J. Liput:** None. **H.L. Puhl:** None. **R. Cai:** None. **J. Duan:** None. **E. Albarran:** None. **J. Ding:** None. **D.M. Lovinger:** None. **B. Li:** None. **I. Soltesz:** None. **Y. Li:** None.

Digital Abstract Session

P377. New Optical Sensors and Imaging Technologies

Program #/Poster #: P377.07

Topic: I.04. Physiological Methods

Support: NIH BRAIN Initiative grant U01NS103558
General Program of National Natural Science Foundation of China (project 31671118)

Title: A family of genetically-encoded GRAB sensors for detecting neuropeptides in vitro and in vivo

Authors: *H. WANG[1], T. QIAN[1], L. WU[1], Y. LI[1,3,4,2];
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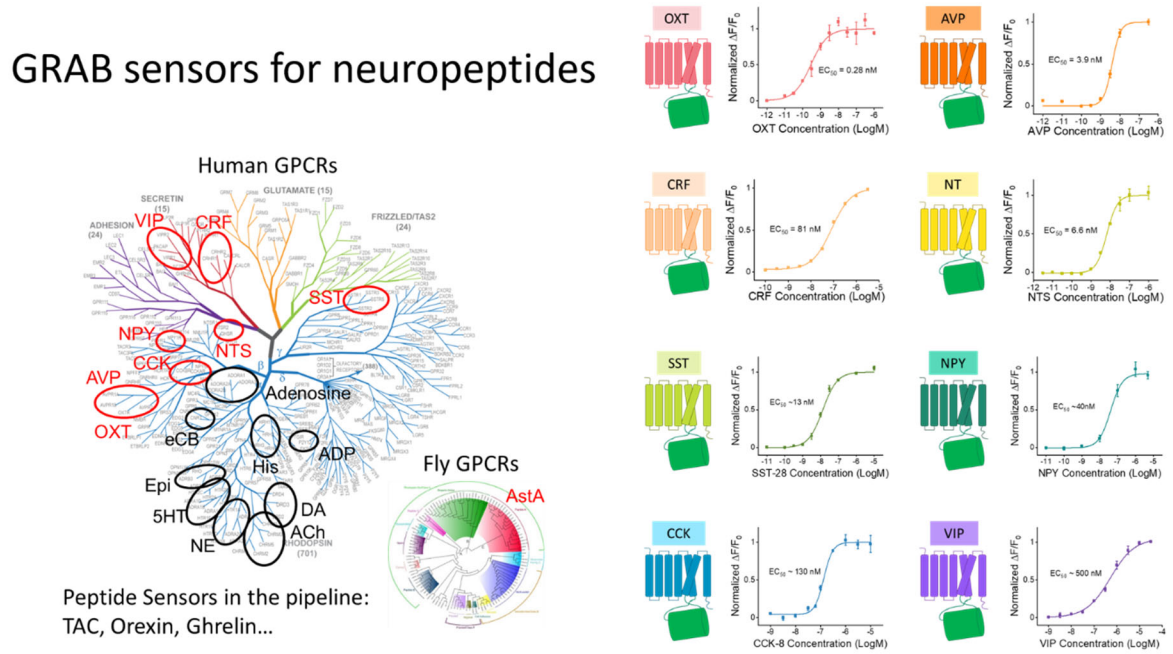
Abstract: Neuropeptides are key signaling molecules in endocrine and nervous system, regulating virtually all physiological processes, ranging from food ingestion, sleep & circadian rhythm, to pain sensation and social behaviors. To understand their important functions in vivo, it is critical to be able to monitor neuropeptide dynamics with selectivity, sensitivity and good spatiotemporal resolution. One major hindrance to the above goal is the lack of direct, sensitive and non-invasive probes to report these bioactive peptides *in vivo.* Here, by tapping into GPCRs

for human neuropeptides, we developed a toolbox of GRAB (GPCR activation based) sensors for neuropeptide Y (NPY), vasopressin (VP), oxytocin (OT), somatostatin (SST), corticotrophin releasing factor (CRF), neurotensin (NT), cholecystokinin (CCK), vasoactive intestinal peptide (VIP) and substance P. These fluorescent sensors utilize native human GPCR ligand binding pockets as neuropeptide sensing modules and circular-permutated GFPs as optical reporters. We have developed sensors with ultra-good selectivity for neuropeptides, with nano molecular affinity, up-to 1500% dF/F fluorescence increase upon cognate ligand application. In addition, these GRAB sensors have minimal downstream G protein coupling, or arrestin coupling, compared to their original GPCR scaffolds. OT sensor and CRF sensor are able to faithfully report electric stimuli induced cognate peptide release in acute brain slices. We are validating these peptide sensors in slice & in vivo and extend the sensor development strategy to cover more neuropeptides in human body. The further development of these sensors and their deployment will likely significantly enhance our understanding of neuropeptide function and regulation in physiological and pathophysiological conditions.



Disclosures: **H. Wang:** None. **T. Qian:** None. **L. Wu:** None. **Y. Li:** A. Employment/Salary (full or part-time):; State Key Laboratory of Membrane Biology, Peking University School of Life Sciences, Beijing 100871, China, PKU-IDG/McGovern Institute for Brain Research, Beijing 100871, China, Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China, Chinese Institute for Brain Research, Beijing 100871, China.

Digital Abstract Session

P377. New Optical Sensors and Imaging Technologies

Program #/Poster #: P377.08

Topic: I.04. Physiological Methods

Support: General Program of National Natural Science Foundation of China (project 31671118)
NIH BRAIN Initiative grant U01NS103558

Title: Development and applications of novel genetically-encoded histamine sensors

Authors: *M. LI[1,3], H. DONG[1,3], T. QIAN[1,3], Y. LI[1,3,2,4];
[1]State Key Lab. of Membrane Biology, Sch. of Life Sci., [2]Peking-Tsinghua Ctr. for Life Sciences, Acad. for Advanced Interdisciplinary Studies, Peking Univ., Beijing, China; [3]PKU-IDG/McGovern Inst. for Brain Res., Beijing, China; [4]Chinese Inst. for Brain Res., Beijing, China

Abstract: Histamine (HA) is an important biogenic monoamine, involved in many crucial physiological processes, including immune responses, sleep-awake regulation and cognitive function. In the central nervous system, HA is mainly released from histaminergic neurons in the tuberomammillary nucleus (TMN), acting on a number of brain regions to execute its function. Despite of undisputable function of histaminergic transmission, how HA release is regulated in the brain is poorly understood, in part due to lacking of sensitive methods to monitor the dynamics of HA non-invasively with good cell specificity and high spatial-temporal resolution, especially *in vivo*. Here, we developed genetically-encoded HA sensors by tapping into cpEGFP and histamine receptors (HRHs), which are G protein coupled receptors (GPCRs). We screened different HRHs and used structure-guided semi-rational screen to yield a set of genetically-encoded HA biosensors with different dynamic ranges. We named these sensors GPCR-Activation-Based sensors for HA, or $GRAB_{HA}$ for short. The first generation of $GRAB_{HA}$, which is based on human histamine H_4 receptor, exhibited specific HA-induced fluorescence response ($\Delta F/F_0$ ~400%) with high ($GRAB_{HA1h}$, EC_{50} ~20 nM) or medium ($GRAB_{HA1m}$, EC_{50} ~200nM) apparent affinity. The $GRAB_{HA}$ sensors were validated in HEK293T cells and cultured cortical neurons regarding the properties of fluorescence response, kinetics, affinities and molecular specificity. Fiber photometry recording in freely moving mice with virus-mediated $GRAB_{HA}$ expression revealed the endogenous HA release during sleep-wake cycles. Further continuous optimization led to the identification of second generation of HA sensors based on water bear histamine H_1 receptor, named $GRAB_{HA2m}$, with more than 2-fold improvement in fluorescence response and faster kinetics. These HA sensors would likely enhance our understanding of complex histaminergic neuromodulation *in vivo* in both physiological and pathological conditions.

Disclosures: **M. Li:** None. **H. Dong:** None. **T. Qian:** None. **Y. Li:** None.

Digital Abstract Session

P377. New Optical Sensors and Imaging Technologies

Program #/Poster #: P377.09

Topic: I.04. Physiological Methods

Support: The Beijing Municipal Science & Technology Commission Grant Z181100001318002
The Beijing Municipal Science & Technology Commission Grant Z181100001518004
NSFC Grant 92032000
National Key R&D Program of China Grant 2020YFE0204000
Grants from the Peking-Tsinghua Center for Life Sciences and the State Key Laboratory of Membrane Biology at Peking University School of Life Sciences

Title: A new genetically-encoded GRAB sensor for monitoring extracellular ATP dynamics in vitro and in vivo

Authors: *Z. WU[1,2], K. HE[1,2], M. JING[3], H. LI[4], K. SONG[4], S. PAN[1,2,5], W. PENG[4], T. LIU[4], B. LI[1], Y. CHEN[3], J. DU[4], M. XU[4], Y. LI[1,2,5];
[1]Sch. of Life Sciences, Peking Univ., Beijing, China; [2]PKU-IDG/McGovern Inst. for Brain Res., Beijing, China; [3]Chinese Inst. for Brain Res., Beijing, China; [4]Inst. of Neuroscience, State Key Lab. of Neuroscience, Ctr. for Excellence in Brain Sci. and Intelligence Technology, Shanghai Res. Ctr. for Brain Sci. and Brain-Inspired Intelligence, Chinese Acad. of Sci., Shanghai, China; [5]Peking-Tsinghua Ctr. for Life Sciences, Acad. for Advanced Interdisciplinary Studies, Peking Univ., Beijing, China

Abstract: The purinergic transmitter adenosine 5' triphosphate (ATP) plays a critical role in both central and peripheral nervous systems, and the ability to directly measure ATP dynamics is essential for understanding its physiological and pathological functions. Here, we developed a fluorescent GPCR-Activation-Based ATP ($GRAB_{ATP1.0}$) sensor. with ultrasensitive fluorescence response to extracellular ATP in multiple cell types ($\Delta F/F_0$ values of 500% to 1000%), and exhibits subsecond kinetics, nanomolar to micromolar affinity and enables subcellular detection of ATP. Using $GRAB_{ATP1.0}$ sensor, we monitored distinct patterns of ATP release, including the spontaneous ATP release, mechanical stimulation evoked ATP release and hypotonic stress evoked ATP release, in the primary hippocampal cultures. In zebrafish *in vivo*, we detected injury induced ATP release, and revealed its correlation with microglia migration after injury. In mice, we characterized a spatially selective ATP releasing event after systemic inflammation induced by lipopolysaccharide (LPS) using two-photon imaging, and we monitored ATP dynamics during sleep-wake cycles using fiber photometry. Together, we developed a new ATP sensor for monitoring extracellular ATP dynamics.

Disclosures: **Z. Wu:** A. Employment/Salary (full or part-time):; Z.W. is supported by the Boehringer Ingelheim-Peking University Postdoctoral Program.. **K. He:** None. **M. Jing:** None. **H. Li:** None. **K. Song:** None. **S. Pan:** None. **W. Peng:** None. **T. Liu:** None. **B. Li:** None. **Y. Chen:** None. **J. Du:** None. **M. Xu:** None. **Y. Li:** None.

Digital Abstract Session

P377. New Optical Sensors and Imaging Technologies

Program #/Poster #: P377.10

Topic: I.04. Physiological Methods

Title: Registration across in vivo neuronal imaging modalities: miniature microscopes to high resolution microscopes

Authors: *S. GULATI, K. ZITELLI, A. STAMATAKIS;
Inscopix, Palo Alto, CA

Abstract: Inscopix's miniscopes allow neuroscientists to record and manipulate large-scale calcium (Ca2+) dynamics in genetically defined populations of neurons at single-cell resolution in freely behaving animals over months. This is crucial to understanding and investigating the correlative and causal link between neural circuits and behavior. Adding the ability to register data from freely moving imaging experiments to data from high resolution confocal or multi-photon imaging will begin to provide crucial links between activity dynamics and anatomical, molecular, and/or connectivity profiles of distinct neuronal populations. To enable this goal, we have developed a Multimodal Image Registration and Analysis (MIRA) platform that fully integrates calcium imaging using miniaturized microscopes with high-resolution laser scanning microscopes. Here, we use the MIRA platform to record and register contralaterally-projecting medial prefrontal cortical (mPFC) neurons (labeled with tdTomato and imaged with a ZEISS Airyscan confocal), within the broader mPFC circuit during free behavior (imaged with an Inscopix nVista miniscope). To ensure we are recording from the same focal plane, we designed a confocal adapter that allows the experimenter to make the nVista system parfocal with the Airyscan. Freely-behaving Ca2+ imaging is first performed with the nVista platform, followed by head-fixed recordings with the Airyscan. After acquiring data from the two imaging modalities, we extract functional cell maps from each Ca2+ imaging data set using Inscopix Data Processing Software. To align the cell maps from both microscopes, we combine cell maps with structure images containing blood vessel patterns and shared landmarks. The different scale, rotation, and elastic deformations between the images are then corrected and aligned using the bUnwarpJ algorithm. After alignment, the same cells are registered from the different imaging modalities. The freely behaving calcium cell map is then overlaid with the static red cell map generated with the Airyscan, allowing for the identification of the subset of contralateral-projection mPFC neurons. Importantly, the registration method described here can be expanded to other high-resolution imaging modalities such as multiphoton microscopes.

Disclosures: **S. Gulati:** A. Employment/Salary (full or part-time):; Inscopix. **K. Zitelli:** A. Employment/Salary (full or part-time):; Inscopix. **A. Stamatakis:** A. Employment/Salary (full or part-time):; Inscopix.

Digital Abstract Session

P377. New Optical Sensors and Imaging Technologies

Program #/Poster #: P377.11

Topic: I.04. Physiological Methods

Support: NIH IDP20D017782-01
NSF NeuroNex Technology Hub 1707316
PECASE
NIH BRAIN RF1MH117069
Caltech BBE postdoctoral department fellowship
the Hebrew University postdoctoral fellowship for women, Israel
Life Sciences Research Foundation Good Ventures Fellowship

Title: 'Light-guided sectioning' for precise localization and 3D visualization of in situ optical implants

Authors: *A. KAHAN, A. GREENBAUM, M. J. JANG, J. E. ROBINSON, J. R. CHO, X. CHEN, P. KASSRAIAN, D. A. WAGENAAR, V. GRADINARU;
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Abstract: In the last two decades, there has been a significant increase in the use of implanted optical devices to control and monitor neuronal activity *in vivo*. However, *post hoc* examination of the implant location is commonly achieved via 2D histology, which often suffers from distortion and loss during tissue processing. To address this, we developed a 3D method that allows *post hoc* labeling and clearing of tissue that preserves the size of the tissue while leaving the optical implant unperturbed. By coupling the implant to a light-emitting diode, the implant itself can serve as a light guide for removal of redundant tissue (light-guided sectioning, LiGS). The residual tissue, including the tissue-implant interface, is then processed as a whole for further investigation. This enables the precise location of the optical implant to be determined as well as the identification of tissue changes at the implant site. LiGS can also be used for accurate cell registration of *ex vivo* histology with single-cell two-photon calcium images obtained through GRIN lenses. We anticipate that LiGS will provide valuable additional information in any experiment that uses optical implants and will increase reproducibility through well-defined fiber-to-target localization.

Disclosures: A. Kahan: None. **A. Greenbaum:** None. **M.J. Jang:** None. **J.E. Robinson:** None. **J.R. Cho:** None. **X. Chen:** None. **P. Kassraian:** None. **D.A. Wagenaar:** None. **V. Gradinaru:** None.

Digital Abstract Session

P377. New Optical Sensors and Imaging Technologies

Program #/Poster #: P377.12

Topic: I.04. Physiological Methods

Support: NSF-NCS Award # 1734887

Title: A Novel Pressure Regulating Brain Imaging Implant For Ultra-Large Field-of-View Microscopic Imaging in NHPs

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Abstract: Several problems challenge mesoscopic imaging in the brain: 1) Difficulty with positioning high-NA objectives near the brain; 2) Creating a flat imaging window against the surface of the brain; 3) Adjusting the imaging window in the face of changes in swelling and pressure in the brain; 4) Preventing growth of dura and biofilms that obscure the imaging window; 5) Follow-on MRI imaging of the animal post-implantation. We propose here an ultra-large window radiolucent implant to address these issues. Our approach provides a 2 cm diameter window for non-human primates (NHPs) that regulates pressure and employs a stable, strong, and thin design. The system is mechanically modeled and stress-tested to achieve access to the brain by large objectives, with design features that allow for manual repositioning of the imaging lens. To optimize the distance between the objective and the brain, we prioritize a thin implant design. A strong radiolucent implant is created using PEEK plastic, a strong, thermoresistant and biostable material. We heighten strength of the chamber's attachment to the skull by using titanium screws that are normal to the surface of the bone at each point. The implant design has several parts and contemplates a potential method to maintain pressure on the brain. This method uses an engineered silicone mount to maintain even pressure of the imaging window on the brain's surface, despite brain motion. The mechanical properties of the silicone are manipulated to closely resemble that of brain tissue to be more biomimetic and act as a cushion for motion. This method also allows for the manual repositioning of the cover slip to create a flat imaging window. Lastly, our approach prevents dural growth by blocking the migration of migratory biofilm-forming cells; we hypothesize that use of dynamic pressure maintenance on the brain is key to this method's success. We are also investigating methods to elongate the longevity of the implant and imaging site, such as silver sputtering on implants and blue light therapy. These methods have produced an ultra-large field of view with 2P image results in <60,000 neurons. As such the chambers are expected to enhance recording window longevity and may prove to be a critical advance in NHP and human brain imaging.

Disclosures:

Digital Abstract Session

P378. Applications of Optogenetic Modulation

Program #/Poster #: P378.01

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH OT2OD023852

Title: Optical vagus nerve modulation of heart and respiration via heart-injected retrograde AAV

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Abstract: Targeting specific subsets of peripheral pathways of the autonomic nervous system will enable new avenues to study organ control and develop new disease therapies. Vagus nerve stimulation (VNS) has shown many therapeutic benefits but current approaches involve imprecise electrical stimulation that gives rise to adverse effects, while the functionally relevant pathways are poorly understood. One method to overcome these limitations is the use of optogenetic techniques, which facilitate highly specific neural communication with light-sensitive actuators (opsins). Opsins can be targeted to cell populations of interest based on the location of viral delivery and genetic control of expression. Here, we tested whether retrograde adeno-associated virus (rAAV2-retro) injected in the heart can be used to selectively express opsins in vagus nerve fibers controlling cardiac function. Furthermore, we investigated whether perturbations in cardiac function could be achieved with photostimulation at the cervical vagus nerve. Viral injection in the heart resulted in robust, primarily afferent, opsin reporter expression in the vagus nerve, nodose ganglion, and brainstem. Photostimulation using both one-photon stimulation and spatially-selective stimulation using two-photon holography was tested on the pilot-cohort of injected mice, using a GRIN-lens incorporated nerve cuff. Changes in heart rate, surface electrocardiogram, and respiratory responses were observed in response to both one- and two-photon photostimulation. The results demonstrate feasibility of retrograde labeling for organ targeted optical neuromodulation.

Disclosures: **A. Fontaine:** None. **G. Futia:** None. **P. Rajendran:** None. **S. Littich:** None. **D. Restrepo:** None. **J. Caldwell:** None. **R. Weir:** None. **E. Gibson:** None. **N. Mizoguchi:** None. **J. Ardell:** None. **K. Shivkumar:** None.

Digital Abstract Session

P378. Applications of Optogenetic Modulation

Program #/Poster #: P378.02

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH/NINDS Grant R01-NS100908
NIH Grant T32-NS096050
NIH Grant OD P51-OD011132

Title: Optogenetic inhibition of the external globus pallidus in normal and parkinsonian monkeys

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Abstract: Based on extracellular recordings obtained in vivo, neurons in the external globus pallidus (GPe) of parkinsonian animals have lower average firing rates and increased bursting

compared to normal animals. However, recent work in rodents has demonstrated differences in the parkinsonian-related changes that occur in arkypallidal and prototypical GPe neurons. It is currently unknown whether these differences are also seen in non-human primates and how such changes in GPe firing rates impact activity in the putamen and subthalamic nucleus (STN).Here, we characterize the effects of inhibitory opsin Jaws in the GPe of rhesus monkeys. Two monkeys received chronic recording chambers aimed at the GPe. We injected AAV5-hSyn-Jaws-EYFP into the GPe (post-mortem histology showed that Jaws were expressed in neuronal terminals and remain stable for many months). To examine the effects of inhibition of GPe terminals in the putamen and STN, we obtained recordings of putamen and STN neurons during 500ms light pulses to activate the inhibitory opsins. Recordings were also performed in the GPe, to examine the efficacy of the opsins. Extracellular electrophysiologic recordings were conducted while the animals were awake, using tungsten electrodes coupled to 200um optic fibers and standard recording procedures, while the monkeys were sitting on a primate chair. In one of the monkeys, recordings were conducted before and after induction of stable parkinsonism with MPTP. For each cell, we generated peri-light-stimulus time histograms to analyze the proportion of light-responsive neurons. We analyzed firing rates for the 500ms before stimulus onset ('control') and for the 500ms of stimulus duration ('light-stimulated'). For each structure we used paired t-tests to compare differences in firing rates between control and light-stimulated periods (for normal and parkinsonian conditions separately).In approximately half of the GPe neurons, changes in firing rate were seen in both normal and MPTP-treated monkeys. The change in firing rates between control and light-stimulated conditions was comparable in normal and parkinsonian conditions. No effects were seen in putamen neurons in normal or parkinsonian conditions and minimal effects were seen in STN neurons in both conditions. Although our results suggest that optogenetic silencing of GPe terminals in STN and putamen has little effect on the firing rates of these structures, other parameters of firing could be modulated by the pallidal inputs.

Disclosures: **K.S. Heffernan:** None. **X. Hu:** None. **T. Wichmann:** None. **A. Galvan:** None.

Digital Abstract Session

P378. Applications of Optogenetic Modulation

Program #/Poster #: P378.03

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 5T32LM012419-05 (BDGN)
DARPA Grant W911NF-14-2-0043
NSF Grant EEC-1028725 (Center for Neurotechnology)
NICHHD-NIH Grant K12HD073945
AHA Post-Doctoral Fellowship

Title: Stimulation-induced functional connectivity change in brain networks is mediated by the existing structure: An optogenetic study in non-human primate cortex

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Abstract: Aberrant functional connectivity underlies many neural disorders from schizophrenia to epilepsy. Neural stimulation has been proposed as a means to rewire brains towards healthier connectivity. Research investigating the stimulation-induced functional connectivity change (SIFCC) has primarily analyzed how connectivity between stimulation sites changes as a function of stimulation delay. Here, we challenge this approach by revealing that the cortical network structure is the primary controlling feature of SIFCC, and that changes it underlies are not localized to the stimulation sites but spread over the surrounding cortical network.
We obtain network-level recordings from a large-scale (1cm^2) array of 96 micro-electrocorticography (μECoG) electrodes in the non-human primate (primary sensorimotor cortex in two subjects. We stimulate the cortex using optogenetics in a paired pulse protocol and quantify the functional connectivity over the network before, during, and after stimulation. We then develop a nonparametric statistical regression model to assay the contribution of a wide set of stimulation and network characteristics for predicting SIFCC. We parameterize the stimulation protocol with features such as delay, anatomical locations of electrodes, and relevant distances between electrodes and stimulation sites. Similarly, we parameterize cortical network structure by extracting from neural recordings a diverse set of graph metrics such as degrees of direct and indirect connectivity between electrodes, average connectivity strength to the network, and covariances of connection patterns.
By testing the prediction accuracy of models fit on subsets of these features we elucidate their importance in controlling the network SIFCC. First, we observe that while stimulation protocol features are statistically significant predictors, they have poor explanatory power (R2~0.05). We then augment the model with the network features and find that the network structure is much more important than the stimulation protocol in determining SIFCC (R2~0.50). Finally, we repeat these analyses with a time-varying model and find that the input-output relationships linking protocol and network features to SIFCC temporally vary as the cortex is being stimulated.
In this work we investigate for the first time how neural stimulation interacts with pre-existing cortical networks to drive network SIFCC. We demonstrate that the structure of the network is the primary controlling factor of SIFCC. This observation opens the door to more powerful and accurate stimulation interventions for the treatment of neural disorders.

Disclosures: J. Bloch: None. **A. Greaves-Tunnell:** None. **E. Shea-Brown:** None. **Z. Harchaoui:** None. **A. Shojaie:** None. **A. Yazdan-Shahmorad:** None.

Digital Abstract Session

P378. Applications of Optogenetic Modulation

Program #/Poster #: P378.04

Topic: I.08. Methods to Modulate Neural Activity

Support: CIHR GRNT: PJT148521

Title: A deep learning platform to quantify in-vivo cerebrovasculature changes in response to neuronal optogenetic activation

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Abstract: Introduction: Neurovascular coupling is frequently compromised in neurodegenerative conditions and contributes to their progression. Two-photon fluorescence microscopy (2PFM) coupled with optogenetic activation allows for controlled neuronal activation and direct vascular observation (Shih et al., 2012). We here present a deep learning platform for high-throughput quantification of neurovascular coupling in mice based on 2PFM data. **Methods:**8 Thy1-ChR2-YFP mice, injected with TexasRed, were imaged on a FVMPE-RS system. Image stacks covered 509x509μm lateral FOV at 0.99 μm resolution from the surface to 500 μm below, at 2.64 μm resolution. Stacks were acquired in pairs, before and after stimulation (458 nm wavelength at 20% power, 250 μm diameter cylinder in center of FOV) for quantification of vascular changes. We trained a 3D Unet CNN (Goubran et al. 2019) on these data. 5 image stacks were used for training and 3 withheld for validation. The model achieved an F1 score of **0.83 (±0.01)**. Segmentation masks were filled and skeletonized using an iterative thinning method. Vessel diameters were mapped onto skeletons via the distance transform of the segmentation mask. Graphical representations of the skeletons were aligned before and after stimulation. Images segmented for analysis were not involved in updating the machine learning model. **Results and Discussion:**Six regions of interest in 12 image stacks from 3 mice whose data were not used for training of the segmentation model were used for analysis **(Fig.1, A):** 1632 vessel segments across the 3 mice were analyzed. The CNN's output aligned well qualitatively to the raw data on these 3 test mice **(Fig. 1, B)**. Photostimulation-induced vessel caliber changes were observed in **(Fig. 1, C, D)**. Viscous resistance () for each vascular segment was calculated at both timepoints. The network showed an increase in vascular volume **(Fig.1, E, F)** and thus an average decrease in vessel resistivity **(Fig.1,G,H,I)**, with 98% of the net volume increase attributed to capillaries, and 53% of the overall volumetric changes.
Figure 1:

Disclosures: M. Rozak: None. **J. Mester:** None. **A. Dorr:** None. **M. Biparva:** None. **P. Mojiri:** None. **M. Goubran:** None. **B. Stefanovic:** None.

Digital Abstract Session

P379. Machine Learning For Automated Patch Clamp Recordings

Program #/Poster #: P379.01

Topic: I.04. Physiological Methods

Support: NIH Grant 1-U01-MH106027-01
NIH Grant R01NS102727
NIH Grant R01EY023173
NIH Grant R01DA029639
NSF Grant CISE 1110947
NSF Grant EHR 0965945

Title: Deep learning-based real-time detection of neurons in brain slices for in vitro physiology

Authors: *M. C. YIP[1], M. M. GONZALEZ[1], C. R. VALENTA[2], M. J. M. ROWAN[3], C. R. FOREST[1];
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Abstract: A common electrophysiology technique used in neuroscience is patch clamp: a method in which a glass pipette electrode facilitates single cell electrical recordings from neurons. Typically, patch clamp is done manually in which an electrophysiologist views a brain slice under a microscope, visually selects a neuron to patch, and moves the pipette into close proximity to the cell to break through and seal its membrane. While recent advances in the field of patch clamping have enabled partial automation, the task of detecting a healthy neuronal soma in acute brain tissue slices is still a challenging step that is commonly done manually. To overcome this obstacle and progress towards full automation of patch clamp, we combined the differential interference microscopy optical technique with an object detection-based convolutional neural network (CNN) to detect healthy neurons in acute slice. Utilizing the YOLOv3 convolutional neural network architecture, we achieved a 98% reduction in training times to 18 minutes, trained on an annotated data set of 1138 neurons. We also compared networks trained on unaltered and enhanced images, achieving up to 77% and 72% mean average precision, respectively. This novel, deep learning-based method accomplishes automated neuronal detection in brain slice at 18 frames per second with a small data set, rapid training time, and high precision. The addition of this technology during live-cell imaging for patch clamp experiments can not only improve manual patch clamping by reducing the neuroscience expertise required to select healthy cells, but also help achieve full automation of patch clamping by nominating cells without human assistance.

Disclosures: **M.C. Yip:** None. **M.M. Gonzalez:** None. **C.R. Valenta:** None. **M.J.M. Rowan:** None. **C.R. Forest:** None.

Digital Abstract Session

P379. Machine Learning For Automated Patch Clamp Recordings

Program #/Poster #: P379.02

Topic: I.04. Physiological Methods

Support: NEI and NIMH 1-U01-MH106027-01
NIH R01NS102727
NIH Single Cell Grant 1 R01 EY023173
NSF (EHR 0965945 and CISE 1110947)
NIH R01DA029639

Title: Machine learning-based pipette correction for automatic patch clamp in vitro

Authors: *M. M. GONZALEZ[1], M. C. YIP[1], C. F. LEWALLEN[1], M. J. ROWAN[2], C. R. FOREST[1];
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Abstract: Patch clamp electrophysiology is a common technique used in neuroscience to understand individual neuron behavior, allowing one to record current and voltage changes with submicron and sub-millisecond spatiotemporal resolution. While patch clamp experiments produce high fidelity electrophysiology data, the technique is onerous and labour-intensive. Despite the emergence of automated patch clamp systems, the crucial step of identifying the pipette location and carefully placing it within 1-2 micrometers of a cell membrane remains a bottleneck for the success rate of whole-cell experiments. Specifically, when patching in acute brain slices, the inherent light scattering from brain tissue makes automatically identifying the pipette tip a non-trivial problem that is not easily solved with traditional image processing methods. Here we used a convolutional neural network (ResNet101) to identify and correct the pipette position during automated patch clamp experiments. This deep-learning based pipette detection method improved the cell detection success rate and whole-cell success rates by 71% and 59% respectively compared to the state-of-the-art cross correlation method, as well as reduced the average time for pipette correction by 81.2%. This improvement has to potential to ultimately enable real-time correction of pipette position during patch clamp experiments with similar accuracy and quality of recording to manual patch clamp.

Disclosures: M.M. Gonzalez: None. **M.C. Yip:** None. **C.F. Lewallen:** None. **M.J. Rowan:** None. **C.R. Forest:** None.

Digital Abstract Session

P380. New Methods For Electrophysiological Recording and Analysis

Program #/Poster #: P380.01

Topic: I.04. Physiological Methods

Support: This work is part of BrainLinks-BrainTools which is funded by the Federal Ministry of Economics, Science and Arts of Baden-Württemberg within the sustainability programm for projects of the excellence initiative II.

Title: Correlations of electrode parameters and recording performance in chronic epicortical implants

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Abstract: Electrocorticography (ECoG) has particularly gained strength in the recent years as ultra-conformable grids featuring neuron-sized electrodes have allowed to extend the frequency band from local field potentials (LFPs) to spike-like activity. Diverse methods have been proposed to effectively improve the performance of microelectrodes, one of them being the use of conductive polymers such as PEDOT to reduce the electrochemical impedance between electrode and tissue. Nonetheless, the relation between electrode impedance and signal quality still remains a topic of debate. To address the issue, we have collected *in vivo* electrode impedance and ECoG recordings from eight rats implanted with micromachined, ultra-conformable grids at implantation day and after 3, 6 and 12 weeks and have assigned electrochemical features and recording features to each single reading. The eight implants - one per rat - had electrodes with 10 µm and 100 µm in diameter and platinum, iridium oxide and PEDOT/PSS as electrode coatings. Spearman correlation coefficients were computed across all features and have shown that the root mean square (RMS) of the recorded signal in the high frequency band (above 300 Hz) strongly correlates with the thermal noise (ρ= 0.73) and anti-correlates with the interface capacitance (ρ=-0.66). Electrode material highly influenced the correlations between the performance parameters. Platinum had the strongest coefficient correlations between thermal noise and high band RMS, followed by iridium oxide. PEDOT had the weakest coefficients. Interestingly, spike counts and peak-mean ratio (PMR) from peri-event histograms showed no significant correlation to electrochemical features, suggesting that, once provided a reliable tissue-electrode contact via ultra-conformability, impedance does not explain high frequency performance. Looking at implantation day only, thermal noise and high band RMS remained highly correlated but, differently from the chronic scenario, both PMR and spike counts showed an anti-correlation to the high band RMS, indicating a larger contribution of thermal noise in the acute scenario. Scarring reactions reduce desired signals, as well as non-desired biological artefacts. Therefore, the signal-to noise ratio might stay constant along the weeks. Thermal noise, which is proportional to the real part of the *in vivo* impedance, seems not to be the main part of signal deterioration at higher frequencies. Reliable material-tissue interfaces matter more.

Disclosures: M.F. Porto Cruz: None. **I.G. Vasilas:** None. **E. Zucchini:** None. **L. Fadiga:** None. **T. Stieglitz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CorTec, Freiburg, Germany, neuroloop, Freiburg, Germany. F. Consulting Fees (e.g., advisory boards); CorTec, Freiburg, Germany, neuroloop, Freiburg, Germany.

Digital Abstract Session

P380. New Methods For Electrophysiological Recording and Analysis

Program #/Poster #: P380.02

Topic: I.04. Physiological Methods

Support: NIH Grant 1RF1NS113303

Title: Monolithically integrated microfabricated stainless steel neural probe platform for high-resolution recording in non-human primates

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Abstract: Non-human primate (NHP) brain is a very helpful model system for translational studies. Electrophysiology recording in non-human primates with high spatio-temporal resolution can facilitate our understanding of the neural basis of brain function and dysfunction in humans. Despite significant recent advancements in developing tools for high resolution neural recording in rodents, progress in developing scalable, high-density, and robust neural interfaces for recording from the larger NHP brain has been comparatively limited. Silicon-based rodent neural probe technologies benefit from the well-established nanofabrication technologies. The large NHP brain requires probes with extremely large aspect ratios, i.e., several centimetres long with small cross-sections to minimize tissue damage. However, the brittleness and fragility of silicon makes longer silicon neural probes vulnerable to breakage in large NHP brains. Stainless steel, on the other hand, is bio-compatible, highly fracture-resistant and has high modulus of elasticity making it a promising material platform for NHP probes. However, microfabrication and micromachining technology for stainless steel is challenging and fairly underdeveloped. As a result, commercially-available stainless-steel probes are mostly manufactured and assembled using expensive manual methods and have low channel-densities and are very expensive. To address these challenges, we have designed a neural probe based on a combination of stainless steel and Parylene C that can be implemented using a scalable microfabrication process . We have demonstrated 8 cm long neural probes with upto 102 channels densely distributed channels over a 300 μm wide shank. The rigid stainless steel shank enables reliable insertion and the flexible polymer cable reduces tethering forces from skull fixtures. We will discuss the design and implementation of these hybrid rigid-flexible neural probes as well as their in vitro characterization. This hybrid Parylene-stainless steel device platform enables the design of robust implantable brain interfaces for large animals with the potential for clinical translation.

Disclosures: Z. Ahmed: None. **J. Reddy:** None. **I. Kimukin:** None. **T. Teichert:** None. **M. Chamanzar:** None.

Digital Abstract Session

P380. New Methods For Electrophysiological Recording and Analysis

Program #/Poster #: P380.03

Topic: D.07. Vision

Support: NIH/NINDS K99/R00NS092972
Moorman-Simon Interdisciplinary Career Development Professorship from Boston University
Whitehall Foundation
Young Investigator Award from the Brain and Behavior Research Foundation
NIH/NIMH K99/R00MH101234
NIH/NIMH R01MH116008
NIH/NINDS Transformative Research Award R01NS076460

Title: Revealing cell types in vivo via dimensionality reduction and graph clustering of spike waveforms

Authors: *E. K. LEE[1], H. BALASUBRAMANIAN[5], A. TSOLIAS[6], S. ANAKWE[2], M. MEDALLA[6], K. V. SHENOY[8,9], C. CHANDRASEKARAN[3,7,4];
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Abstract: Anatomical, physiological, and transcriptomic studies suggest a diverse range of neuronal cell types. However, current *in vivo* methods—such as clustering on waveform features—only differentiate between broad- (BS) and narrow-spiking (NS) neurons. This gap between 'known' and 'observable' *in vivo* diversity limits our understanding of how cell types shape behavior. Here, we developed a new method (WaveMAP) that combines non-linear dimensionality reduction (UMAP) with graph clustering on spike waveforms and show that it better reveals candidate cell classes *in vivo*. We applied WaveMAP to extracellular waveforms recorded with U-probes from macaque dorsal premotor cortex (PMd) during a decision-making task. WaveMAP revealed that BS and NS classes are comprised of at least three and five sub-classes of neurons. These sub-classes had distinct physiological, functional, and laminar distribution properties. First, the BS neuron sub-classes had low firing rates (FR), late choice-selectivity, and broad laminar distributions concentrated in middle layers—hallmarks of excitatory pyramidal cells. Second, two sub-classes of NS neurons had high FR, early choice-selectivity, and strong decision-related responses. The laminar distribution of these neurons was also consistent with our layer-specific histological counts of calbindin-/calretinin- and parvalbumin-positive inhibitory interneuron densities respectively. Third, an NS sub-class had identical FR and functional properties as candidate excitatory neurons—consistent with findings that PMd excitatory neurons possess biophysical machinery capable of producing narrow spikes.

Fourth, another NS sub-class had FR, functional properties, and triphasic waveforms consistent with excitatory axons in deep layers. Fifth, WaveMAP sub-classes explained heterogeneity in decision-related properties of PMd neurons over and above our previously reported laminar differences. Notably, candidate cell types were not identifiable with traditional Gaussian mixture models that operate on derived features of the waveform (e.g., trough-to-peak and waveform width). WaveMAP produced more candidate cellular sub-classes and these sub-classes were also better separable. In summary, WaveMAP provides previously unavailable access to candidate cell types *in vivo*. Combining opto-tagging with WaveMAP will provide further insight into whether these candidate cell types are consistent with populations of excitatory and inhibitory neurons. WaveMAP will therefore enable more precise investigation of microcircuit dynamics during behavior in species where genetic access to cell types is difficult.

Disclosures: E.K. Lee: None. **H. Balasubramanian:** None. **A. Tsolias:** None. **S. Anakwe:** None. **M. Medalla:** None. **K.V. Shenoy:** F. Consulting Fees (e.g., advisory boards); Neurlink Corp., CTRL-Labs Inc., MIND-X Inc., Inscopix Inc., Heal Inc.. **C. Chandrasekaran:** None.

Digital Abstract Session

P380. New Methods For Electrophysiological Recording and Analysis

Program #/Poster #: P380.04

Topic: I.04. Physiological Methods

Support: DSF Charitable Foundation

Title: A Multiplexed Active Digital Implantable Neural Probe

Authors: *X. HE[1], S. LIU[2], S. KARGARRAZI[1], V. CHEN[1], M. CHAMANZAR[1], L. PILEGGI[1];
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Abstract: Implantable neural probes have been extensively used as an important tool in neuroscience research. Implantable probes can record single unit action potentials and local field potentials from brain tissue with amplitudes ranging from tens of micro-volts to hundreds of micro-volts. Recently, silicon-based neural probes have been designed based on complementary metal-oxide-semiconductor (CMOS) technology. With the feature sizes that are possible with existing CMOS technology, neural probes can be designed to incorporating active elements such as low noise amplifiers and multiplexers within the area of a single electrode site on the probe. Neural signals can then be amplified and multiplexed at hundreds of sites along the probe shank. The traces along the probe shank are shared to transmit the recorded neural signals. Although this approach heavily expands the total number of recordable channels, the number of simultaneous recording channels is still limited. Therefore, only a sub-group of channels can be recorded simultaneously. In this work, we designed and fabricated a novel active digital neural probe in which the analog neural signals in the spikes band (i.e., 300 Hz - 10 kHz) are detected at the recording sites and then digitized and multiplexed over a common transmission line. The

probe integrates analog-front-end (AFE) circuitry under each recording electrode for instant neural signal quantization. Only one signal trace is needed to transmit the aggregate digitized neural data. Our prototype design features 113 recording electrodes over a 2.2 mm long shank that can all be recorded simultaneously. Each recording channel takes an area of 75 μm × 68.4 μm on the probe shank and each electrode is 15 × 15 μm^2. Each channel can be individually selected to be in the ON or OFF state, as needed. Measurements show that the recording system achieves an input referred noise of 12.43 μV_{rms} in the spikes frequency band, with the dynamic range of ±750 μV. The power consumption is measured to be 12.76 μW/channel. The number, density and the arrangement of the recording sites can be scaled and customized in our design. The design and in vitro characterization of these neural probes are discussed.

Disclosures: X. He: None. **S. Liu:** None. **S. Kargarrazi:** None. **V. Chen:** None. **M. Chamanzar:** None. **L. Pileggi:** None.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.01

Topic: I.04. Physiological Methods

Title: Axontracking assay: automatic identification and functional characterization of axons by high-density microelectrode array technology

Authors: B. CLEMENT, ***M. FISCELLA**, N. BIYANI, E. GRONSKAYA, D. JÄCKEL; MaxWell Biosystems, Zurich, Switzerland

Abstract: Axons are the output projections of neurons that conduct action potentials towards other neurons in a network[1]. Axonal dysfunction plays a central role in neuronal pathophysiologies such as Parkinson's Disease[2] and Amyotrophic Lateral Sclerosis[3]. Therefore, access to axonal physiology is crucial to study information processing in neuronal networks and to discover cures for human neurological disorders.High-density microelectrode array (HD-MEA) technology[4,5] enables in-vitro recordings of extracellular axonal action potentials in primary rodent neurons[6] and human iPSC-derived neurons[7], long-term and label-free. In this work, we present the MaxLab Live[8] AxonTracking Assay, a software package for automatic detection and functional characterization of subcellular axonal physiological signals, across hundreds of neurons within a neuronal network.
MaxLab Live AxonTracking Assay features toolsets to record and analyze axonal signals, identify individual cells and to compute and export physiological neuronal metrics. In a first step, tens to hundreds of neurons are simultaneously recorded while the HD-MEA sensor area is scanned for signals of axonal outgrowth. Next, the detected spikes of the recorded neurons are sorted by clustering signal features from close-by electrodes spanning the action potential initiation site. The spike sorting results are used to reconstruct the spike-triggered average extracellular waveform over the entire array area for every identified cell. An unsupervised object tracking algorithm is then applied to detect the traveled path of action potential signal

propagation, thus identifying individual axonal branches and the morphology of neuronal outgrowth per cell. Finally, identified signal paths are used to compute branch-level and single-cell assay metrics, such as axonal conduction velocity and total axon length.
In conclusion, the presented MaxLab Live AxonTracking Assay combined with HD-MEA technology enables to access novel electrophysiological parameters of neurons, which can be used as metrics for new functional phenotype studies and drug screenings.
References: *(1)*.Rama, S. et al. Curr. Opin. Neurobiol. 51, 37-44 (2018). *(2)*.Chu, Y. et al. Brain 135, 2058-2073 (2012). *(3)*.Moloney, E. B. et al. Front. Neurosci. 8, (2014). *(4)*.Müller, J. et al. Lab. Chip 15, 2767-2780 (2015). *(5)*.Yuan, X. et al. Nat. Commun. 11, 4854 (2020). *(6)*.Bakkum, D. J. et al. Nat. Commun. 4, 2181 (2013). *(7)*.Ronchi, S. et al. bioRxiv 2020.09.02.271403 (2020) doi:10.1101/2020.09.02.271403. *(8)*.https://www.mxwbio.com/products/maxone-mea-system-microelectrode-array/maxlab-live-software/.

Disclosures: **B. Clement:** A. Employment/Salary (full or part-time):; MaxWell Biosystems. **M. Fiscella:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MaxWell Biosystems. **N. Biyani:** A. Employment/Salary (full or part-time):; MaxWell Biosystems. **E. Gronskaya:** A. Employment/Salary (full or part-time):; MaxWell Biosystems. **D. Jäckel:** A. Employment/Salary (full or part-time):; MaxWell Biosystems.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.02

Topic: I.04. Physiological Methods

Support: This work is part of BrainLinks-BrainTools which is funded by the Federal Ministry of Economics, Science and Arts of Baden-Württemberg within the sustainability programm for projects of the excellence initiative II.

Title: Recording performance of low-impedance coatings for neural probes: an in vitro and in vivo comparison

Authors: *C. BOEHLER[1], R. LILJEMALM[2], C. LEWIS[3], T. STIEGLITZ[2], M. ASPLUND[1]; [1]Dept. of Microsystems Engin. - Bioelectronic Microtechnology, [2]Dept. of Microsystems Engin. - Biomed. Microtechnology, Albert-Ludwigs-University, Freiburg, Germany; [3]Brain Res. Inst., Univ. of Zurich, Zurich, Switzerland

Abstract: Implantable microprobes enable researchers and clinicians to tap into the signalling pathways of the nervous system to diagnose and treat diseases, understand basic functions, or offer neuroprosthetic rehabilitation. In order to provide high quality recordings, a variety of electrode materials has been developed, focussing in particular on low-impedance coatings to improve the performance of small electrode sites. Whether the lowest possible impedance, as

determined *in vitro*, also translates to the best possible recording performance *in vivo*, has to date not been clarified. We here address this question by implementing four electrode materials on the same flexible shank, comparing *in vitro* characterization with chronic performance measures *in vivo* (16 weeks). Flexible probes (polyimide 10µm thick, electrodes 12µm diameter/ pitch 45µm) were used as test platform to evaluate the recording performance of different low-impedance materials. Iridium Oxide (IrOx), nanostructured platinum (nanoPt) and PEDOT were deposited in an alternating pattern on the probes, allowing direct comparison among all materials including smooth Pt (Fig. 1). The electrochemical properties were determined before the coated probes were implanted into mouse cortex for recording evaluation. PEDOT electrodes had the overall lowest impedance followed by IrOx and nanoPt (Fig. 1), all orders of magnitude lower than bare Pt. For single unit activity (SUA), yield and amplitude were found to be substantially higher for the low-impedance materials indicating a direct correlation between recording quality and electrode impedance. Low-impedance coatings moreover greatly reduced movement related artefacts in local field potential (LFP) recordings, contributing to improved robustness in experiments with awake and moving animals. Last, but not least, low impedance materials allow for electrode area to be reduced so that few electrodes can be replaced with many, offering better opportunities for spike sorting and correlative analysis and improving prospects for clinical use of precision bioelectronics.

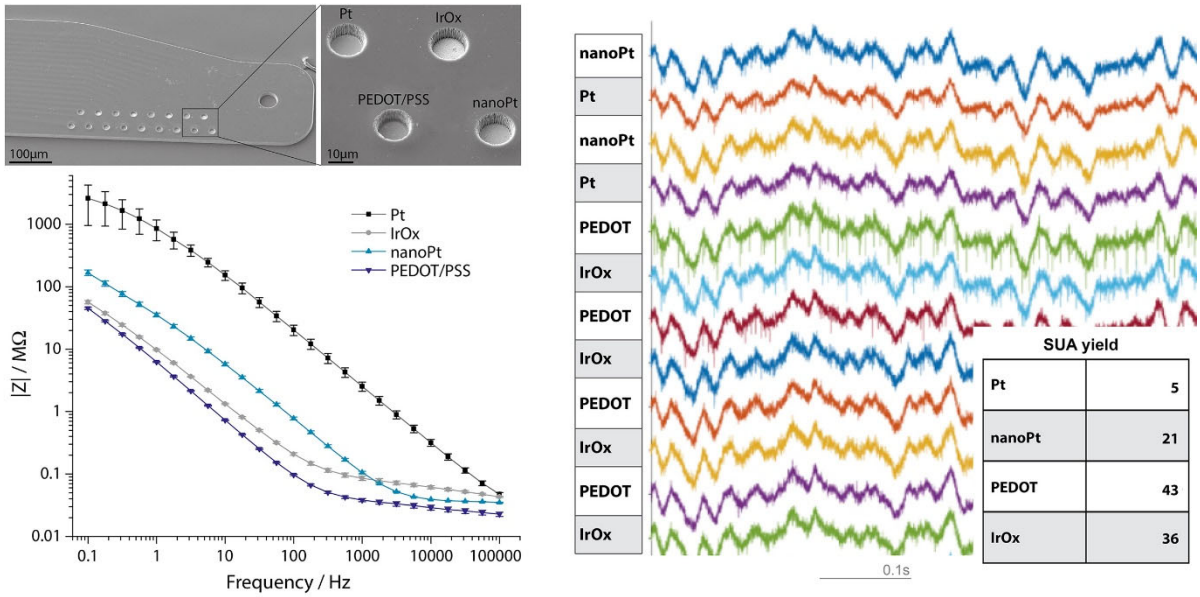

Figure 1: SEM image of a flexible polyimide thin-film probe providing a dense array of electrode sites for performance evaluation of different low-impedance materials on the same probe (top left). Impedance spectroscopy data (measured *in vitro*) shows a significant difference among individual electrode materials (bottom left). Representative *in vivo* data verifying high recording quality when using low impedance materials for SUA and LFP recordings (right).

Disclosures: **C. Boehler:** None. **R. Liljemalm:** None. **C. Lewis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ernst Strüngmann Institute, Frankfurt Germany / Blackrock Microsystems, Salt Lake City, USA. **T. Stieglitz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cortec, Germany, neuroloop, Germany. F. Consulting Fees (e.g., advisory boards); Cortec, Germany, neuroloop, Germany. **M. Asplund:** None.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.03

Topic: I.04. Physiological Methods

Support: RGPIN 2020-0675

Title: Exploring the feasibility of simultaneous cortical and muscle mapping using TMS and high-density EMG: A case study in the context of muscle fatigue.

Authors: *S. L. TOEPP[1], A. J. NELSON[2];
[2]Kinesiology, [1]McMaster Univ., Hamilton, ON, Canada

Abstract: Mapping the excitability of motor cortex muscle representations using transcranial magnetic stimulation (TMS) and surface electromyography (EMG) has been used to study motor function in clinical and healthy human populations since the early 1990s. Recently, the development of a rapid TMS mapping technique, enabling the assessment of a cortical map in as little as two minutes, has greatly improved the time resolution that is attainable in TMS mapping studies. This may be particularly useful in studies where tonic or phasic muscle contraction introduces myoelectric manifestations of muscle fatigue to the surface EMG signal that is used to obtain TMS maps. The purpose of the present study is to determine whether it is feasible to simultaneously assess TMS map characteristics and EMG features during a prolonged isometric fatiguing task using high-density surface EMG (HDsEMG). The participant was a right-handed female volunteer (age = 20 yrs). At three discrete timepoints during a 23-minute isometric biceps brachii contraction at 5% of maximum voluntary EMG level, fast TMS maps and muscle HDsEMG maps were simultaneously obtained. Each map set was obtained over a 3-minute period at 0, 10 and 20 minutes. Cortical maps were assessed by delivering 100 TMS stimuli at random locations within a 6x6cm region of the scalp over the primary motor cortex at a rate of 0.5 Hz. The muscle potential evoked by each TMS stimulus was recorded by a 64-channel HDsEMG patch placed over the muscle belly. Relative to 0 minutes, the area of the cortical excitability map increased by 591mm^2 and 511mm^2 at 10 minutes and 20 minutes, respectively. The TMS map center of gravity also moved laterally at 10 minutes (1.5 mm) and 20 minutes (3.2 mm), while shifting less than 1 mm in the anterior-posterior direction (10 min: anterior 0.8 mm, 20min: posterior <0.1 mm). The spatial distribution of background muscle EMG activity was also assessed. The distribution of the mean rectified signal across the HDsEMG grid during the 250 - millisecond interval prior to each TMS pulse was averaged across the 100 map trials. The center of the EMG activity distribution along the medial-lateral and proximal-distal axes of the HDsEMG patch was located by taking the weighted average of the activities from each of the grid coordinates. The center of the distribution shifted medially (10 min: 0.1mm, 20min: 0.4 mm) and distally (20min: 0.2 mm) relative to 0 minutes. This case study highlights the feasibility and potential utility of combined HDsEMG and rapid TMS mapping for studies involving muscle fatigue. Future studies with sufficient sample sizes will be necessary to interpret the observations made in this case study.

Disclosures: **S.L. Toepp:** None. **A.J. Nelson:** None.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.04

Topic: I.04. Physiological Methods

Support: NSF Grant CBET-1343193

Title: Large-scale recordings from the rat hippocampus using a 3D Parylene-based multi-electrode array

Authors: ***W. JIANG**, X. WANG, H. XU, E. MENG, D. SONG;
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Abstract: Large-scale monitoring of resolvable neuronal activities from multiple hippocampal sub-regions of behaving animals provides valuable insights into neurobiological mechanisms underlying learning and memory. However, current state-of-the-art probes such as microwire and silicon devices are much stiffer than brain tissue, which largely increases the chance of inflammation. Parylene C is a promising alternative structural and insulation material for building neural interfaces with reduced mechanical mismatch between tissue and implants. Furthermore, it can achieve high density of recording contacts as silicon devices. With high spatial and temporal resolution, Parylene-based multi-electrode array advances the possibilities for studying the anatomically distributed sub-circuits in the hippocampus. In our previous work, a 64-channel Parylene-based multi-electrode array with 8 shanks was developed and applied to chronic recording of the hippocampus in behaving rats. In our current work, a novel 3D Parylene-based probe consisting of 16 (2x8) shanks with a 250um spacing was developed. Each shank contains 8 platinum electrodes and thereby has a total of 128 electrodes. To aid insertion, dissolvable polyethylene glycol (PEG) brace was employed to temporarily support the flexible probe. One 3D Parylene probe was successfully implanted at 2.6mm posterior to the bregma and 2.4mm lateral to the midline at a depth of 4.4mm, and it was angled ~30 degrees from the midline to match the septal-temporal axis of the hippocampus. On the day of implantation, 12 out of 16 shanks from 2 arrays recorded single and complex neuronal signals from hippocampus. 59 units were captured from hippocampus through 4 electrode clusters arranged on the probe. 6 shanks recorded 8 units from CA1 while 9 shanks recorded 51 units from CA3. The histology showed that the shanks were straightly inserted in the first 2.5mm. They began to deviate from the midline of the probe when they were further advanced, which was due to the different dissolving rates on the surface of the PEG block. Severe bleeding around the implantation site was shown in the brain slices. It suggested the shanks might hit blood vessels during the insertion. This could explain why the captured units were less around the CA1 region. This preliminary result demonstrated the feasibility of implanting 3D flexible polymer probe to deep brain structures for large-scale recordings. In the next step, different insertion methods will be investigated to ensure straight insertion of the probes. The large-scale 3D Parylene probe will be

tested both acutely and chronically for recording from multiple hippocampal regions in behaving rats.

Disclosures: **W. Jiang:** None. **X. Wang:** None. **H. Xu:** None. **E. Meng:** None. **D. Song:** None.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.05

Topic: I.04. Physiological Methods

Title: Mea measurement in cerebral organoid

Authors: R. YOKOI, Y. ISHIBASHI, N. MATSUDA, ***I. SUZUKI**;
Tohoku Inst. of Technol., Sendai, Japan

Abstract: We have been developing a method for predicting seizure liability of drugs using in vitro cultured human iPSC-derived neurons. However, it is necessary to approach the in vitro to in vivo extrapolation (IVIVE). As one approach to IVIVE, an assessment method using a human brain organoid that mimics the three-dimensional structure is considered to be effective. In this study, we attempted to detect the response to convulsants by MEA measurement using human cerebral organoids. We detected the responses to convulsants in cerebral organoids and found that features appear in low frequency components of MEA data. Wavelet analysis revealed that the frequency intensity from the θ wave to the β wave component significantly increased in a dose-dependent manner. This is a result that enables comparison with in vivo brain waves. This study demonstrated that MEA measurement using human brain organoids may be a method that can approach in vitro to in vivo extrapolation in prediction of seizure liability of drugs.

Disclosures: **I. Suzuki:** None. **R. Yokoi:** None. **Y. Ishibashi:** None. **N. Matsuda:** None.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.06

Topic: I.04. Physiological Methods

Support: The Lundbeck Foundation

Title: Diamond electrodes for minimally invasive and long-term recordings of neuronal network activity

Authors: ***N. BERTRAM**[1], B. CHANG[2], A. HAN[3], R. W. BERG[1];
[1]Dept. of Neurosci., Univ. of Copenhagen, Copenhagen, Denmark; [2]Nanolab, [3]Mechanical Engin., Tech. Univ. of Denmark, Lyngby, Denmark

Abstract: The coordinated activity of neurons gives rise to all output of the nervous system, yet the network logic is still an elusive scientific topic. Electrodes can be used to investigate this network logic as well as an interface for neuroprosthetic devices. Such electrodes should demonstrate longevity, mechanical strength, and preserve sensitive tissue, hence the material biocompatibility is of utmost importance. Diamond as a material has many extraordinary properties for tissue interfacing: extreme durability, chemical inertness, low surface friction and superior mechanical strength, which allows design of very small physical dimensions. Despite a great interest in artificially grown diamond thin films, the use of diamond is limited in the field of neuroscience. The aim of this study is therefore to produce long-term durable and minimally invasive diamond electrodes, while still capturing the extracellular activity of hundreds of individual neurons. Specifically, we aim to decrease the state-of-the-art probe dimension by an order of magnitude from few tens of microns to below 8 μm and down to 2 μm i.e. the scale of mammalian cell nuclei. The first prototype aims for 16 shanks with 8 electrodes on each (128 electrodes total). Hot filament chemical vapor deposition provides polycrystalline diamond on a silicon base as our starting point. We adapt techniques from the microchip industry, so that diamond can easily fit into a long history of silicon processing. The challenge is partially to overcome the chemical inertness of diamond; we have established an oxygen plasma etching scheme for freely manipulating diamond into CAD-drawn shapes and micron-sized thicknesses. We have functionalized diamond surfaces with platinum electrodes in well-defined patterns of 5 μm widths via lift-off photolithography and e-beam evaporation. However, deposition parameters such as temperature must be carefully considered for defect-free depositions. Full diamond-encapsulation of platinum patterns has also been demonstrated with etched electrode access. Results are verified via optical microscopy, scanning electron microscopy, and atomic force microscopy. This experimental work is ongoing, but these prototype results show promise for future in-vivo testing of functionality and biocompatibility. Beyond assisting in fundamental neuroscience, this work has many interesting perspectives for long-term durable brain-computer interfaces and neuroprosthetics.

Disclosures: **N. Bertram:** None. **B. Chang:** None. **A. Han:** None. **R.W. Berg:** None.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.07

Topic: I.04. Physiological Methods

Support: NIH Grant U24NS113647

Title: Advances in Polymer Microelectrode Array Technology

Authors: ***K. SCHOLTEN**[1], J. ORTIGOZA-DIAZ[1], D. SONG[2], E. MENG[1];
[1]Biomed. Engin., [2]Ctr. for Neural Engin., Univ. at Southern California, Los Angeles, CA

Abstract: Microwire arrays and silicon-based microelectrode arrays (MEAs) made large-scale recording of single neural units possible, and standardization and commercialization of these technologies led to their integration into neuroscience research worldwide. Mounting evidence suggests the rigid structure of these devices can compromise the electrode-tissue interface, limiting their usefulness for chronic recordings. Flexible MEAs made from compliant polymers can mitigate tissue damage and offer greater compatibility with optical and magnetic imaging; adoption of this technology has been slow, due to long-standing limitations of existing polymer MEA technology, including low channel count, bulky packaging, and lack of standardization in fabrication and implantation. By adapting semiconductor manufacturing methods to the biocompatible polymer Parylene C we have developed a new generation of polymer-based MEAs for neural recording. Our devices consist of a transparent polymer backbone, 100× softer than silicon or metal, supporting micropatterned metal recording sites. These Parylene MEAs have been validated *in vivo* in free-moving models for >1 year. Here we describe several advances in design, fabrication capabilities, and packaging methods, and present *in vitro* performance data. Our 'Standard Probe' design features 64 Pt recording electrodes (700μm^2, 50kΩ mean impedance at 1khz) supported across 4 Parylene C penetrating shanks (5mm length, 210µm max width), for cortical/subcortical recording in small-rodent models. MEAs are ultrasonically welded to custom-built PCBs for external connection, and dip-coated in a thin layer of polyethylene glycol as a temporary stiffener to aid insertion. Using this platform we can build Parylene MEAs of arbitrary shape and configuration. We have launched the Polymer Implantable Electrode Foundry, a shared technology resource, to proliferate custom polymer MEAs to academic users. Examples of recent MEA devices built in this manner include a 32 channel array for targeted recordings in mouse hippocampi, and 16 channel MRI compatible probes for recording in cat cortices and lateral geniculate nuclei. By disseminating these devices and the underlying technology we aim to establish polymer MEAs as a reliable tool for the neuroscience researcher.

Disclosures: K. Scholten: None. **J. Ortigoza-Diaz:** None. **D. Song:** None. **E. Meng:** None.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.08

Topic: I.04. Physiological Methods

Support: National Science Foundation Graduate Research Fellowship
University of Washington Royalty Research Fund
Weill Neurohub
Center for Neurotechnology, a National Science Foundation Engineering Research Center, EEC-1028725
Washington Research Foundation
NICHHD-NIH Grant K12HD073945
NIH P51 OD010425

Title: Large-scale multi-modal dura for non-human primates

Authors: *D. J. GRIGGS, J. ZHOU, K. KHATEEB, W. K. S. OJEMANN, A. YAZDAN-SHAHMORAD;
Univ. of Washington, Seattle, WA

Abstract: We aim to develop large-scale multi-modal cortical interfaces for non-human primates for the purpose of studying large-scale neural phenomena including neural disease, damage, and recovery. Non-human primates are critical for the development of translational neural technology because of their neurological and neuroanatomical similarity to humans, and their big brains serve as valuable models for studying large-scale neural processes. Interfaces which can capitalize on these opportunities, and particularly interfaces with multiple modalities for stimulation and data collection at a large-scales, pose us to unveil network-scale dynamics of both healthy and unhealthy neural systems.

We present a transparent electrocorticographic array useful for multi-modal experiments in non-human primates in both acute and chronic scenarios. The array is composed of conductive traces printed into medical grade polymer to produce an electrocorticographic array embedded into a transparent artificial dura. This array provides simultaneous neurophysiological recordings and optical access to the cortex and it has been designed to provide an effective window of optical access on the order of months. The array is the centerpiece of the interfaces we have designed to support electrocorticographic recording and stimulation, cortical imaging, and optogenetic experiments, all at the large-scales afforded by the brains of non-human primates. We have made improvements to our past work in the areas of array flexibility, corrosion resistance, and photoelectric artifact mitigation.

Electrical and optical experiments were performed bench-side and acutely *in vivo* with macaques to validate the utility of our array. To provide representative data of the *in vivo* performance, we present optical coherence tomography angiography cortical data collected through the array and electrocorticography recordings and stimulation with the array.

The results indicate that our interfaces are suitable for large-scale electrocorticography, large-scale cortical imaging techniques and, by extension, large-scale optogenetic experimentation. These interfaces prepare the way for both acute and long-term chronic experiments with complimentary data collection and stimulation modalities. When paired with the complex behaviors and cognitive abilities of non-human primates, these assets position us well to study large-scale neural phenomena including neural disease, damage, and recovery.

Disclosures: D.J. Griggs: None. **J. Zhou:** None. **K. Khateeb:** None. **W.K.S. Ojemann:** None. **A. Yazdan-Shahmorad:** None.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.09

Topic: I.04. Physiological Methods

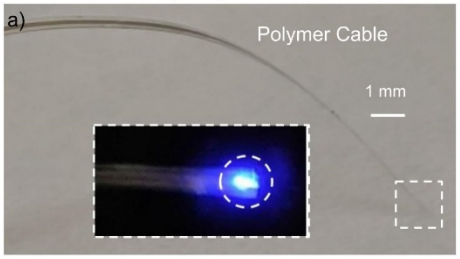
Support: Carnegie Mellon ProSEED Grant

Title: Micro-led neural probes on flexible polymer substrates for bi-directional electrical and optical neural interfaces

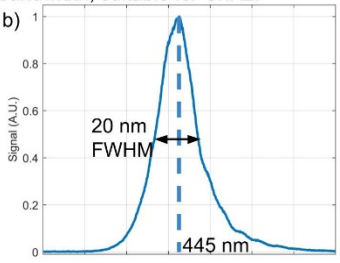
Authors: *J. W. REDDY, I. KIMUKIN, L. T. STEWART, Z. AHMED, A. L. BARTH, E. TOWE, M. CHAMANZAR;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: To enable high-resolution neural interfaces for closed-loop optogenetic experiments in deep tissue, optical stimulators and microelectrode arrays must be integrated into a single implantable device. Additionally, a fully-flexible architecture using thin-film microfabrication and flexible polymer substrates reduces tissue damage from implantable neural probes during chronic experiments. Micro-light-emitting diodes (micro-LEDs) are traditionally fabricated on rigid silicon and sapphire substrates and then either thinned to make an implantable shank, or released and transferred to a new flexible substrate. However, a monolithic fabrication process can achieve a very high device density with any desired arrangement of light sources. Here, we demonstrate our integrated neural probe platform for compact 22 µm x 22 µm micro-LEDs monolithically integrated with recording microelectrodes on a flexible Parylene C polymer substrate (Figure 1a). Parylene C acts as a flexible, biocompatible, and highly impermeable insulation and substrate material. We demonstrate gallium nitride micro-LEDs with an emission wavelength of 445 nm, suitable for stimulating Channelrhodopsin-2 (ChR2) (Figure 1b). The micro-LEDs can generate power densities up to 12.4 mW/mm2, significantly above the optogenetic threshold of stimulation for ChR2. LED arrays are shown in 1D and 2D configurations in densities of up to 400 LEDs/mm2. The high-density, high-power micro-LED stimulation raises concerns of tissue heating, since unlike rigid silicon substrates, polymers are less effective at dissipating heat. We present a thermal simulation framework to identify thermally-safe stimulation paradigms (Figure 1c). We will discuss the fabrication process, simulation results, and device validation using transgenic mice.

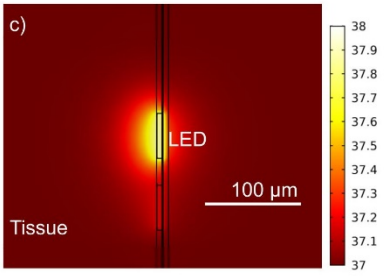
a) Microfabricated polymer cable containing GaN micro-LEDs in a Parylene C substrate, with single device illuminated (inset).



b) LED emission spectrum centered at 445 nm with 20 nm FWHM bandwidth, suitable for ChR2.



c) Tissue heating (°C) after 5 ms LED optical stimulation at 4.8 µW power.



Disclosures: J.W. Reddy: None. **I. Kimukin:** None. **L.T. Stewart:** None. **Z. Ahmed:** None. **A.L. Barth:** None. **E. Towe:** None. **M. Chamanzar:** None.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.10

Topic: I.04. Physiological Methods

Support: Marie Sklodowska-Curie grant agreement No 798836
Marie Sklodowska-Curie grant agreement No 842804

Title: Label-free functional characterization of human brain organoids at single-cell resolution

Authors: S. SAJGO, G. ZORZI, ***M. J. OBIEN**;
MaxWell Biosystems AG, Zurich, Switzerland

Abstract: Three dimensional organ-like cell aggregates (organoids) that originate from human induced pluripotent stem cells (h-iPSCs) are emerging as promising tools for investigating development and disease progression, as well as for drug discovery. Organoids from h-iPSC-derived neurons recapitulate the architectures and characteristic functions of different brain areas that can be used to model human disease in-vitro. In order to adopt brain organoids for rapid and cost-effective drug screenings, it is necessary to assess their cell type composition, gene expression patterns and physiological function.
The electrical activity of brain, retina or muscle organoids can now be easily captured, label free, at single-cell resolution by using MaxWell Biosystems' high-density microelectrode array (HD-MEA) technology—MaxOne (single-well) and MaxTwo (multi-well)[1]. The HD-MEA's large sensor array, featuring 26,400 electrodes at high-resolution enables recording of neuronal activity across different scales, from the network level, single-cell level, down to the sub-cellular level.
Three different neuronal assays have been implemented in the software, MaxLab Live, and used to evaluate the spontaneous activity of brain organoids. (A) The ActivityScan Assay allows detection and identification of all active areas in the organoid. Firing rate and amplitude of the action potentials can be extracted. (B) The Network Assay enables the analysis of network bursts and synchronicity, indicating formation of connectivity between neurons. (C) The AxonTracking assay identifies single neurons and provides metrics such as axonal conduction velocity.
In this work, we present results from organoids modeling different brain compartments and will demonstrate the potential of HD-MEA technology for characterizing the physiological function of human brain organoids and for testing compounds.
1. https://www.mxwbio.com

Disclosures: S. Sajgo: None. **G. Zorzi:** None. **M.J. Obien:** None.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.11

Topic: B.09. Network interactions

Title: Development and characterization of an in vitro synaptic propagation assay using optogenetics and multiwell microelectrode array technology

Authors: *D. C. MILLARD, A. NICOLINI, F. GOODFELLOW, H. HAYES;
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Abstract: Synaptic connections are a fundamental building block of neuronal function, enabling neuronal circuits to process and relay information downstream via action potential propagation. Indeed, many neurological disorders involve impaired connectivity between distinct brain regions. However, traditional in vitro "disease-in-a-dish" neuronal models comprise only a single neuronal circuit, whereas animal models are too costly and complicated to facilitate a screen on compounds or genetic edits that affect synaptic propagation. Here, we describe the development and characterization of a simple in vitro assay of synaptic propagation between two distinct neural circuits. First, an easy-to-use, two-compartment silicone insert was added to each well of a 6-well microelectrode array (MEA) plate. Cryopreserved rodent primary cortical neurons were prepared and seeded into each compartment of the silicone inserts. An adeno-associated virus was added to transduce the cells of each compartment with a distinct optogenetic construct (Chronos vs. Chrimson). After two days in vitro, the silicone insert was removed and the networks allowed to mature. The development of functional network activity (e.g., activity, synchrony, oscillations) was monitored every 2-3 days throughout the cell culture period. Activity and synchrony developed first (~7-10 days) within the networks originally defined by the two-compartment silicone insert, followed by the propagation of network activity from one compartment to the other (~14-17 days). Optogenetic stimulation was used to selectively stimulate one network at a time within each well of the plate while the electrophysiological activity was monitored from the second network. The evoked synaptic propagation was evaluated for each well at baseline, after treatment with either the vehicle control or a cocktail of synaptic blockers (CNQX, APV, Bicuculline), and then following washout. The effect of the synaptic blockade was quantified as the probability and delay of synaptic propagation in response to the optogenetic stimulus. These results support the continued development and use of in vitro neuronal models and MEA technology for drug toxicity and safety assessment, evaluation of phenotypic disease-in-a dish models, and cell development.

Disclosures: D.C. Millard: A. Employment/Salary (full or part-time):; Axion Biosystems. **A. Nicolini:** A. Employment/Salary (full or part-time):; Axion Biosystems. **F. Goodfellow:** A. Employment/Salary (full or part-time):; Axion Biosystems. **H. Hayes:** A. Employment/Salary (full or part-time):; Axion Biosystems.

Digital Abstract Session

P381. Novel Approaches With Electrode Arrays

Program #/Poster #: P381.12

Topic: B.09. Network interactions

Support: JSPS Grant 20J22686
JSPS Grant 19H04437
JSPS Grant JP18H05465
JSPS Grant 19H05323
JSPS Grant JPJSBP120194809

Title: Observing neuronal ensemble based on synaptic connectivity estimation on high-density microelectrode array

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Abstract: Neuronal ensemble is important because it is an essential unit of information processing in the brain. One of the major problems about neuronal ensembles is difficulty of observation. Neuronal ensemble consists of tens of thousands of neurons and the large number of neurons makes it difficult to record the whole activity of neuronal ensemble. In this study, we developed a novel method to estimate activities of unobserved neurons from local spike recordings. Using the proposed method, we observed multiple patterns of neuronal ensembles from extracellular spike recording. A computational model was established to estimate summation of functional synaptic connectivity to a single recorded neuron from many other neurons. When a neuronal ensemble is activated, single recorded neuron receives synaptic inputs from many surrounding neurons of which the neuronal ensemble consists. Under the assumption that every recorded neuron receives inputs simultaneously from all neurons in the active neuronal ensemble, the summation of synaptic connection to each recorded neuron was estimated based on membrane potential calculations. By using this estimation method, optimal connectivity summation patterns can be obtained reflecting activities of all recorded neurons for a long term. The proposed method was applied to spike trains which were recorded from cultured neuronal networks to distinguish multiple neuronal ensembles. Spike trains were recorded from rat cortex neurons cultured on high-density microelectrode array. Neuronal activities were recorded from four samples around a month of their culturing. Spike trains were recorded from 1,024 electrodes for more than twelve hours for each sample. Synchronized bursts were detected from spike trains and the synaptic connectivity summations were estimated for each detected burst. The estimated synaptic connectivity summation patterns were regarded as feature values of the bursts. As a result, transitions from one active neuronal ensemble to another active neuronal ensemble were observed in the form of synaptic connectivity summation pattern changes. Moreover, the observation of multiple neuronal ensembles from only 50 electrodes using the proposed method was as clear as the observation from 1,024 electrodes using a simple conventional method (clustering based on basic statistics of bursts). And the observation of multiple neuronal ensembles from 1,024 electrodes using the proposed method was clearer. From this result, we conclude that the proposed method is useful for estimating neuronal ensemble activity in wide areas from local recordings.

Disclosures: **T. Asahina:** None. **K. Shimba:** None. **C. Chang:** None. **K. Kotani:** None. **Y. Jimbo:** None.

Digital Abstract Session

P382. Angelman and Other Developmental Disorders

Program #/Poster #: P382.01

Topic: A.07. Developmental Disorders

Support: NIH R21 HD091823-01
NIH R01 HD094953
Angelman Syndrome Foundation (ASF)
Connecticut Regenerative Medicine Research Fund

Title: Morphological and electrophysiological phenotypes in Angelman syndrome iPSC/hESC-derived neurons

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Abstract: Angelman Syndrome (AS) is a neurodevelopmental disorder characterized by motor dysfunction, intellectual disability, severe seizures, absent speech, and a happy demeanor. The disorder is caused by a deletion or mutation of the maternally inherited allele of *UBE3A*. The paternal copy in the brain cannot compensate for this loss because it is subject to silencing via genomic imprinting by the *UBE3A* antisense transcript (*UBE3A-ATS*). Using AS patient specific derived induced pluripotent stem cells (iPSCs) and their neuronal derivatives as a model system, we sought to identify phenotypic differences between human AS neurons and healthy control neurons to pinpoint the primary cellular deficits in AS in order to potentially reveal how neuronal physiology contributes to the neurological and behavioral deficits in individuals with AS. Using CRISPR/Cas9, we have generated two distinct isogenic AS and control human pluripotent stem cell pairs. For the first pair, we correct a C to T transition resulting in a phenylalanine to serine missense mutation in the maternally-inherited copy of *UBE3A* in iPSCs derived from a 7-year old male with AS. This missense mutation kills the enzymatic activity of *UBE3A* but causes no reduction in RNA or protein levels. The second isogenic pair was generated by deleting the maternal copy of *UBE3A* in H9s, a female human embryonic stem cell (hESC) line. Whole cell patch clamping was used to assess electrophysiological phenotypes in forebrain cortical neurons derived from these two isogenic pairs. Both isogenic pairs showed that AS neurons displayed a more depolarized resting membrane potential and impaired action potential firing compared to their isogenic control. Confocal imaging to interrogate morphological differences in the isogenic pairs found that mature AS neurons have a decreased density of dendritic protrusions, decreased soma size, and decreased dendritic branching compared to their isogenic control. Altogether, these findings identify novel cell intrinsic and network-dependent cellular phenotypes in human AS neurons and provide a framework for identifying the neuronal deficits that contribute to the human AS symptomology. These data also

provide a phenotypic readout that may be able to more accurately predict the effectiveness of different drug treatments in ameliorating AS-related deficits.

Disclosures: D. Gorka: None. **J. Bloom:** None. **C. Sirois:** None. **L. Loew:** None. **E. Levine:** None. **S. Chamberlain:** None.

Digital Abstract Session

P382. Angelman and Other Developmental Disorders

Program #/Poster #: P382.02

Topic: A.07. Developmental Disorders

Support: NIH Grant 1R01DA051893-01

Title: Impaired Neural Induction and Neuronal Differentiation in a Novel Cerebral Organoid Model of NIBP Syndrome

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Abstract: NIBP (NIK-and-IKK2-binding protein; also known as TRAPPC9), is an important mediator of both NFκB signaling and protein transport/trafficking. Increasing clinical evidence shows that homozygous mutations of *NIBP/TRAPPC9* has been linked to a novel autosomal recessive intellectual disability syndrome, NIBP Syndrome. Patients with NIBP Syndrome exhibit various symptoms including intellectual disability, developmental delays, microcephaly, dysmorphic facial features, and obesity. While clinical findings suggest this is due to absent or decreased levels of functional NIBP protein, the cellular mechanisms underlying this phenotype is still unclear. In order to elucidate the pathogenesis resulting from NIBP deficiency, we generated cerebral organoids (COs) from induced pluripotent stem cells (iPSCs) derived from three NIBP Syndrome patients and two healthy subjects as controls. While there were no significant differences in iPSC propagation or embryoid body formation between patient and control lines, there were robust differences in CO formation and maturation. Control COs readily formed neuroepithelial tissue and neural stem/progenitor cells (NSC/NPC) during neural induction and matured in a typical fashion, but patient COs often formed numerous abnormal sizes and shapes, had decreased neuroepithelial tissue, and grew more slowly or died off, suggesting an impairment of neural induction. Intriguingly, there was varying degree of severity of impairment and structural change among different patient COs. To further examine the impact of NIBP deficiency on neural lineage differentiation, some COs were dissociated after neural induction to generate monolayer NSCs/NPCs. The control NSC/NPCs readily formed neurospheres or differentiated into neural cells including neurons with long thin processes projecting outwards; however, patient NSC/NPCs displayed very little morphological changes, with most projections being bipolar or unipolar, suggesting impaired neural differentiation. Long-term neuronal differentiation/maturation studies showed impaired neural network and

synaptic plasticity, with the majority of patient NSCs/NPCs failing to differentiate into mature neurons. In conclusion, we find evidence of neurogenic deficits related to NIBP Syndrome in an innovative, novel, and clinically relevant model in an effort to help elucidate pathogenic mechanisms underlying a serious neurodevelopmental disorder.

Disclosures: B. Bodnar: None. **Y. Lin:** None. **Y. Zhu:** None. **W. Hu:** None.

Digital Abstract Session

P382. Angelman and Other Developmental Disorders

Program #/Poster #: P382.03

Topic: C.10. Brain Injury and Trauma

Support: Tiny Blue Dot Foundation (UCLA award number: 20163896:2)
US NIH AS Natural History Study [NCT00296764]
US NIH Grant No. U54RR019478
US NIH Grant No. 4HD061222

Title: Paradoxical cortical dynamics in Angelman syndrome as a crucible for testing biomarkers of consciousness

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Abstract: Across many contexts, including sleep, anesthesia, and the vegetative state, EEG delta waves (1 – 4 Hz) are a signature of unconsciousness. How is it then that children with Angelman syndrome (AS), a genetic disorder caused by dysfunction of *UBE3A*, display extremely high voltage delta activity while awake and conscious? AS is highly penetrant for intellectual disability, epilepsy, and motor delays, yet children with AS display a rich spectrum of behaviors that indicate conscious awareness. Understanding this paradox (an EEG signature of sleep accompanying wakeful behavior) is important for deriving robust EEG markers of covert consciousness in unresponsive brain-injured patients with disorders of consciousness (i.e., what EEG features, if not delta power, reliably indicate that a person is unconscious?). To investigate this mystery, we looked at EEG signal complexity as an alternative marker of conscious state. We compared the modified multiscale entropy (mMSE) and the generalized multiscale Lempel-Ziv complexity (gMLZ), two measures of signal complexity, between wakefulness and sleep (within-subject) in 23 children with AS and 23 age matched TD controls (mean age of 68 months, both groups). 19 channel EEGs were bandpass filtered 0.5 – 45 Hz and artifact reduced. We averaged both complexity measures across 19 channels and 20 timescales. mMSE and gMLZ

both showed a significant ($p < 0.005$) main effect of sleep and group x sleep interaction (two-way repeated measures ANOVA), but only gMLZ showed a significant main effect of group. Complexity values indeed differentiate sleep from wakefulness in AS, but the separation (as measured with mMSE) is not as large as TD, and the mean awake level of complexity in AS (as measured with gMLZ) is still less than the sleeping level of complexity in TD, suggesting that complexity does not benchmark conscious level consistently across groups. Next, using regularized logistic regression, we trained a binary classifier on complexity values from 35 AS EEGs and then validated the model on 41 TD EEGs (these were larger, non-age matched cohorts). The classifier yielded 71.4% sleep/wake accuracy for AS and 92.7% accuracy for TD; the higher accuracy for TD was likely due to a simpler feature space boundary that differentiated sleep/wake for this group. We conclude that although the absolute level of complexity in AS may erroneously suggest that children are unconscious while awake, biomarkers of conscious state in AS nonetheless appear to generalize to healthy controls, as demonstrated using logistic regression. The paradoxical cortical dynamics in AS may therefore serve as a crucible for validating biomarkers of conscious state.

Disclosures: **J. Frohlich:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **L.M. Bird:** D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); F. Hoffmann-La Roche Ltd. **J.F. Hipp:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **C. Chu:** F. Consulting Fees (e.g., advisory boards); Biogen, SleepMed. **M.M. Monti:** None.

Digital Abstract Session

P382. Angelman and Other Developmental Disorders

Program #/Poster #: P382.04

Topic: A.02. Postnatal Neurogenesis

Support: CIHR

Title: Chronic intermittent theta burst stimulation (iTBS) effects on adult hippocampal neurogenesis in males and females

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Abstract: Intermittent theta burst stimulation (iTBS) is a promising form of non-invasive stimulation therapy for a variety of psychiatric illnesses. Currently, iTBS is administered similarly across males and females, but it is not known whether iTBS's mechanism of action is affected by patient sex. As hippocampal neurogenesis plays a critical role in stress adaptation and antidepressant course of action, we examined adult hippocampal neurogenesis in male and female mice following a chronic iTBS protocol similar to those used in the clinics. Although chronic iTBS does not exert dramatic effects on neurogenesis, we found that there is a trend for a

greater population of immature neurons after iTBS in males, but not females. Females do not respond to iTBS with respect to any hippocampal neurogenesis related measures. There is no effect on iTBS on the survival of neurons born one week before stimulation for either sex. We also examined the morphology of new-born neurons and found that iTBS produced a trend for a slight decrease in spines in stimulated animals compared to sham stimulated animals. New neurons generated in either sex have normal morphology, similar to non-stimulated control animals, with the exception of females overall have lower total dendritic length compared to males. Otherwise, all comparisons between male and female new-born neurons are consistent with each other, indicating that baseline neurogenesis levels and new-born neuron morphological features are comparable between sexes. Our findings suggest that iTBS's primary target on neuroplasticity may not be adult hippocampal neurogenesis, however, there may be physiological differences of new-born neurons that are influenced by iTBS that remains to be further explored.

Disclosures: T. Zhang: None. **B. Askari:** None. **A. Kesici:** None. **E.M. Guilherme:** None. **F. Vila-Rodriguez:** None. **J.S. Snyder:** None.

Digital Abstract Session

P383. Techniques in Neurodegenerative and Neuropsychiatric Diseases' Research

Program #/Poster #: P383.01

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: U54 AG065187

Title: The Emory-Sage-SGC TREAT-AD Center: Developing tools and reagents for emerging targets in Alzheimer's Disease

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Abstract: Drug development in Alzheimer's disease (AD) has been marked by repeated failures. Increasing evidence supports the need for a diverse portfolio of therapeutic and diagnostic targets in order to effectively reduce the burden of AD. The Emory-Sage-SGC TREAT-AD Center was established with the aim at developing drugs for a diverse portfolio of therapeutic approaches to break the cycle of failure, by focusing on research advances in two key areas. One focus is to cast a wider net and develop drugs that target the multifaceted dysregulation in the brains of AD patients, rather than two key pathological hallmarks. The second is using open scientific

practices to speed up drug development for new therapies. The Emory-Sage-SGC TREAT-AD Center has identified a diverse set of novel AD targets derived from systems biology studies within the Accelerating Medicines Partnership in AD (AMP-AD) consortium and further evaluated using data from multiple AD consortia and existing literature. Targets nominated through the AMP-AD consortium were mapped to mechanistic hypotheses and prioritized based on unbiased bioinformatic assessments across multiple lines of evidence. Hypotheses prioritized in the initial evaluation included those relating to immune function, endocytosis, and retromer-mediated endosomal protein sorting. To engage the research community to pursue emerging targets in AD, the center is generating and openly distributing experimental tools for use in target validation to advance or reject therapeutic hypotheses. Distribution of all data and Target Enabling Packages that include expression constructs, knockout cell lines, assays, antibody validation data, and crystal structures for 20 proteins identified within these mechanisms will be publicly shared through standard repositories and cataloged on the Agora website, a data visualization portal funded by the NIA and developed by Sage Bionetworks. The open drug discovery approach of TREAT-AD is aimed to de-risk potential AD therapeutics to support industry investment by catalyzing robust and independent evaluation of a diverse portfolio of promising yet untested AD therapeutic hypotheses.

Disclosures: L.M. Mangravite: None. **R. Betarbet:** None. **H. Fu:** None. **A.I. Levey:** None. **G.W. Carter:** None. **S.V. Frye:** None. **K.H. Pearce:** None. **O. Gileadi:** None. **A.M. Edwards:** None. **L. Omberg:** None. **A.K. Greenwood:** None. **K. Leal:** None. **F.M. Longo:** None.

Digital Abstract Session

P383. Techniques in Neurodegenerative and Neuropsychiatric Diseases' Research

Program #/Poster #: P383.02

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Melbourne Neuroscience Institute Interdisciplinary Seed Fund
Melbourne Research Fellowship

Title: Retinal hyperspectral changes in a mouse model of Parkinson's disease

Authors: *C. T. O. NGUYEN[1], P. TRLIN[1], K. K. N. TRAN[1], V. H. Y. WONG[2], D. ZHAO[1], J. K. H. LIM[3], D. I. FINKELSTEIN[4], A. METHA[1], B. V. BUI[1], P. BEDGGOOD[1];
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Abstract: Purpose: The retina is an accessible out-pouching of the central nervous system and may reflect cortical changes that occur with Parkinson’s disease (PD). In vivo hyperspectral imaging is a non-invasive means to image the retina and has shown promise as an indicator for central nervous system conditions in animal models and human patients. This is the first study to evaluate whether hyperspectral imaging is altered in a well-characterized mouse PD model of

alpha-synuclein overexpression and whether this changes with advancing age. **Methods:** A transgenic mouse model of alpha-synuclein deposition (A53T) and wild-type littermates were assessed at 6 and 14 months old (n = 10-24 / group). Hyperspectral imaging of the retina centred around the optic nerve head was conducted from 320 to 680nm in 5 nm steps (TILL Polychrome V light source, Andor Neo cMOS camera). Images were registered and blood vessels and optic nerve head were masked to isolate retinal regions of interest. Normalized spectral reflectivity was analysed using two-way ANOVA with Bonferroni correction for multiple comparisons (Prism, GraphPad), and with principle components analysis (PCA; MATLAB, MathWorks). **Results:** Six-month-old A53T mice exhibited statistically significant wavelength-selective changes using two-way ANOVA ($p < 0.05$), and a genotype effect on spectrum shape using PCA ($p < 0.05$; sensitivity 0.88, specificity 0.59). Fourteen-month-old A53T mice showed statistically significant interaction effects (two-way ANOVA; $p < 0.001$) with increased reflectivity at shorter wavelengths (< 500nm) and decreased reflectivity at longer wavelengths (> 600nm). Spectral shape differences bordered on significance using PCA ($p = 0.08$), with specificity and sensitivity of 0.75 and 0.40, respectively. Healthy ageing changes to hyperspectral imaging were examined by comparing the 6- and 14-month-old wild-type control mice. A different pattern of hyperspectral reflectivity was observed in healthy aging, with a statistically significant interaction effect ($p < 0.001$) indicating greater reflectivity at longer wavelengths (> 600nm) with advancing age. This healthy aging effect was confirmed in a separate control cohort of aging mice. **Conclusion:** A transgenic mouse model of alpha-synuclein overexpression exhibits an alteration in spectral reflectivity of the retina at mid and advanced stages of PD. The hyperspectral signature exhibited with advancing alpha-synuclein deposition is distinct from normal healthy ageing.

Disclosures: C.T.O. Nguyen: None. **P. Trlin:** None. **K.K.N. Tran:** None. **V.H.Y. Wong:** None. **D. Zhao:** None. **J.K.H. Lim:** None. **D.I. Finkelstein:** None. **A. Metha:** None. **B.V. Bui:** None. **P. Bedggood:** None.

Digital Abstract Session

P383. Techniques in Neurodegenerative and Neuropsychiatric Diseases' Research

Program #/Poster #: P383.03

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Melbourne Neuroscience Institute Interdisciplinary Seed Fund
Research Initiatives Fund Collaborative Equipment Grant Scheme
Australian Research Council Linkage grant (LP160100126)

Title: Ultra-reflectivity as a novel ocular biomarker in mice models of Parkinson's and Alzheimer's diseases

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Abstract: Purpose: Early biomarkers for neurodegenerative diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD) are needed. Optical coherence tomography (OCT) has the capability to detect changes within the eye, where retinal nerve fibre thickness alteration is present in PD and AD. This study explores the possibility that in addition to changes in tissue thickness, retinal alpha-synuclein (α-syn) and amyloid beta (Aβ) deposition may change OCT reflectivity or "ultra-reflectivity".
Methods: Two transgenic murine models were used: a human α-syn overexpression model of PD (A53T mice) and an Aβ accumulation model of AD (5xFAD mice). Sedated (ketamine: xylazine mix 80:10 mg/kg, i.p.) A53T mice were assessed at 6 and 14 months of age (n=15-17/group) and 5xFAD mice were assessed at 3, 6 and 12 months of age (n=11-12/group) and compared with their respective wild-type (WT) littermates. Three adjacent OCT B-scans (OCT2, Spectralis) across the optic nerve head (768 A-scans, axial resolution: 3.87µm, lateral: 10.29µm) were analysed to measure ultra-reflectivity at the retinal nerve fibre layer (RNFL) and the outer nuclear layer (ONL). Two-way ANOVA with Sidak correction for multiple comparisons (Prism, GraphPad) was used to compare groups.
Results: ONL reflectivity was elevated in A53T mice compared to WT_{PD} littermates ($p = 0.026$), particularly at 6 months of age. RNFL reflectivity was also significantly reduced in A53T mice compared to WT_{PD} littermates ($p = 0.011$), particularly at 14 months of age ($p = 0.003$). As such, when the difference between RNFL and ONL reflectivity is calculated ("ultra-reflectivity" = RNFL reflectivity - ONL reflectivity) there was a significant decrease in reflectivity in A53T mice at both 6 and 14 months of age ($p = 0.035$ and 0.014, respectively). There was no genotype difference in RNFL and ONL reflectivity between 5xFAD and WT_{AD} mice ($p = 0.785$). Ultra-reflectivity profiles changed in both 5xFAD and WT_{AD} ($p = 0.041$) when comparing across 3 and 12 months of age.
Conclusion: Our study demonstrates that RNFL and ONL reflectivity are useful tools in following the neurobiological changes within the eye. A53T mice exhibited changes in ultra-reflectivity measures, whereas 5xFAD mice did not. Further studies are required to better understand these reflectivity changes in relation to α-syn related pathology and normal healthy aging.

Disclosures: V.H.Y. Wong: None. **H. Lee:** None. **D. Zhao:** None. **K.K.N. Tran:** None. **B.V. Bui:** None. **D.I. Finkelstein:** None. **C.T.O. Nguyen:** None.

Digital Abstract Session

P383. Techniques in Neurodegenerative and Neuropsychiatric Diseases' Research

Program #/Poster #: P383.04

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NiH Grant K08DA037465
NIH Grant R43DA044050
NIH Grant R44MH108481

Title: Neurotheranostic for MR imaging of dopamine transporters and treatment of dopaminergic neurodegeneration

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[3]McLean Hosp., Belmont, MA; [4]ExQor Technologies, Boston, MA

Abstract: Neurotheranostics (NTs) show great promise for diagnostic imaging and treatment of neurodegenerative disorders. NTs consist of combined CNS diagnostic and therapeutic agents and can be used to enhance MRI sensitivity and molecular targeting by delivering superparamagnetic iron oxide (SPIO) contrast agents along with antibodies. However, NTs cannot cross an intact blood-brain-barrier (BBB) limiting their use in neurology/psychiatry. Our goal was to develop a novel NT for targeted, noninvasive delivery of DAT-antibody (DAT-Ab), SPIO, and brain-derived neurotrophic factor (BDNF) to dopamine brain regions.
NTs were synthesized by conjugating DAT-Ab and SPIO for MRI (plus BDNF for treatment) to Clathrin carrier using polyethylene glycols (PEGs) at 1:1:1:1 molar ratio, and NT's size and structure were characterized. For 7 days, iTat mice received intraperitoneally doxycycline (100 mg/kg/day) to induce neurotoxic HIV-Tat protein expression. Concurrently, Tat+ mice received intranasally (i.n.) saline or NTs (BDNF, 300 µg/kg/d). For MRI, wild-type C57BL/6J mice received one dose of i.n. saline or NTs (SPIO, 68pmol, 50µL). Four hours after the last NT/saline dose, mouse brains were prepared for immunohistochemistry or *ex-vivo* MRI. Voxel-wise R2* relaxation rates were obtained using a series of gradient-echo images (TR=1.5 s, TE=3.2, 4, 5, 6, 7, 8, 9, 10 ms; 128x128 in -plane matrix; 0.2 mm resolution; 64 slices at 0.5 mm thickness; 7 averages). R2* values in the striatum (STR), substantia nigra (SN) and visual cortex (vCTX), a control region with low DAT expression, were calculated.
The iron-stained brain slices contained NTs in brain regions rich in DAT (e.g., STR). Striatal tyrosine hydroxylase densities were higher (P=0.0467) in NT vs. saline treated Tat+ mice. MRI studies of NT with DAT-Ab-SPIO revealed that R2* values were significantly higher in the STR (p=0.0010) and SN (p=0.0007) compared to vCTX in animals that received NTs, but not in saline treated animals. NTs significantly increased R2* in the STR (p<0.0001) and SN (p=0.0002) compared to saline without significantly altering R2* in the vCTX.
NTs successfully bypassed the BBB and delivered adequate concentrations of SPIO and BDNF to neurons expressing DAT in the mouse brain. NTs enabled DAT detection using MRI, and rescued striatal tyrosine hydroxylase-positive fibers from HIV/Tat neurotoxicity. Thus, Clathrin-based NTs could assist in early detection and treatment of neurodegeneration and in monitoring disease progression and recovery processes.

Disclosures: **G. Vitaliano:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ExQor technologies. **C. Adam:** None. **G. Zeballos:** None. **F. Vitaliano:** A. Employment/Salary (full or part-time):; ExQor Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ExQor Technologies.

Digital Abstract Session

P383. Techniques in Neurodegenerative and Neuropsychiatric Diseases' Research

Program #/Poster #: P383.05

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Design and Development of Proteolysis Targeting Chimera against Cytoplasmic TDP-43 in Neurodegeneration

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Abstract: Neurodegenerative disorders (ND) are increasingly recognized as the major cause of disability and deaths, currently affecting 47.5 million people globally with dementia in people aging 60 or above. One common feature linked to mostly all ND is the misfolding and aggregation of certain proteins, which generates the cascade of pathological events leading to failure to protein homeostasis in the physiological state. TAR DNA binding protein 43 (TDP-43), is one such protein responsible for Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal lobar degeneration (FTLD). Physiologically TDP-43 is a regulator of gene expression, RNA processing, etc. However, the mutation in TDP-43, leads to the excess deposition and formation of misfolded toxic protein aggregates in the cytoplasm of the cell, leading to disease. The aggregation-prone proteins are pharmacologically difficult to target because of their misfolded and disordered structure. To tackle this stumbling block, we contemplated the Targeted Protein Degradation approach and developed a peptide-based Proteolysis Targeting Chimera (PROTAC) against aggregated TDP-43 degradation. PROTAC is a heterobifunctional molecule, composed of two different binding domains connected by a linker. One is for engaging an E3 ubiquitin ligase and the other for the binding to targeted protein. E3 ligase recruitment leads to the ubiquitination of targeted protein, and recognition by the Ubiquitin proteasome system for the degradation. As a protein quality control system, the UPS plays a key role in the degradation of misfolded or aggregated proteins and maintaining protein homeostasis. Our objective is to study the degradation potential of cytoplasmic mutated TDP-43 utilizing synthesized peptide-PROTAC Synthesis of the peptide-PROTACs was done via Fmoc-base solid-phase peptide synthesis methodology. The purity and characterization of the molecule were checked using RP-HPLC and MALDI mass spectrometer. TDP-43 recombination protein expressed and purified in *E.Coli* bacterial culture was performed. The Cell penetration study of the synthesized PROTAC was performed in the SH-SY5Y neuroblastoma cell line using confocal microscopy. Further, the cell viability assay was done using the MTT Assay and analyzed. Further experiments including the protein expression in SH-SY5Y cell lines are currently in process. The preliminary studies performed on peptide-PROTAC were seen to be promising and further evaluation needs to be done. Conclusion: We believe that the synthesized novel PROTAC will regulate the aggregated cytosolic TDP-43 towards the selective degradation, utilizing inherent cellular UPS.

Disclosures: K.G. Bhavsar: None.

Digital Abstract Session

P383. Techniques in Neurodegenerative and Neuropsychiatric Diseases' Research

Program #/Poster #: P383.06

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH R01NS079153
NIH R01NS075035
NIH R01NS097000
NIH R01NS101718
NIH NS106950
AHA 16BGIA27250263

Title: Location-specific gene co-expression modules in peripheral blood implicate neutrophilic, monocytic and lymphocytic response to spontaneous human Intracerebral Hemorrhage

Authors: B. KNEPP[1], A. YEE[1], G. JICKLING[1,2], F. RODRIGUEZ[1], K. NG[1], X. ZHAN[1], N. ALOMAR[1], M. HAKOUPIAN[1], H. AMINI[1], P. CARMONA-MORA[1], H. HULL[1], B. P. ANDER[1], F. R. SHARP[1], ***B. STAMOVA**[1];
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Abstract: Intracerebral Hemorrhage (ICH) occurs within the cortical (lobar) or deep intraparenchymal brain regions. Deep ICH tends to be caused by hypertension, while Lobar ICH tends to be caused by cerebral amyloid angiopathy. Since peripheral immune cells respond to ICH and contribute to damage and repair, we examined whether peripheral blood gene co-expression networks differed between Deep and Lobar ICH patients. Whole-genome gene expression for 28 ICH patients (19 Deep, 9 Lobar) and 33 Vascular Risk Factor Controls (VRFC) was examined on HTA 2.0 microarrays. Weighted Gene Co-Expression Network Analysis was performed on 10,200 protein-coding genes and generated 31 co-expressed gene modules. Of these, 7 were significant ($p < 0.05$) in a Lobar ICH vs VRFC contrast and 6 in Deep ICH vs VRFC (Fig. 1). There were 3 significant modules (Fig. 1) common between the two. They were significantly enriched in monocyte- and neutrophil-specific genes. Additionally, one of these modules was also enriched in T Cell-specific genes. The Lobar ICH-specific modules were enriched in monocyte, neutrophil and B-cell specific genes, while one of the Deep ICH-specific modules was enriched in T cell specific genes. The Lobar- and Deep ICH-common modules were overrepresented (adj. $p < 0.05$) in inflammatory pathways such as Interleukin-(1,2,6,10,12, and 17A), Inflammasome, Neuroinflammation, Acute Phase Response, and B Cell Receptor Signaling. Lobar unique modules were also overrepresented in Phagosome Formation, Granulocyte Adhesion and Diapedesis, and PI3K Signaling in B Lymphocytes. Deep ICH-unique modules were overrepresented in T Cell pathways, such as Th1 and Th2 Activation, T Cell Exhaustion, and T Cell Receptor Signaling. Our current data suggest common and specific monocyte, neutrophil, T-cell, and B-cell involvement in Lobar and Deep ICH. Collectively, these results give additional evidence for a specific pathophysiology and transcriptome architecture

based on ICH location. Our exploratory findings may guide the search for location-specific potential therapeutic targets.

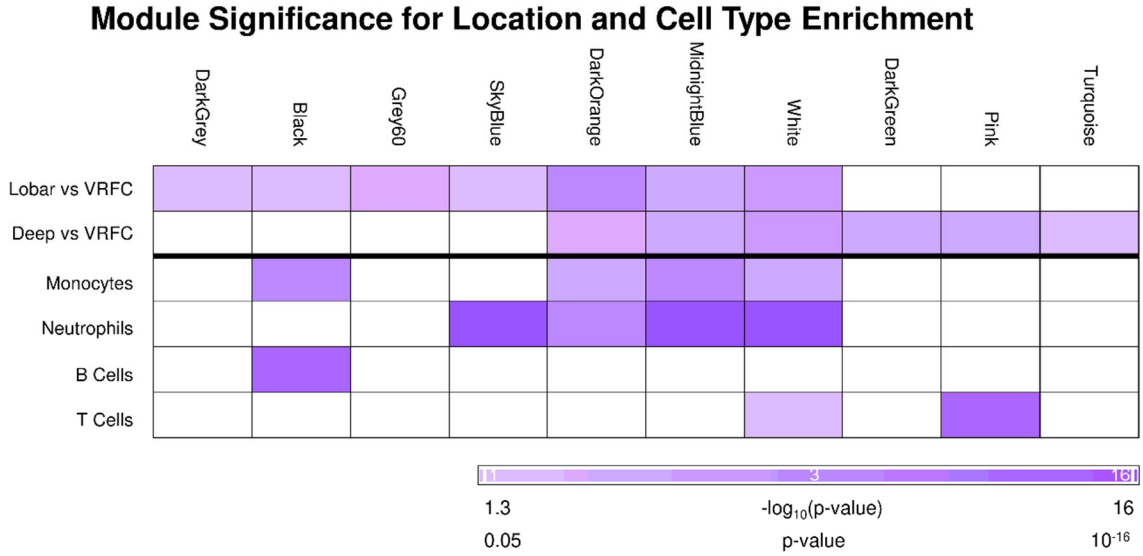

Figure 1 Module significance for ICH location as well as cell type enrichment of all modules significant to at least one location comparison. Shaded boxes show -$\log_{10}$(p-value); white boxes represent non-significance. Location comparisons were done on an ANCOVA that accounted for the covariate Age. Cell type enrichment was found using hypergeometric probability testing based on module's overlap with known cell type specific gene lists.

Disclosures: B. Knepp: None. **A. Yee:** None. **G. Jickling:** None. **F. Rodriguez:** None. **K. Ng:** None. **X. Zhan:** None. **N. Alomar:** None. **M. Hakoupian:** None. **H. Amini:** None. **P. Carmona-Mora:** None. **H. Hull:** None. **B.P. Ander:** None. **F.R. Sharp:** None. **B. Stamova:** None.

Digital Abstract Session

P383. Techniques in Neurodegenerative and Neuropsychiatric Diseases' Research

Program #/Poster #: P383.07

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Artificial intelligence assisted feature selection for novel and translatable quantitative electroencephalography biomarker discovery

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Abstract: Electroencephalography (EEG) is a noninvasive neuroimaging technique that records small voltage fluctuations at the scalp that result from the summed activity of the underlying cortical neurons. EEG biomarkers play a valuable role in the early diagnosis of many neurological disorders, in the monitoring of disease progression, and for determining the effects

of pharmacological treatment on the brain. However, canonical quantitative EEG (qEEG) biomarkers are often limited to pre-defined frequency bins (designated as Greek letters such as alpha, theta, and gamma waves) that impose constraints and bias data analyses. As such, there is an urgent need for identifying more sensitive and robust qEEG biomarkers. In this analysis, we built a machine learning model to enable the unbiased detection of novel EEG biomarkers that takes into account the temporal dynamics of the EEG signal. This is especially important in cases where spectral differences are not expected to be static, such as for pharmacological biomarker discovery, whereby pharmacodynamic effects are dependent on the drug's pharmacokinetic profile, and in cases of brain-state dependent spectral changes. Given the temporal dynamic of EEG signals, we used a recurrent neural network (RNN) model to ingest and capture the time-series EEG data and to automatically select optimal qEEG features. We developed and implemented various time-series data augmentation techniques to increase the size and diversity of training data for robust and generalizable training of the RNN models. We also evaluated different model architectures and hyperparameters. As a result, we achieved a reliable model with high classification performance. By analyzing the feature importance, specific frequency bins were obtained as potential qEEG biomarkers in a genetic mouse model with relevance to bipolar disorder and schizophrenia. We are currently integrating our models with an Amazon Web Services-based Streamlit processing and visualization pipeline to identify novel EEG biomarkers over hundreds of mice with an easy to use, scientist-friendly interface.

Disclosures: L. Gou: None. **D.J. Graziano:** None. **M.M. Sidor:** None. **B. Magnusson:** None.

Digital Abstract Session

P383. Techniques in Neurodegenerative and Neuropsychiatric Diseases' Research

Program #/Poster #: P383.08

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: PAPIIT IA202120

Title: Structural neuroadaptative changes as a consequence of alcohol use disorder: A pilot study.

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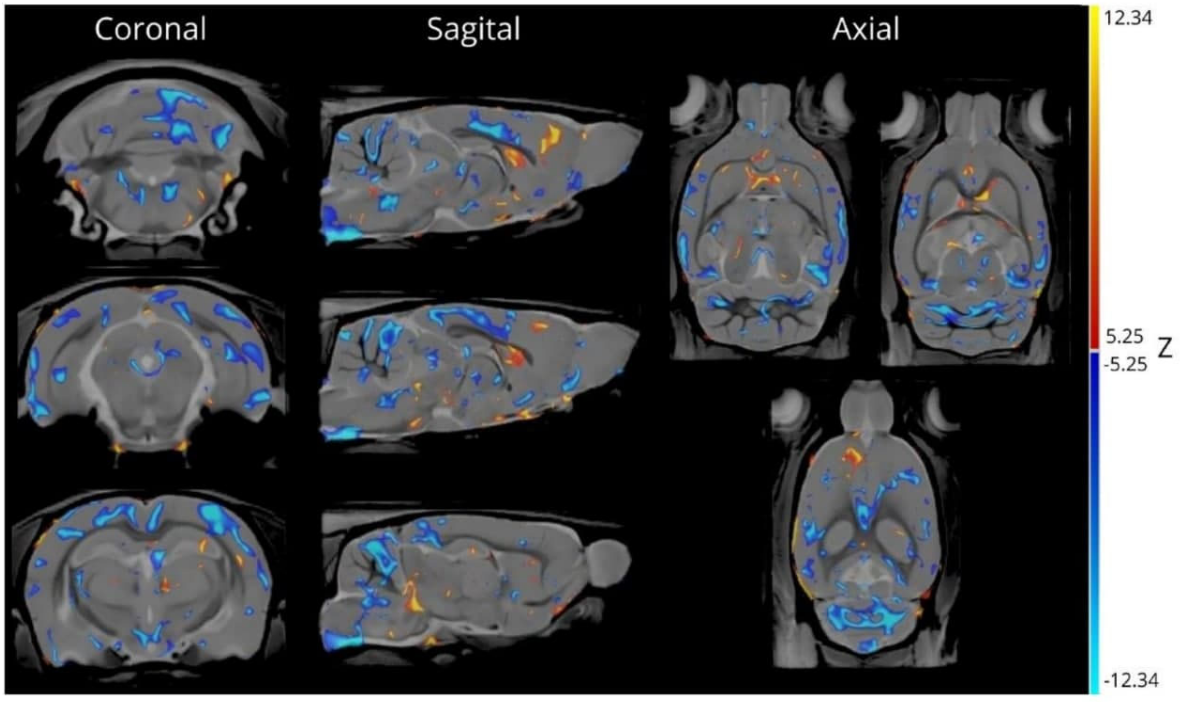
Abstract: Alcohol use disorder (AUD) is a complex neuropsychiatric condition that combines behavioral, neurobiological, and psychosocial alterations. The development of AUD has been characterized as a three-stage cycle. We need behavioral models that capture AUD aspects. These three stages can modeled in animals with the assumption that they emulate the human AUD characteristics. Longitudinal neuroimaging analysis of structure may help us understand

the neuroadaptive changes in the brain as a consequence of AUD. We implemented a pre-clinical AUD, the Intermittent-Access Ethanol 2-Bottle-Choice Drinking Paradigm (IA2BC). The model started in P45 with n=3 (2 female) Wistar rats with continuous-Access 10%-20% ethanol on a 12-hour reversed light/dark cycle (7 am). Brain structutral images were acquired using a Bruker 7T MRI scanner with a 2x2 surface array rat coil using a FLASH sequence. First acquisition was before the IA2BC model as baseline, and the second MRI acquisition was after finishing the 20 sessions (45 days) of ethanol intake. All images were preprocessed by: center image, denoising, and N4-Bias Field Correction. We used deformation-based morphometry to create Jacobian maps per subject and time point using an in-house pipeline, Two Level DBM based on ANTS based on Fisher atlas parcellation, with mixed model GLM and correction for multiple comparisons using an FDR 5%. The main alcohol intake (g/kg/24hrs) was 3.64 ± 0.66 (mean/sd). We found local structural changes after 20 sessions of alcohol consumption in several brain regions. When evaluating the changes in deformation-based morphometry in rats, in both logistic models we did not find any significant coefficients of brain changes related to a specific stage. Conclusions We found robust local neuroadaptive structural changes related to chronic voluntary alcohol use in a small sample of Wistar rats. Our future study will include a sample of 48 rats as well as functional connectivity analysis and immunofluorescence techniques to understand the functional microstructural neuroadaptive changes related to AUD.



Disclosures: **D.A. Valdez:** None. **A. López-Castro:** None. **E. Garza-Villarreal:** None. **S. Alcauter:** None.

Digital Abstract Session

P383. Techniques in Neurodegenerative and Neuropsychiatric Diseases' Research

Program #/Poster #: P383.09

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Clinical predictions in CNS drug discovery based on in vivo systems response profiles and non-linear machine learning methodology

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Abstract: Background
The Integrative screening Process, ISP, is a CNS drug discovery platform based on in vivo phenotypic profiling (Waters et al, ACS Chem Neurosci, 2017). Systems response profiles capturing pharmacological effects on neurochemistry, behaviour and gene expression are uniformly collected for all test compounds of interest, and analysed by linear dimensionality reducing methods such as principal component analysis (PCA) and partial least squares regression (PLS). This approach enables visualization of trends and clusters among therapeutic classes, as well as prediction of therapeutic class for novel compounds, while maintaining full transparency regarding underlying biological effects and differences in response profiles. However, non-linear features, such as non-monotonous dose response patterns are less well modelled. In this work we wanted to explore if non-linear methods could capture more information and improve the models and predictions.
Methods
Nonlinear analysis methodology was applied for the analysis of multidimensional in vivo pharmacological dose-response data (post mortem neurochemistry in several brain regions, behavioural patterns) on a broad array of CNS therapeutics and exploratory compounds from different therapeutic or mechanistic classes. Kernel PLS and t-distributed stochastic neighbor embedding (tSNE) were applied for dimensionality reduction and visualization. Classification of compounds into clinical classes based on therapeutic usage was performed using multinomial logistic regression as well as random forest and multi-layer perceptron networks. Based on performance metrics (precision, recall, F1 score), the best performing classifier was applied on a separate set of 85 compounds.
Results and conclusion
Results from nonlinear dimensionality reduction methods yielded similar results, compared to linear methods. However, in particular t-SNE modelling resulted in somewhat different clustering and appears promising to build upon in further applications. The best performing classification method, the perceptron network, shows results corresponding to well known effects for 71% of the compounds evaluated. Moreover, classifications of 12% of the compounds indicate potentially unknown effects, which are interesting and could be a springboard for further

analysis. In conclusion, based on in vivo phenotypic response profiles, non-linear classification methods, in particular multilayer perceptrons, performed well in terms of correct classifications, and further, indicated potential new classes of interest in several cases, suggesting use eg for drug repurposing purposes.

Disclosures: K. Granbom: None. **F. Wallner:** A. Employment/Salary (full or part-time):; Integrative Research Laboratories. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrative Research Laboratories. **P. Svensson:** A. Employment/Salary (full or part-time):; Integrative Research Laboratories. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrative Research Laboratories. **S. Holm Waters:** A. Employment/Salary (full or part-time):; Integrative Research Laboratories. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrative Research Laboratories. **J. Kullingsjö:** A. Employment/Salary (full or part-time):; johan.kullingsjo@irlab.se. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrative Research Laboratories. **N. Waters:** A. Employment/Salary (full or part-time):; Integrative Research Laboratories. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrative Research Laboratories. **A. Andersson:** None.

Digital Abstract Session

P383. Techniques in Neurodegenerative and Neuropsychiatric Diseases' Research

Program #/Poster #: P383.10

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: COH-0013 from Steven A. Cohen for the RAPID-DX program
COH-0003 from Steven A. Cohen for the RAPID-DX program

Title: Driving progress in biomarkers of neuropsychiatric disease: RAPID-Dx's metabolomic cross-platform comparison study

Authors: *L. CHABY[1], H. C. LASSETER[2], A. THOMPSON[1], T. VAUGHAN[1], L. LANCASHIRE[1], M. HAAS[3], A. JEROMIN[1];
[2]Dept. Psychology, [1]Cohen Veterans Biosci., Cambridge, MA; [3]Cohen Veterans Biosci., New York, NY

Abstract: *Objectives*: Biomarker discovery and validation will require large scale 'omics investigations across diverse populations, yet the integration and replication of 'omics data remains a key challenge in neuropsychiatric research. To determine replicability and reliability of analytical approaches in leading biomarker modalities and facilitate analysis at the scale needed for biomarker discovery, Cohen Veterans Bioscience - through its Research Alliance for

Precision Therapeutics, Innovation and Diagnostics (RAPID-Dx) Program - is conducting cross-platform comparisons in multiple domains of interest for neuropsychiatric disease, with findings in inflammation recently published (Lasseter, et al. *Cytokine X* 2020). Current efforts are directed at metabolomics, which provides a global assessment of metabolites resulting from biochemical activity and can be a powerful tool for understanding complex mechanisms of disease.
Methods: We conducted a systematic literature review of metabolomics efforts in PTSD to structure an evaluation of technical performance and dynamic range of metabolomics assay platforms. Clinical and healthy control biospecimens as well as technical standards were utilized for comparisons across and within platforms. Coverage and platform performance was mapped for metabolites implicated in PTSD through (i) case-control studies and (ii) genome-wide association studies (GWAS) of PTSD.
Results: Our systematic review revealed that prior efforts in PTSD have largely relied on discovery-based approaches and workflows. PTSD findings varied across metabolomics techniques, but emphasized essential and long chain fatty acids. Of metabolites implicated in PTSD, platform coverage ranged from 25-80%. For metabolites associated with PTSD GWAS loci, e.g. PARK2, coverage varied from 5-90%. Technical performance varied across analytical workflows; for example, coefficients of variation (CV%) within assays for duplicate PTSD samples ranged from 0.4-76.1%.
Conclusion: The metabolomics cross-platform comparison will help identify the best assay platforms to discover the metabolomic signature of PTSD. Further, this work has generated a metabolomics database to facilitate recommendations across clinical states. Together with findings from the RAPID-Dx inflammatory-markers cross-platform comparison, this study highlights the importance of considering assay performance in interpreting and designing biomarker studies. RAPID-Dx will continue conducting cross-platform comparisons across domains of interest for neuropsychiatric disease, with planned evaluations for transcriptomics and epigenetics.

Disclosures: **L. Chaby:** None. **H.C. Lasseter:** None. **A. Thompson:** None. **T. Vaughan:** None. **L. Lancashire:** None. **M. Haas:** None. **A. Jeromin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Quanterix.

Digital Abstract Session

P384. Cellular Models

Program #/Poster #: P384.01

Topic: I.06. Computation, Modeling, and Simulation

Title: Development and characterization of in vitro perineural invasion model to study peripheral nerve-cancer cell interaction

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Abstract: Perineural invasion (PNI) is the invasion of cancer cells in and around nerves influencing the pathological characteristics of malignant tumors, usually associated with low survival rate and poor prognosis of patients. PNI is often characterized by neuropathic pain, sensory and motor dysfunction, paralysis and disfigurement. The mechanism associated with PNI is not well-understood due to lack of relevant *in vitro* models. We developed for the first time a 3D culture model that recapitulates the PNI features observed *in vivo*. Our approach involves co-culture of thoracic or lumbar dorsal root ganglia (DRG)-nerve explant with cancer cells in Matrigel. Using the breast cancer cells MDA MB 231 and prostate cancer cells DU145, we demonstrated that seeding the cells at specific orientation and distance from the nerve ending promotes PNI *in vitro*. Visualization of nerve invaded cancer cells was done by immunostaining of cancer cells using cytokeratin or by using e-GFP tagged cancer cells. Characterization of PNI events over two weeks showed selective migration of cancer cells towards nerve endings by interacting with neurites and glial cells during the initial days of culture. By 7-10 days, the cancer cells surrounded the entire DRG-nerve. Fluorescence microscopy of cytokeratin stained/eGFP labelled cancer cells in DRG-nerve sections demonstrated the presence of cancer cells within nerve layers. The number of cells within the nerve layers increased in a time-dependent manner over two weeks. The changes in sensory axons and glial cells that are in contact with cancer cells within nerves were visualized using NF200 and GFAP/S100beta staining. We also confirmed the proliferation status of nerve-invaded cancer cells in contact with axons/glial cells using the cell proliferation marker Ki67 and by visualizing BrdU uptake. Overall, this new model will serve as a tool to study the fundamental mechanism/s of PNI. This model will also serve as a screening tool for developing PNI therapeutics for the management of cancers.

Disclosures: S. Dwivedi: None. **M. Bautista:** None. **A. Krishnan:** None.

Digital Abstract Session

P384. Cellular Models

Program #/Poster #: P384.02

Topic: I.06. Computation, Modeling, and Simulation

Title: Modeling and Characterization of Single Cytoskeleton Filaments

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Univ. of Texas At San Antonio.

Abstract: A variety of biological functions of cytoskeletal filaments involve the formation of high order structures such as bundles and network arrangements which mainly depend on their individual electrostatic, semi-flexibility, and polymerization degree properties, as well as, the biological environment. In this presentation, we introduce an unprecedented modeling and characterization of single cytoskeleton filaments. We combined accurate models and multi-scale theories to characterize polyelectrolyte properties of actin filaments including the electric potential and charge, electrophoretic mobility, filament length distribution, semi-flexibility, and

hydrodynamic size.
Additionally, we performed Dynamic Light and Electrophoresis Scattering experiments on actin filaments in solution at dilute concentrations to measure their diffusivity (hydrodynamic sizes) and electrophoretic mobility (surface electric potentials) in physiological conditions. We optimized the formulation by adjusting some, otherwise unknown, atomistic-scale parameters to reproduce the experimental results. We used several polymerization buffers and pH electrolyte solutions to characterize these actin filament properties in a variety of biological conditions. Our preliminary results on the diffusion coefficient show a correlation between the nucleation/elongation and the ATP concentration. A buffer with high concentration of ATP resulted in a high diffusion coefficient of, i.e. 6.44 ± 0.153 um^2/s, in contrast to a lower diffusion coefficient of, i.e. 1.39 ± 0.0714 um^2/s, using less amount of ATP. Based on the theoretical approach, we concluded that high ATP concentration results in faster nucleation of G-actins followed by a faster formation of short actin filaments. This, in turn, generates a high diffusion coefficient. Whereas, lower concentrations of ATP result in longer nucleation times of G-actins. This forms longer actin filaments which lead to lower diffusion coefficient values.

Disclosures:

Digital Abstract Session

P384. Cellular Models

Program #/Poster #: P384.03

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH R01MH118930
NIH R01NS109498
NIH RF1MH117428
BMBF 01GQ1804A
BMBF 031L0229
von Behring Röntgen Foundation

Title: Multi-scale modeling of morphologically realistic neurons under transcranial magnetic stimulation

Authors: *S. SHIRINPOUR[1], N. HANANEIA[2], J. ROSADO[3], C. GALANIS[4], A. VLACHOS[4], P. JEDLICKA[2], G. QUEISSER[3], A. OPITZ[1];
[1]Univ. of Minnesota Twin Cities, Minneapolis, MN; [2]Justus Liebig Univ., Giessen, Germany; [3]Temple Univ., Philadephia, PA; [4]Neuroanatomy, Med. Faculty, Univ. of Freiburg, Freiburg, Germany

Abstract: Transcranial Magnetic Stimulation (TMS) of the dorsolateral prefrontal cortex is currently utilized as a non-invasive neuromodulation tool for the treatment of depression. Nevertheless, the basic mechanisms underlying TMS effects at the neuronal level are largely unknown. Since the recording of single-unit activity *in vivo* during TMS is challenging in

animals, and typically not possible for humans, modeling is an important tool to investigate the neuronal response to TMS.Here, we develop a new multi-scale pipeline (*NeMo-TMS*) for modeling TMS effects across spatial scales. On the macro-scale, we generate a realistic head model from MRI that includes multiple tissue types (white matter, grey matter, cerebrospinal fluid, skull, and scalp) and use the SimNIBS software to simulate the electric field induced in the brain for a given TMS coil position and orientation using Finite Element Method (FEM). Afterward, simulated electric fields from the previous step are coupled with morphologically realistic neuronal models. These models are solved by discretizing the neuronal branches into small compartments and by numerically solving the cable equation and channel dynamics in the NEURON environment. These neuron-scale simulations allow investigating membrane voltage (depolarization/hyperpolarization), action potential initiation and propagation, field intensity, and orientation necessary for modulating neuron response, etc. Then, we incorporate the membrane voltage data to simulate the calcium concentration induced by voltage-gated calcium-channels at the subcellular scale by solving the calcium dynamics equations. This step is important for understanding neural plasticity due to rTMS protocols. In this study, we also provide results to demonstrate how different neuron types behave distinctly for the same external electric field and therefore make a case for the importance of such multi-scale realistic modeling of neurons. Our pipeline can facilitate research as a tool for hypothesis testing and prediction technique for experiments. Additionally, modeling has the potential to be used for calculating the dose needed for efficient treatment in clinical applications.

Disclosures: S. Shirinpour: None. **N. Hananeia:** None. **J. Rosado:** None. **A. Vlachos:** None. **P. Jedlicka:** None. **G. Queisser:** None. **A. Opitz:** None. **C. Galanis:** None.

Digital Abstract Session

P384. Cellular Models

Program #/Poster #: P384.04

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF GRFP
ONR Grant 7020124

Title: Towards biophysically-based neuromorphic computing at scale: Markov abstractions of electochemical reaction-diffusion in synaptic transmission

Authors: *M. WAGNER[1,2], T. M. BARTOL[2], T. J. SEJNOWSKI[2], G. CAUWENBERGHS[1]; [1]Dept. of Bioengineering, UCSD, La Jolla, CA; [2]Salk Inst., La Jolla, CA

Abstract: Progress in computational neuroscience towards understanding brain function is challenged both by the complexity of molecular-scale electrochemical interactions at the level of individual neurons and synapses, and the dimensionality of network dynamics across the brain covering a vast range of spatial and temporal scales. Our work abstracts the highly detailed, biophysically realistic 3D reaction-diffusion model of a chemical synapse to a compact internal

state space representation that maps onto parallel neuromorphic hardware for efficient emulation on very large scale, and offers near-equivalence in input-output dynamics while preserving biologically interpretable tunable parameters.Methods: We initially built a physically realistic stochastic 3D reaction-diffusion system in containing all major components for presynaptic vesicle release variability in response to a stimulus input using MCell. The model included realistic geometry for a CA3-CA1 Schaffer collateral en passant synapse with parameters set from experimental data. The CA3-CA1 Schaffer collateral was chosen as it is highly studied experimentally and is important for learning and memory [5, 6].
We modelled an action potential input followed by the stochastic opening and closing of voltage-dependent calcium channels (VDCCs) located on the membrane resulting in calcium influx into the presynaptic bouton and calcium diffusion, binding to calbindin and calcium sensors, and removal due to plasma membrane calcium pumps (PMCA pumps). These factors directly determine the quantity of neurotransmitters released and thus the strength of the synaptic connection. Abstracting this for computational efficiency, we created a series of Markov state transitions to fully realize the system with multiple internal states done in Python allowing for a biophysically tunable model of synaptic connectivity implementable in neuromorphic architectures.

Disclosures: **M. Wagner:** None. **T.M. Bartol:** None. **T.J. Sejnowski:** None. **G. Cauwenberghs:** None.

Digital Abstract Session

P385. Network Modeling: Experimentation and Theory

Program #/Poster #: P385.01

Topic: I.06. Computation, Modeling, and Simulation

Support: HGF young investigator's group VH-NG-1028
European Union’s Horizon 2020 research and innovation program under Grant agreements No. 785907 (Human Brain Project SGA2) and No. 945539 (Human Brain Project SGA3)
ANR grant GRASP
Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - 368482240/GRK2416

Title: Long-range coordination patterns in cortex change with behavioral context

Authors: ***D. DAHMEN**[1], M. LAYER[1], L. DEUTZ[2], P. A. DABROWSKA[1], N. VOGES[3], M. VON PAPEN[1], T. BROCHIER[3], A. RIEHLE[3], M. DIESMANN[1], S. GRÜN[1], M. HELIAS[1]; [1]Juelich Res. Ctr., Juelich, Germany; [2]Univ. of Leeds, Leeds, United Kingdom; [3]Inst. de Neurosciences de la Timone (INT), CNRS - Aix-Marseille Univ., Marseille, France

Abstract: The cerebral cortex is a network of subnetworks that is organized on various spatial scales. Understanding how neurons communicate at the different scales is crucial for

understanding brain dynamics and function. On the microscopic scale the connectivity stems mostly from local axonal arborizations, suggesting coordination is strongest between nearby neurons in the range of a few hundred micrometers. Yet recent studies found activity of neurons across much larger distances to be organized in manifolds. The emergence of such manifolds relies on complex coordination patterns between neurons. We here analyze multi-electrode recordings of resting-state activity in macaque motor cortex that indeed show strong positive and negative spike-count covariances between neurons that are millimeters apart. To understand the origin of such coordination we develop a conceptually novel network theory that combines the spatial extent and heterogeneity of the connectivity with fluctuations of activity treated beyond the mean-field approximation. This quantitative theory uncovers a simple and ubiquitous mechanism that generates long-range correlation patterns despite short-range connections: the heterogeneity of connections causes a dynamical network state that emphasizes cooperation of neurons by multi-synaptic interactions. The mechanism does not rely on specific connectivity structures, but emerges in spatially organized networks with even random connectivity. The theory not only explains the experimentally observed shallow exponential decay of the width of the covariance distribution at long distances, but also predicts that neuronal coordination patterns can change in a state-dependent manner. We confirm this prediction by comparing activity in macaque motor cortex across different behavioral epochs of a reach-to-grasp experiment. Our results explain how spatially extended neural manifolds can emerge from the local network connectivity.

Disclosures: **D. Dahmen:** None. **M. Layer:** None. **L. Deutz:** None. **P.A. Dabrowska:** None. **N. Voges:** None. **M. von Papen:** None. **T. Brochier:** None. **A. Riehle:** None. **M. Diesmann:** None. **S. Grün:** None. **M. Helias:** None.

Digital Abstract Session

P385. Network Modeling: Experimentation and Theory

Program #/Poster #: P385.02

Topic: I.06. Computation, Modeling, and Simulation

Support: MOST Grant 107-2218-E-007-033
The Higher Education Sprout Project funded by the Ministry of Science and Technology and Ministry of Education in Taiwan

Title: Interplay between feedback and mutual Inhibition increases network flexibility via a multi-cusp bifurcation.

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Abstract: One of the most intriguing properties of recurrent neural circuits is their flexibility. This flexibility extends far beyond the ability to learn, but includes the ability to use learned procedures to respond to novel situations. Here using a various sized spiking neuron models, we

show how networks with recurrent inhibition are key in expanding the functionality of the circuit, far beyond what feedback inhibition alone can accomplish. By adding mutual inhibition to small neural motifs, decision-like functionality is off-loaded onto the inhibitory sub-network. This frees up recurrent excitation for working memory. More importantly, feedback inhibition and mutual inhibition work synergistically to allow a plethora of central pattern generators to coexist with decision networks. This allows for quick, robust and flexible external control, without changing any synaptic weights. Taking advantage of dynamical system theory and bifurcation analysis we show mutual inhibition adds to the number of cusp bifurcations that can coexist in the system. This multi-functionality allows robust control of the underlying multi-cusp bifurcation structure by using bias current to push the system through lower co-dimension bifurcations. Thus changes in bias current can quickly switch between different functionalities including 8 different logical operations with 3 different classes of inputs, distinguishing between differences in magnitude, timing, and phase. Furthermore we uncovered several types of central pattern generators, working memory, and chaos. Finally, we showed that the underlying multi-cusp explains why mutual inhibition increases the information storage (Shannon entropy) of larger neural networks. Moreover, the location of the multi-cusp bifurcation in parameter space is near the optimal information capacity for the network.

Disclosures: **A.J. White:** None. **B. Liu:** None. **C. Lo:** None.

Digital Abstract Session

P385. Network Modeling: Experimentation and Theory

Program #/Poster #: P385.03

Topic: I.06. Computation, Modeling, and Simulation

Title: Systematic assessment of cell numbers in the mouse hippocampus

Authors: ***S. SUDHAKAR**[1], N. VELLA[2], S. KALI[3,4], H. MARKRAM[1], A. ROMANI[1], S. RAMASWAMY[1];
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Abstract: The hippocampus is a brain region involved in a spectrum of tasks such as learning, memory and spatial navigation. Cellular and network dysfunction of the hippocampus is implicated in a number of neurological disorders. In order to better understand the computations performed by the hippocampus and how those computations become abnormal in pathological conditions, it is essential to construct a biophysically realistic, large-scale circuit model of the hippocampus and study its spatiotemporal activity patterns. As a first step, we undertook a massive literature search to compute the total number and the distribution of diverse neuron types in the CA1, CA3 and dentate gyrus of the mouse hippocampus. Most of our assumptions were derived from experimental literature on the mouse hippocampus. We overcame missing

data by adapting relevant literature on the rat hippocampus. Our results indicate that the molecular make up and the morphology of a significant number of GABAergic neurons in the mouse CA1, CA3 and dentate gyrus remain uncharacterized. We also report that the mouse CA1 is much denser than the rat CA1 and identified significant cell type-specific differences between the two species. In conclusion, our work could provide a solid foundation for constructing a large-scale model of the mouse hippocampus and enable predictions on its involvement in brain function and dysfunction.

Disclosures: **S. Sudhakar:** None. **N. Vella:** None. **S. Kali:** None. **A. Romani:** None. **H. Markram:** None. **S. Ramaswamy:** None.

Digital Abstract Session

P385. Network Modeling: Experimentation and Theory

Program #/Poster #: P385.04

Topic: I.06. Computation, Modeling, and Simulation

Title: A firing-rate computational model of the mouse dorsal lateral geniculate nucleus: the effects of cortical feedback

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Abstract: The lateral geniculate nucleus (LGN) has traditionally been viewed as a passive relay station transmitting visual information from the retina to the cortex. However, recent literature suggests it may modulate retinal signals before reaching the cortex and thus play a key role in information processing. To better understand the role of cortical feedback in the LGN, we constructed a firing rate model based on experimental mouse LGN data. We created two instances of a high-level firing-rate model of the early visual system based on the extended difference-of-gaussian model, with only one including a thalamocortical loop. The cell populations, comprising of retinal ganglion cells (RGC), LGN relay cells, and primary visual cortex (V1) cells, were modelled as 2-dimensional layers using the python package pyLGN. Connecting kernel functions transmitted visual information between cell populations. Receptive field (RF) data collected experimentally in mice was used as the feedforward kernel between the RGCs and the relay cells. The RFs were modelled to have two spatially shifted and rotated bivariate Gaussian components, each varying temporally in strength according to a Gaussian function. Fullfield grating patterns were fed into the RGCs as Stimuli and we compared the spatiotemporal tuning curves of the model's relay cells with their experimental counterparts by calculating their Pearson correlation coefficient. Cells with correlation values of greater than +0.5 were considered to have been accurately modelled. Without cortical feedback, 149 of 417 cells had their spatial tuning properties accurately predicted; the addition of cortical feedback reduced this to 144 of 417. The model predicted temporal properties more accurately, with 207 of 417 cells being well modelled without cortical feedback, and 204 cells being accurately

modelled with feedback. Due to the blanket application of cortical feedback, accuracy reduced overall, though many individual cells' behaviour converged to the experimentally observed behaviour. This could evidence literature suggesting cortical feedback may neither be uniformly nor constantly applied to all relay cells. Alternatively, this may highlight our model's limitations, and future work should incorporate the addition of multiple thalamocortical loops.

Disclosures: **S. Chawla:** None. **J.D. Goodwin:** None. **R.H.L. Natarajan:** None. **S.R. Schultz:** None.

Digital Abstract Session

P385. Network Modeling: Experimentation and Theory

Program #/Poster #: P385.05

Topic: I.06. Computation, Modeling, and Simulation

Support: "This study was supported by funding to the Blue Brain Project, a research center of the École polytechnique fédérale de Lausanne (EPFL), from the Swiss government's ETH Board of the Swiss Federal Institutes of Technology."

Title: Input representation and processing in a computational model of the rat primary somatosensory cortex

Authors: *S. BOLAÑOS-PUCHET, A. ECKER, C. POKORNY, J. ISBISTER, V. SOOD, M. GEVAERT, P. KUMBHAR, A. ARNAUDON, L. KANARI, W. VAN GEIT, H. MARKRAM, J.-D. COURCOL, M. REIMANN, S. RAMASWAMY;
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Abstract: In recent years, simulation of large-scale computational models of the cortex has emerged as a promising way to study neural processing. However, due to computational costs and difficulty of parametrization, detailed reconstructions have so far been restricted to small volumes of tissue representing isolated cortical columns. In this work, we present a data-driven computational model of the primary somatosensory cortex of the rat built with the aim of gaining insights into how sensory inputs are represented and processed in the cortex at a region-wide level. The model consists of 4.2 million biophysically detailed neurons of 60 different morphological types and 212 different morpho-electrical types, distributed across 8 sub-regions encompassing the entirety of the non-barrel primary somatosensory cortex, and connected with 9.1 billion synapses featuring short-term dynamics. This circuit was built using our previously established methodology, now extended to reproduce the actual geometry of tissue based on the representation of somatosensory regions in a digitized rat brain atlas. Beyond local connectivity, two projection systems have been built: thalamocortical afferents that allow for extrinsic controlled input, and between-region connectivity which provides intrinsic feed-forward and feedback pathways between the various somatosensory sub-regions. After an initial exploration of the emerging dynamical behavior of the circuit with different simulation parameters, an *in vivo*-like working state was found. This state is characterized by asynchronous activity but sits

close to the transition to the synchronous regime, and is where *in vivo*-like behavior has been previously observed. Under these conditions, we analyzed the response of the circuit to realistic thalamocortical input spike trains in different spatial configurations to study phenomena such as input representation, distributed cortical processing and horizontal propagation of activity. Additionally, we investigated the interactions between the various somatosensory sub-regions by comparing simulations with and without between-region connectivity. These initial results provide a starting point for further studies in sensory cortical processing using this circuit to test hypotheses, compare with experimental findings, and make useful predictions.

Disclosures: **S. Bolaños-Puchet:** None. **A. Ecker:** None. **C. Pokorny:** None. **J. Isbister:** None. **V. Sood:** None. **M. Gevaert:** None. **P. Kumbhar:** None. **A. Arnaudon:** None. **L. Kanari:** None. **W. Van Geit:** None. **H. Markram:** None. **J. Courcol:** None. **M. Reimann:** None. **S. Ramaswamy:** None.

Digital Abstract Session

P385. Network Modeling: Experimentation and Theory

Program #/Poster #: P385.06

Topic: I.06. Computation, Modeling, and Simulation

Support: JSPS KAKENHI: 15KK0010
JSPS KAKENHI: 16K00330
JST CREST: JPMJCR1914

Title: Data-driven analysis of information transfer in cortical neural circuit model with extracellular electric field

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Abstract: Recent experimental studies suggest that the extracellular electric field influences information processing in neural systems. Previous theoretical studies showed that neuronal responses in single neurons are controlled by the effect of extracellular electric field. However, it is still unclear what kind of effect the extracellular electric field has on signal transmission in neuronal networks of the cortex.
In this study, we propose a mathematical model of cortical neural circuits that introduces a mechanism that can provide electric fields from outside the cell, and investigated the effects of extracellular electric fields on the neural circuits by numerical simulations. The neural circuits in the proposed model are designed to represent the layered structure of the cerebral cortex. We apply the mechanism of extracellular electric field to the cortical neural circuit and develop a model that can simulate the neural circuit by considering the extracellular electric field. We consider not only a uniform (constant) DC electric field but also a periodically oscillating AC electric field with a specific frequency.
In order to investigate the effect of extracellular electric field on cortical neural circuit, we

performed simulations using the proposed model. To quantify information transfer between cortical layers, we utilized an information theoretic index called normalized transfer entropy. The simulation result showed that the normalized transfer entropy between some layers decreased, whereas the normalized transfer entropy between other layers increased. We also found in specific cortical layers that the change in normalized transfer entropy depends on the frequency of applied extracellular electric field; there may exist optimal frequency for enhancing the normalized transfer entropy, and the effect of extracellular electric field on normalized transfer entropy is modulated depending on internal connectivity of the neural circuits. Our results suggest that the extracellular electric field may control the information transfer between layers in cortical networks.

Disclosures: **N. Matsumoto:** None. **T. Omori:** None.

Digital Abstract Session

P385. Network Modeling: Experimentation and Theory

Program #/Poster #: P385.07

Topic: I.06. Computation, Modeling, and Simulation

Title: Multiple bumps enhance robustness to noise in continuous attractor networks

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Abstract: A central function of continuous attractor networks is encoding continuous variables and accurately updating their values through path integration. To do so, these networks produce localized bumps of activity that move coherently in response to inputs. In the brain, continuous attractors are believed to underlie grid cells and head direction cells that maintain periodic representations of space. However, path integration and various types of periodic tuning can be achieved with any number of activity bumps, and the consequences of producing more or fewer bumps are unclear. To address this problem, we construct continuous attractor networks with different bump numbers and assess their ability to path-integrate in the presence of noise. Single-bump networks with more neurons exhibit greater diffusive motion, which would lead to increased error. However, accuracy can be rescued by introducing multiple activity bumps, which suppresses noise-induced diffusion. Our findings may be particularly salient for large, noisy biological systems, such as the mammalian grid cell network, which could employ multiple attractor bumps to avoid massive error.

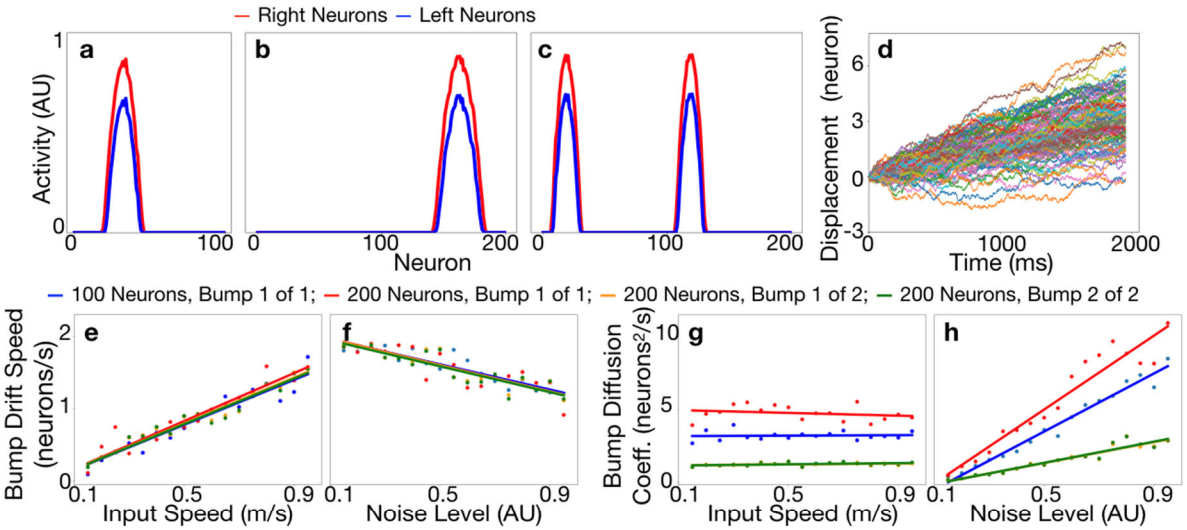

Figure 1: (**a-d**) Bump profiles and motion. (**a-c**) Activity snapshots of networks with (**a**) 100 neurons per population and one bump, (**b**) 200 neurons per population and one bump, (**c**) and 200 neurons per population and two bumps. (**d**) Bump trajectories for 100 replicate simulations that demonstrate noise-induced diffusion relative to the average drift. (**e-h**) Path integration and robustness to noise. Points indicate averages over 100 simulations and lines indicate linear fits. (**e**) At fixed noise level 0.5 AU, bump drift speed is proportional to input speed, which allows for unbiased path integration. (**f**) At fixed input speed 1 m/s, bump speed decreases at higher noise levels. As in **e**, all networks share the same bump speeds. (**g**–**h**) Noise-induced diffusion of bumps increases with neuron number but decreases with multiple bumps. Values for each bump in the two-bump network are almost equivalent. (**g**) Fixed noise level 0.5 AU. (**h**) Fixed input speed 1 m/s.

Disclosures: **R. Wang:** None. **L. Kang:** None.

Digital Abstract Session

P385. Network Modeling: Experimentation and Theory

Program #/Poster #: P385.08

Topic: I.06. Computation, Modeling, and Simulation

Support: KEP-5/2019 (Hungarian Academy of Sciences)

Title: Reliability of EEG functional connectivity based modular network organization

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[2]Dept. of Measurement and Information Systems, Budapest Univ. of Technol. and Econ., Budapest, Hungary

Abstract: A large number of fMRI studies have reported that large-scale brain networks are organized into separated but interacting modules, typically studied in resting-state. Modularity is assumed to reflect functional segregation within the integrated network and is thought to confer robustness and adaptability to brain connectivity networks. However, fMRI data capture functional relations among brain regions on a timescale of seconds, making it difficult to link networks with cognitive functions, while MEG data remains relatively scarce. The present study aims to explore the reliability of large-scale network modularity as measured with resting-state

EEG rhythms (0.5-80 Hz) on a large sample of young healthy adults (N=202; mean age= 22.4). We tested whether stable resting-state networks could be identified with EEG on the individual and the group-level and if those networks could be characterized with a shared modular structure. Phase synchrony (PLV; iPLV) and amplitude envelope correlation (AEC) was calculated to estimate functional connectivity between reconstructed cortical signals in five frequency bands (delta 0.5-4Hz; theta 4-8 Hz; alpha 8-13 Hz; beta 13-30 Hz; gamma 30-80 Hz). Modularity was defined using multislice community detection, with resolution parameters estimated against randomized (null) data. Individual reliability was assessed by halving the resting state data and group-level reliability by randomized subgroups. Our results reveal a stable modular organization occurring during rest irrespective of connectivity measures, with moderate correspondence to structures reported with different modalities. Observed modularity of connectivity might play an important role in state and trait processes of cognitive functioning.

Disclosures: P. Nagy: None. **B. Tóth:** None. **I. Winkler:** None. **Á. Boncz:** None.

Digital Abstract Session

P385. Network Modeling: Experimentation and Theory

Program #/Poster #: P385.09

Topic: I.06. Computation, Modeling, and Simulation

Support: Facial Pain Research Foundation

Title: Imaging Trigeminal Neuralgia Pain: Insights from Combining AI and Granger Causality

Authors: *Y. LIANG[1], Q. ZHAO[1], J. K. NEUBERT[2], M. DING[1];
[1]Biomed. Engin., [2]Orthodontics, Univ. of Florida, GAINESVILLE, FL

Abstract: Imaging Trigeminal Neuralgia Pain: Insights from Combining AI and Granger Causality

Yun Liang[a], Qing Zhao[a], John K. Neubert[b], Mingzhou Ding[aa]*J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida [b] Department of Orthodontics, University of Florida*

AbstractTrigeminal neuralgia (TN) is a severe and disabling chronic facial pain condition. It is considered one of the most severe pain conditions, often called the “suicide disease,” but presently available therapies for TN are mostly ineffective or inadequate. Both the unpredictable response to treatment and variability in long-term clinical outcomes in TN strongly suggest that a range of peripheral and central mechanisms underlying TN pain remain to be understood. This study aims to achieve two objectives. Objective 1 is to identify the regions in the brain that underlie TN pain. Objective 2 is to characterize how these regions interact to produce TN pain perception. Functional MRI (fMRI) data were recorded from TN patients (N=54) while they rated their pain levels in real-time using a tracking ball. Analytically, recognizing the limitations of traditional data analysis techniques (e.g., fMRI activation analysis), we turned to AI-inspired technology. In particular, a deep neural network (DNN) was designed and implemented, which

was able to take fMRI data as input features to predict the moment-to-moment fluctuations of pain levels with high accuracy (R=0.84). Importantly, from the DNN model, a set of brain regions, including the thalamus and the dorsal anterior cingulate cortex (dACC), were derived as signature centers of TN pain. Applying Granger causality analysis to fMRI data from the thalamus and dACC, we further showed that thalamus->dACC causal influence, but not dACC->thalamus causal influence, predicted daily experienced pain levels (R=0.55), consistent with the known physiology that the thalamus is a key brain structure in linking nociceptive sensory input to pain perception and dACC is a key brain structure for perceiving pain.

Disclosures: Y. Liang: None. **Q. Zhao:** None. **J.K. Neubert:** None. **M. Ding:** None.

Digital Abstract Session

P385. Network Modeling: Experimentation and Theory

Program #/Poster #: P385.10

Topic: I.06. Computation, Modeling, and Simulation

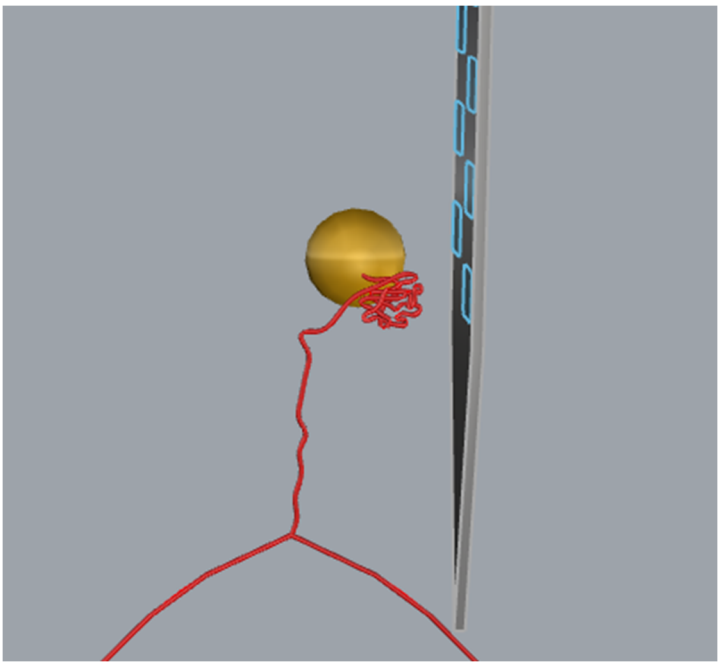
Support: NSF grant 1653080

Title: Microelectrode recordings from an A-delta fiber in dorsal root ganglion: A computational study

Authors: L. R. MADDEN, R. D. GRAHAM, S. F. LEMPKA, *T. M. BRUNS;
Biomed. Engin., Univ. of Michigan, Ann Arbor, MI

Abstract: Dorsal root ganglia (DRG) are discrete targets for neuromodulation to treat various conditions, such as bladder incontinence and chronic pain, due to the presence of sensory neurons that innervate specific dermatomes. DRG neurons are pseudounipolar, meaning they have a stem axon that protrudes from the cell body that splits into a peripheral branch that extends out to the body and a central branch that enters the spinal cord. The stem axons of larger neurons in the DRG have been found to have a long, tortuous path known as a glomerulus. The electrophysiological impact of the glomerulus on extraneural recorded waveforms of DRG neurons is unknown. Understanding the unique electrophysiology of DRG neurons would inform neuromodulation therapies that target them, such as for closed-loop monitoring. Here, we created a computational recording model of a DRG and a sample A-delta fiber within it. We constructed an anatomically-accurate multi-compartment model of an A-delta fiber that included the soma, glomerulus, and stem axon (Figure). We built a finite element model (FEM) of the DRG, surrounding tissues, and a microelectrode shank with multiple recording sites. We then used a two-step reciprocity-based approach to simulate an extraneural recording from the DRG. We varied the active electrode site in order to explore the effect of distance between the recording electrode and the neuron. The results of this model may provide key insights into how the electrophysiological properties of DRG neurons influence neural recordings. Additionally, this model may inform electrode development and closed-loop neuromodulation techniques for both

in-vivo experiments and clinical applications by providing a method of examining DRG electrophysiology under various therapeutic conditions.



Disclosures: **L.R. Madden:** None. **R.D. Graham:** None. **S.F. Lempka:** None. **T.M. Bruns:** None.

Digital Abstract Session

P385. Network Modeling: Experimentation and Theory

Program #/Poster #: P385.11

Topic: I.06. Computation, Modeling, and Simulation

Support: NIMH 1R01 MH117991-01

Title: Deconvolution and Analysis of Responses in Alternating Event Related fMRI Designs

Authors: ***S. DAS**[1,2], M. DING[3], G. R. MANGUN[1,2];
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Abstract: Functional magnetic resonance imaging (fMRI) uses blood oxygen level-dependent (BOLD) signals to index brain activity. Event-related fMRI approaches are especially useful in experimental studies because it enables the measure of different events of interest in studies of perception, cognition, and action. However, because the time course of the BOLD signal is slow (being on the order of several seconds) compared to the millisecond time course of the underlying neural activity, significant challenges arise for fMRI design and analysis, especially when events (e.g., stimuli) are presented closely in time. One key goal is to improve the

efficiency with which the underlying hemodynamic response evoked by one event type can be detected and estimated, and distinguished from other events. The use of experimental methods such as event randomization and strategic sequencing of events can alleviate some of these challenges, they are more difficult to employ in some experimental designs, where the event sequences follow a non-random order, as occurs in typical cued attention paradigms, separating cue events from target and response events is more complicated. We address these issues using simulation, by manipulating design parameters to determine optimal design structures. Our model includes nonlinear and transient properties of fMRI signals that might be expected in certain experimental designs. We also included a more realistic noise component in our model, using the package *fmrisim* (Ellis et al., 2020, PeerJ). Our study investigated the dependence of fMRI signal optimization on several design parameters: Inter-Stimulus-Interval (ISI), different transient properties, and proportion of null events. Our results show optimal ISI ranges for alternating event sequences. We found that it is difficult to simultaneously optimize both detection power (ability to detect a signal) and estimation efficiency (the ability to model the hemodynamic function). We demonstrate how the inclusion of null events is critical for detection power at shorter ISIs. In contrast, there is a direct relationship between the proportion of null events and estimation efficiency. Our study provides additional information to be used to design optimal experimental paradigms for alternating event sequences. Through our assumption of a practical nonlinear model for the refractoriness of the blood flow, implementation of a realistic noise model, and taking into account the various transient properties, we have presented a theoretical structure that can provide insight into how the performance of an alternating event sequence in fMRI studies varies based on different parameters of the design.

Disclosures: S. Das: None. **M. Ding:** None. **G.R. Mangun:** None.

Digital Abstract Session

P386. Computational Tools: Experimental

Program #/Poster #: P386.01

Topic: I.06. Computation, Modeling, and Simulation

Title: Estimating neuronal and glial counts non-invasively using diffusion weighted imaging

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[1]Univ. of California, Irvine, Irvine, CA; [2]Univ. of Bordeaux, Bordeaux, France; [3]The French Natl. Ctr. for Scientific Res., Paris, France

Abstract: Diffusion-weighted imaging (DWI) has uncovered important white matter characteristics tied to development, aging, disease, and even individual differences. These discoveries suggest the massive potential of DWI to non-invasively detect explicit neurobiological properties, beyond what is possible within the realm of conventional neuroimaging. Moreover, recent advances in both DWI acquisition and analysis have enabled us to detect *in vivo* microstructural features of gray matter as well. However, little is known about

how different cellular landscapes may contribute to these metrics, especially in gray matter. Understanding the possible cytoarchitectural signatures of these measures would enable us to non-invasively estimate type-specific cell counts, potentially resulting in a very powerful clinical diagnostic tool as well as a mechanism for longitudinal tracking of effects in animal models. Here, we used a combination of diffusion-weighted imaging, brain clearing, light-sheet microscopy, and a high-resolution cell atlas of the mouse brain (Erö et al., 2018), to determine the link between diffusion images and the underlying neurobiology. We observed that different regions display unique, reliable relationships between cell counts and diffusion metrics. Moreover, we found that these relationships remained consistent across both *in vivo* and *ex vivo* diffusion imaging. We then used the robustness and exclusivity of these associations to create region-specific models to predict counts of both neurons and glial subtypes using the Extra Trees machine learning algorithm.

Disclosures: H. Radhakrishnan: None. **C. Stark:** None. **A. Obenaus:** None. **K. Wendel:** None. **T. Thomas:** None. **B. Hiba:** None.

Digital Abstract Session

P386. Computational Tools: Experimental

Program #/Poster #: P386.02

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant GM127251 awarded to AMK
UTEP Doctoral Excellence Fellowship awarded to AA

Title: Effects of resolution and scale on segmentation of Nissl-stained rat brain tissue images via convolutional neural networks

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Abstract: There are numerous efforts to automate the delineation of rat brain regions from rat brain tissue images. A leading approach is to use convolutional neural networks (CNNs) to model anatomical variability and, ultimately, to determine cytoarchitectonic boundaries. Currently, it is not clear what scale or resolution of the input tissue images offer the most information for these models to exploit. In this work, we test a fully convolutional architecture, U-Net, with inputs of different scales and resolutions of Nissl-stained rat brain tissue images to segment the fornix at the level of the lateral hypothalamic area. Specifically, we reduce either the resolution or the scale of the original images, which results in brain tissue images with less detailed cytoarchitectural information. We consider the intersection over union metric between the model prediction and ground truth segmentation to determine the best model from these data-reduced images and show that the network attains the highest intersection over union when it has access to cytoarchitectural information. Further, our results are consistent with recent work on

CNNs in that our trained models are more sensitive to texture by predicting boundaries that follow the fornix's texture and which deviate from the ground-truth elliptical shape determined by a human expert. Our work provides valuable insight into the optimal input needed to train convolutional neural networks designed to automate the delineation of cytoarchitectural boundaries from Nissl-based images of the brain.

Disclosures: **A. Arnal:** None. **A.M. Khan:** None. **O. Fuentes:** None.

Digital Abstract Session

P386. Computational Tools: Experimental

Program #/Poster #: P386.03

Topic: I.06. Computation, Modeling, and Simulation

Support: NIBIB R01:EB022889/EB022889-02S1
NIMH R01:MH106147
NIH U01EB017695
NYS DOH01-C32250GG-3450000
NINDS 2R01NS011613-42A1
Carney Institute for Brain Science
Providence VA CfNN

Title: Human Neocortical Neurosolver: A User-Friendly Software Tool for Cellular and Circuit Level Interpretation of EEG/MEG

Authors: *B. CALDWELL[1], S. NEYMOTIN[3], M. JAS[4], N. TOLLEY[1], S. DURA-BERNAL[5], M. CANTARELLI[6], N. PELED[4], R. A. MCDOUGAL[7], N. T. CARNEVALE[8], C. I. MOORE[1], M. L. HINES[8], M. HÄMÄLÄINEN[4], S. R. JONES[2];
[1]Neurosci., [2]Dept. of Neurosci., Brown Univ., Providence, RI; [3]Nathan Kline Inst., Orangeburg, NY; [4]MGH / Harvard Med. Sch., Boston, MA; [5]Physiol. and Pharmacol., State Univ. of New York Downstate Med. Ctr., Brooklyn, NY; [6]MetaCell, Inc., Cambridge, MA; [7]Ctr. for Med. Informatics, [8]Neurosci., Yale Univ., New Haven, CT

Abstract: EEG/MEG are currently the only methods to non-invasively record human neural dynamics with millisecond temporal resolution. These signals are correlated with nearly all healthy and pathological brain functions. However, it is still extremely difficult to infer the underlying cellular and circuit level origins without simultaneous invasive recordings. This limits the translation of EEG/MEG signals into novel principles of information processing, or into new treatment modalities for pathologies. As such, there is a pressing need to relate the macro-scale signals to their underlying meso-scale generators. To address this limitation we built the Human Neocortical Neurosolver (HNN): an open-source software tool to help researchers and clinicians without formal computational modeling or coding experience interpret the neural origin of their human EEG/MEG data (https://hnn.brown.edu). HNN provides an intuitive graphical user interface (GUI) to an anatomically and biophysically detailed model of a neocortical brain

circuit, with layer specific thalamocortical and cortical-cortical drives. Unique to HNN is an underlying neural model that accounts for the biophysics generating the primary electric currents underlying EEG/MEG signals, enabling visual and statistical comparison of model output to source localized data (in nAm). Users can change model parameters in the GUI for testing hypotheses on signal differences under varied experimental conditions or in patient populations. Further, visualizations are shown of detailed circuit activity including layer specific responses, cell spiking activity, and membrane voltages. We describe the development and application of HNN, including updated online resources for learning and installing HNN on all major platforms. We give an overview of ongoing expansions, including conversion of the model to the NetPyNE specification (allowing circuit architecture modifications in the GUI), visualization of LFPs, and model parameter optimization to match data features. We highlight the uniqueness of HNN among other software for EEG/MEG modeling. In total, HNN represents an unprecedented tool for the EEG/MEG community to translate their human signals to underlying neural dynamics.

Disclosures: **B. Caldwell:** None. **S. Neymotin:** None. **M. Jas:** None. **N. Tolley:** None. **S. Dura-Bernal:** None. **M. Cantarelli:** A. Employment/Salary (full or part-time):; MetaCell, Inc.. **N. Peled:** None. **R.A. McDougal:** None. **N.T. Carnevale:** None. **C.I. Moore:** None. **M.L. Hines:** None. **M. Hämäläinen:** None. **S.R. Jones:** None.

Digital Abstract Session

P386. Computational Tools: Experimental

Program #/Poster #: P386.04

Topic: I.06. Computation, Modeling, and Simulation

Title: Development of an automated high-throughput machine learning pipeline for phenotypic classification based on rodent quantitative electroencephalography

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Abstract: Electroencephalography (EEG) is a non-invasive and translational method to record brain activity across species, with robust alterations in the EEG frequency spectra found across several neurological diseases. Indeed, such alterations are being increasingly explored in transgenic rodent models to identify disease-associated biomarkers. Conventional analysis of EEG frequency spectra, however, often divides the spectra into arbitrary and discrete frequency bins (i.e. alpha, theta, gamma) whereby subtle differences that span the spectrum or that straddle traditional frequency bounds can be missed. In this context, machine learning (ML) offers an advantage that enables the unbiased selection of features for classification purposes. Here, we present an automated high-throughput ML pipeline for rodent EEG spectral analysis. The

pipeline was divided into three parts: feature extraction, feature selection, and classification. Continuous EEG data was collected from 32 wild-type and transgenic mice using wireless telemetry. Power spectral density (PSD) estimates were computed from 0.5-90Hz using the fast-fourier transform (Hanning window, 1024 nfft samples, 50% overlap). Both unbinned (~2Hz resolution) and traditional frequency bin (e.g. alpha, theta, gamma) PSD estimates were used for model training. Performance across random forest, linear discrimination analysis, support vector machine, and hierarchical clustering models were compared using 5-fold cross validation with 1000 iterations on stratified training and test data. The best-performing model, a combination of random-feature elimination with an underlying SVM model combined with a decision tree classifier, correctly classified rodent genotype using unbinned PSD estimates with 93% accuracy and a F1 score of 89% using data from a representative resting state EEG phenotyping experiment. Moreover, novel biomarkers were identified using a ML approach. Parallel computing optimization in a High-Performance Computing (HPC) environment, reduced processing time to a few minutes on a standard personal computer. The pipeline is highly modular, whereby additional feature selection or classification models can be included. Our results demonstrate that ML can uncover novel biomarkers and achieve a speed, scope, and scale that would be unachievable using conventional EEG analytical methods.

Disclosures: D.J. Graziano: None. **F. Sheng:** None. **M.M. Sidor:** None. **H. Hoefling:** None. **E.J. Ma:** None. **M. Xiao:** None.

Digital Abstract Session

P386. Computational Tools: Experimental

Program #/Poster #: P386.05

Topic: I.06. Computation, Modeling, and Simulation

Title: Quantifying changes in complexity of the event related response to sensory stimuli in different states of arousal

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Abstract: Complexity measures have traditionally been applied to resting state electroencephalography (EEG) as a way to quantify the dynamic, nonlinear changes in the signal. While there has been some interest in porting these techniques into the event-related response (ERP) space, the computational and temporal requirements for complexity measures have limited their spread into this field. However, a new measure has been developed, called the *perturbational complexity index* (PCI), that quantifies the complexity of an EEG response to single pulse transcranial magnetic stimulation (TMS). While this new measure originated in TMS-EEG, new work has already applied a modified version of this measure to multisensory and unisensory ERPs. It is still unclear however how well this measure, along with its new iteration (PCIst), tracks consciousness and arousal when applied to sensory ERPs as compared to TMS evoked responses. Importantly, PCIst and the original PCI differ in how the complexity of

the data is measured, with the former measuring the distance between the baseline and response signals, and the latter using Lempel-Ziv to measure the compressibility of the data. In this preliminary study, left median nerve somatosensory evoked potentials (SEPs) and auditory evoked potentials (AEPs) were delivered while subjects rested with eyes open and eyes closed in a within-subjects design to test the sensitivity of a variety of PCI measures to slight changes in arousal. Here, we report that while cluster permutation paired t-tests showed ERP level changes only for AEPs, there was a significant reduction in complexity of the responses as measured by Lempel-Ziv PCI for both ERP conditions ($F(1,16)=25.92$, $p<0.001$) and only for SEPs with PCIst (post-hoc comparison, Scheffe corrected, $p<0.001$) in low arousal. Therefore, while the Lempel-Ziv PCI appears to follow the expected direction of effect for both ERPs—complexity decreases as arousal decreases, as seen in the TMS-EEG experiments, PCIst appears to be sensitive to these changes only for one ERP condition (SEPs). In conclusion, we recommend using Lempel-Ziv based PCI when attempting to measure differences in arousal using the complexity of sensory evoked responses.

Disclosures: A. Dallavecchia: None. **F. Micheli:** None. **J. Frohlich:** None. **D. Toker:** None. **M.M. Monti:** None.

Digital Abstract Session

P386. Computational Tools: Experimental

Program #/Poster #: P386.06

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF Grant 1935749
NSF Grant 1935771
NIH Grant U24EB029005
NIH Grant R24MH120037

Title: Neuroscience Gateway - Large Scale Data Processing and Modeling Using High Performance and High Throughput Computing Resources

Authors: S. SIVAGNANAM[1], K. YOSHIMOTO[1], A. DELORME[1], R. MARTINEZ[1], D. TRUONG[1], M. KANDES[1], S. YEU[1], S. MAKEIG[1], T. CARNEVALE[2], ***A. MAJUMDAR**[1]; [1]Univ. of California San Diego, La Jolla, CA; [2]Yale Univ., New Haven, CT

Abstract: Since its inception in 2013, the Neuroscience Gateway (NSG) reduces technical and administrative barriers that neuroscientists face in accessing and using high performance computing (HPC) resources needed for large scale neuronal modeling projects. NSG provides about twenty neuroscience software that require and run efficiently on high performance computing (HPC) resources. Since around 2017 experimentalists such as cognitive neuroscientists, psychologists, biomedical researchers started to use NSG for their neuroscience data processing, analysis and machine learning work. Data processing workloads are more suitable on high throughput computing (HTC) resources where single core jobs run to process

individual data sets of subjects. NSG is adapting to the needs of experimental neuroscientists by providing HTC resources, in addition to already successfully serving the computational neuroscience community. To support data processing workload NSG is adding new functionalities such as transfer/store of large data, processing of data by multiple users, visualization of data, HTC of data etc. In addition NSG is becoming an environment where neuroscience software developers can test, benchmark, and scale their newly developed software and eventually disseminate their software via the NSG. NSG is also being integrated with the NEMAR (NeuroElectroMagnetic data Archive and tools Resource) project which is a data and tools archive focused on EEG data. It will extract EEG data deposited on the OpenNeuro archive and then allow researchers to search, visualize and process EEG data. NSG's EEGLAB data processing pipeline, will be used for large scale EEG data processing on high throughput supercomputing and cloud resources. The presentation will describe: (i) how NSG has been successfully serving both the computational neuroscience community, and the experimental neuroscience researchers; (ii) new features that are added to make it a suitable and efficient dissemination environment for lab-developed neuroscience software. NSG has a growing user base, and provides an easy to use and an open environment, and allows large scale computing on powerful compute resources. It has a well-established training and outreach program, and a functioning user support system.

Disclosures: **S. Sivagnanam:** None. **K. Yoshimoto:** None. **A. Delorme:** None. **R. Martinez:** None. **D. Truong:** None. **M. Kandes:** None. **S. Yeu:** None. **S. Makeig:** None. **T. Carnevale:** None. **A. Majumdar:** None.

Digital Abstract Session

P386. Computational Tools: Experimental

Program #/Poster #: P386.07

Topic: I.06. Computation, Modeling, and Simulation

Title: Convolutional Neural Network Predicts Impairment Scores for Repetitive Movement Measurements

Authors: *A. SURESH[1], J. R. BRASIC[1], M. E. HERNANDEZ[2];
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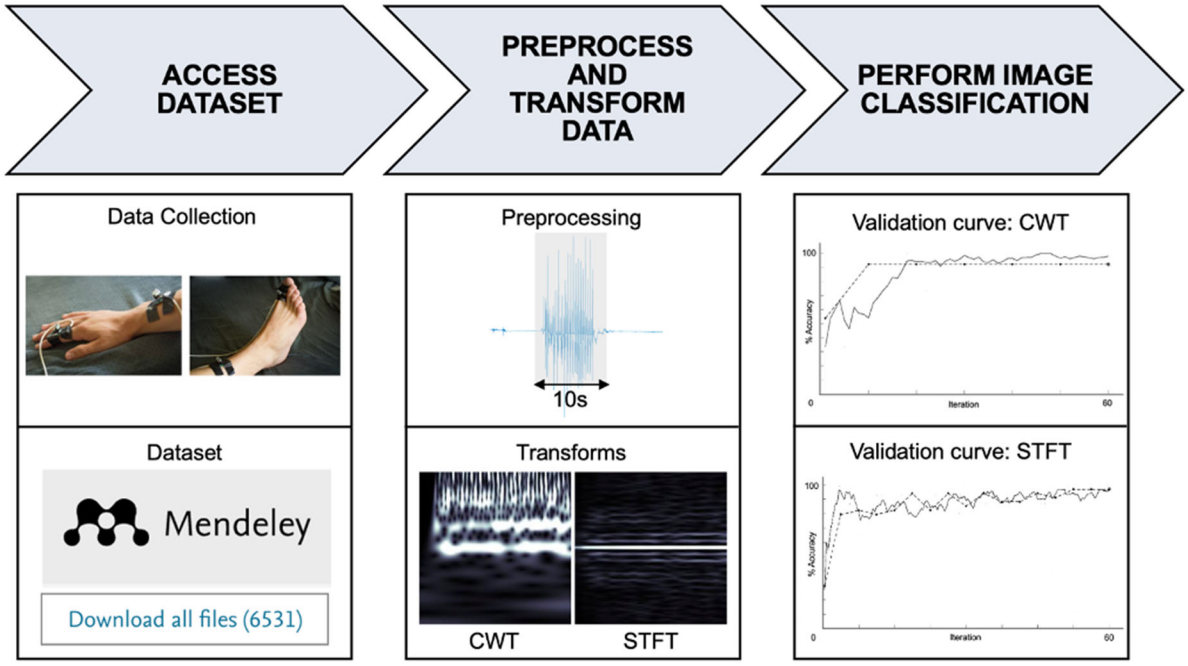
Abstract: Objective: To develop a machine-learning based method to predict motor impairment scores of repetitive movement measurements.
Methods: A dataset containing repetitive movement measurements from patients with Parkinson's disease (PD) and age- and sex-matched control participants was used (Harrigan et al., 2020). Movements included finger tapping, toe tapping, and pronation-supination. A low-cost, accelerometer-based protocol (McKay et al., 2019) was administered to obtain the movement measurements from the extremities of participants. A trained examiner scored the movements live (McKay et al., 2019; Goetz et al., 2008). For each trial, ten-second segments of accelerometer signals were selected and images were generated using their continuous wavelet

transforms (CWTs) and short-time Fourier transforms (STFTs). Image classification was performed using a convolutional neural network, GoogLeNet in MATLAB (The Math Works, Inc., 2020). The CWT images and STFT images were divided into two classes: low (corresponding to ratings 0-1) and high (corresponding to ratings 3-4). An equal number of images were selected randomly from each class and used for classification. An 80-20 split for training and validation images was chosen. The network's ability to correctly classify validation images determined its accuracy score.

Results: The network had a 92% accuracy in predicting new CWT images and a 97% accuracy in predicting new STFT images into low (0-1) and high (3-4) classes.

Conclusion: Image classification performed by a GoogLeNet generates a high level of accuracy in predicting the impairment score of repetitive movements measured by accelerometers. This technique has significant potential for the evaluation of movements in patients in telemedicine and for the tracking and monitoring of movements through embedded accelerometers in wearable devices. It also provides the foundations for the identification of electronic signatures pathognomonic for PD to facilitate the diagnosis, monitoring, and treatment of PD and other movement disorders.



Disclosures: **A. Suresh:** None. **J.R. Brasic:** None. **M.E. Hernandez:** None.

Digital Abstract Session

P386. Computational Tools: Experimental

Program #/Poster #: P386.08

Topic: I.04. Physiological Methods

Support: NICHHD-NIH Grant K12HD073945
NSF Grant EEC-1028725
University of Washington Royalty Research Funds
BRAIN Initiative NSF-NCS Grant 1734887
NSF Grant 1523614
NSF Grant 1829474
SUNY Empire Innovator Scholarships

Title: A magnetic resonance imaging based toolbox for neurosurgical planning in non-human primates

Authors: *W. OJEMANN[1], D. GRIGGS[2], Z. IP[1], O. CABALLERO[5], H. JAHANIAN[3], S. MARTINEZ-CONDE[6], S. MACKNIK[5], A. YAZDAN-SHAHMORAD[4];
[1]Bioengineering, [2]Electrical Engin., [3]Radiology Dept., [4]Bioengineering and Electrical Engin., Univ. of Washington, Seattle, WA; [5]Dept. of Ophthalmology, SUNY Downstate Hlth. Sci. Univ., New York, NY; [6]Dept. of Ophthalmology, State Univ. of New York Downstate Med. Ctr., Brooklyn, NY

Abstract: Primate research is a pivotal step in the progression of medical research from animal models to human trials. This is especially true in neuroscience and neural engineering where nonhuman primates (NHP) have been highlighted as an ideal preclinical model for understanding brain function. In most NHP neuroscience experiments, neurosurgical measures are required for the implantation of devices such as head posts, stimulation and recording chambers, electrode arrays, and optical windows. Current practices for headpost implantation, surgical craniotomy, and fluid injection use methods for surgical preparation that carry a degree of unavoidable uncertainty. This comes from an inability to visualize and test the physical compatibility of complex components and anatomy prior to neurosurgery.In this work, we outline a method for surgical preparation that allows for the practical planning of a variety of neurosurgeries in NHPs solely using data extracted from magnetic resonance imaging (MRI). This protocol allows for the generation of 3D printed anatomically accurate physical models of the brain and skull, as well as an agarose gel model of the brain that replicates some mechanical properties of the brain. These models can be obtained from MRI using brain extraction software for the brain model, and custom code for the skull. The preparation protocol takes advantage of state-of-the-art 3D printing technology to combine models of the brain and skull with neuroprostheses. With the addition of a craniotomy using the custom code, the skull and brain models can visualize brain tissue inside the skull, enabling better preparation for surgeries.Using the methods outlined in the protocol, the accuracy of the 3D printed brain, skull, and craniotomy placement were successfully validated through a comparison to the original MRI scan. The gel brain was additionally used to visualize delivery of a mock viral vector through the craniotomy of a skull model. By preoperatively fitting a headpost to the physical model of the skull we successfully reduced the time required for the headpost implantation surgery by 40% and greatly reduced the risk of operative complications. Unlike previous publications in surgical preparation, this protocol offers a novel, unimodal method requiring only an MRI scan that can additionally visualize and prepare for surgical injection using the various gel molding components. The

applications of these methods are designed for surgeries involved in neurological stimulation and recording as well as injection in NHPs, but the versatility of the system allows for future expansion of the protocol, extraction techniques, and models to a wider scope of surgeries.

Disclosures: W. Ojemann: None. **D. Griggs:** None. **Z. Ip:** None. **O. Caballero:** None. **H. Jahanian:** None. **S. Martinez-Conde:** None. **S. Macknik:** None. **A. Yazdan-Shahmorad:** None.

Digital Abstract Session

P387. Data Analysis and Statistics: Human Data

Program #/Poster #: P387.01

Topic: I.07. Data Analysis and Statistics

Support: ERC Grant 670325, Advanced grant BRAVIUS
CIFAR senior fellowship
ANR-17-EURE-0017

Title: Evaluation of different statistical procedures to estimate coupling between oscillatory phase and behavioral response

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Abstract: A growing number of studies report a link between the phase of an oscillation and behavior. For instance, the phase of brain alpha oscillations at which a stimulus is presented correlates with the likelihood to detect a near-threshold stimulus. Phase-effects have been observed not only for neural oscillations but also within the cardiac or respiratory cycle. A large variety of methods has been employed for the statistical evaluation of phase effects on behavior, without rigorous investigation to determine which method is optimal for the question under study. Additionally, most studies relied on the assumption that phase-behavior would take a 1:1 relationship, i.e. with one preferred portion of the phase for each behavioral outcome (e.g., "hits" and "misses"). Here, we compare the peformance (sensitivity, False Positive rate) of five circular tests commonly used in the field of neuroscience to quantify relationships between the phase of an oscillation and a binary behavioral outcome, namely Phase Opposition Sum, Circular Logistic Regression, the Watson test, Modulation Index, and the Rayleigh test. We created semi-artificial datasets mimicking real experiments with 30 participants, where we imposed a link between a simulated behavioral outcome with the phase of a physiological oscillation. We systematically varied the strength of phase-outcome coupling, the coupling mode (1:1 to 4:1), the overall number of trials, the relative number of trials in the two outcome conditions, and evaluated different strategies to estimate phase-outcome coupling chance level, as well as significance at the individual or group level. We found that Circular Logistic Regression, Phase Opposition Sum and the Watson test are the most sensitive tests when coupling mode is 1:1. Modulation index, and to a lesser extent the Watson test, is sensitive to higher coupling modes. The Watson test is

thus a good first intention test, with a good sensitivity and low False Positive rate, some sensitivity to 2:1 coupling mode and low computational load. Modulation index, intitially designed for continuous variables but that we find useful to estimate coupling between phase and a binary outcome, should be preferred if coupling mode is higher than 2:1. Phase opposition Sum, coupled with a resampling procedure, is the only test retaining a good sensitivity in the case of a large imbalance in the number of occurrences of the two behavioral outcomes. The results of this systematic evaluation directly generate recommendations to analyze data for phase-behavior coupling in future studies.

Disclosures: **N. Wolpert:** None. **C. Tallon-Baudry:** None.

Digital Abstract Session

P387. Data Analysis and Statistics: Human Data

Program #/Poster #: P387.02

Topic: I.07. Data Analysis and Statistics

Support: Samuel F. Hulbert Chair Endowment

Title: Memory-tasks using rapid-serial visual presentation paradigm to inform brain-computer interface error correction

Authors: ***A. W. CHIU**, D. H. HUGHES, L. M. JILEK, M. E. JACOBS;
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Abstract: One of the applications of the brain-computer interface (BCI) is to help people with neuromuscular deficiencies to communicate effectively. Many of these BCI speller systems employ various visual presentation paradigms that allow users to construct and edit sentences but are limited by the oculomotor deficiencies of the target population. In this study, we implemented a modified rapid-serial visual presentation (RSVP) paradigm and evaluated features to allow for an intuitive means for BCI spellers to quickly identify and correct errors. Fifteen healthy individuals with no prior experience with RSVP software or similar speed-reading tools were recruited. OpenBCI 16-channel Mark IV system with Cyton and Daisy was used for data acquisition. Subjects were asked to complete 240 trials of a modified working memory free recall task to assist in identifying relevant features that can be used to illustrate possible errors during BCI spelling tasks. Each trial involved a presentation phase (PP), where subjects were shown a list of words, and a response phase (RP), where subjects were shown a single word. The PP lists contained 4 words (120 trials) or 10 words (120 trials) and subjects were asked to memorize the words as they flashed on the screen. Words were presented at 200 to 420 words per minute (WPM) (3.3 to 7 Hz) and the flash rate was randomly chosen for each trial with an equal number of trials being presented via each flash rate. During the RP subjects were instructed to press different buttons depending on whether the RP word had appeared on the PP list. Only responses within 4 seconds are considered. Although event-related potentials (ERPs) such as N400 and P600 are often used in language comprehension tasks, they are more aligned with

sensitive to the degree of semantic expectancy, and comprehension, and are not relevant features for BCI error identification. Time-dependent Generalized Hurtz Exponent (GHE) for each EEG channel was used to identify the difference between conditions where RP mismatch occurred. Significant changes in both alpha and theta activity were found depending on the number of words in PP. Taken independently, none of these features were sufficient in classifying whether an error occurs. However, using machine learning techniques, the accuracy of identification could be enhanced up to 65% at 200 WPM but down to 50% at 420 WPM, suggesting that presentation speed also played a huge factor.

Disclosures: **A.W. Chiu:** None. **D.H. Hughes:** None. **L.M. Jilek:** None. **M.E. Jacobs:** None.

Digital Abstract Session

P387. Data Analysis and Statistics: Human Data

Program #/Poster #: P387.03

Topic: I.07. Data Analysis and Statistics

Support: NSF Grant 1823889

Title: A comparison of active marker and deep learning based marker-less tracking of human motion in 3D

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Abstract: Measuring quantitative information about human motion is fundamental to understand how our central nervous system controls and organizes movements and is essential to many fields including motor control/learning, rehabilitation, and sport biomechanics. Currently, for scientific use, the study of human motion is commonly done through marker-based techniques and motion capture systems. These methods are the gold standard because they are precise and reliable. However, they are expensive, time consuming and the use of markers or wires can affect the naturalness of the motion in some real-world contexts. Marker-less video-based techniques are an alternative to these methods. They are less expensive and can be used to record performers in their natural settings. Unfortunately marker-less techniques appear to be less precise, although a systematic and detailed comparative analysis of recent models have yet to be explored. The aim of this work is to implement and evaluate a video-based marker-less system to quantitatively study human motion in three dimensions (3D).To examine performance in a real-world context, we studied violin players of a wide range of ages and capabilities. We acquired the kinematics of these violinists repeatedly playing a G scale arpeggio. For the marker-less system, the setup included three RGB cameras (Mako G125). We used a motion capture system (Optotrak 3020) with 6 active markers placed on the violin and 4 placed on the bow to validate the marker-less

system.We first trained a state-of-the-art deep learning algorithm (DeepLabCut) to automatically detect the (x,y) coordinates of landmark points in the image plane for each frame in all the videos. Then, we filtered the obtained trajectories in order to enforce spatio-temporal consistency. We reconstructed the 3D positions of the actual markers placed on the violin and on the bow combining the information from different viewpoints (stereopsis). At the end, we measured the Euclidean distances between different couples of markers in the 3D space with both actual markers and their analogues reconstructed from videos and we compute the mean and the standard deviation for each distance across different time instants. We obtained similar results in term of mean distances between marker-based and marker-less techniques (average difference < 4 mm) with a standard deviation lower than 1 mm for marker measures and around 5 mm for marker-less ones. These results suggest that marker-less systems can successfully track performance in real-world settings, although their higher variability may potentially limit their use in certain contexts.

Disclosures: **M. Moro:** None. **M. Casadio:** None. **L. Mrotek:** None. **F. Odone:** None. **R. Ranganathan:** None. **R. Scheidt:** None.

Digital Abstract Session

P387. Data Analysis and Statistics: Human Data

Program #/Poster #: P387.04

Topic: I.07. Data Analysis and Statistics

Support: Leverhulme Trust Research Leadership Award

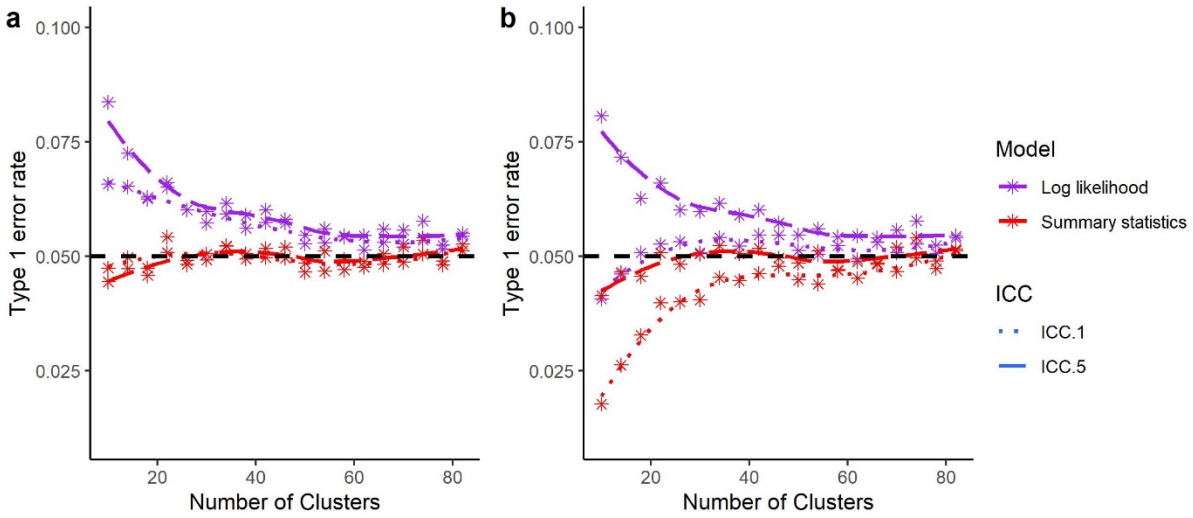
Title: Unnecessary reliance on multilevel modelling to analyse nested data: when a traditional summary statistics approach excels

Authors: *C. B. MCNABB, K. MURAYAMA;
Sch. of Psychology and Clin. Language Sci., Univ. of Reading, Reading, United Kingdom

Abstract: Nested data structures (common in neuroscience) create dependence in the data that influences the effective sample size and statistical power of a study. Several methods are available for dealing with nested data, including the summary statistics (SS) approach and multilevel modelling (MM). Recent publications have heralded MM as the best method for analysing nested data, claiming benefits in power over SS approaches (e.g. the *t*-test). However, when data are not cross-nested and cluster size is equal, conventional analysis with the SS approach is actually mathematically equivalent to MM. This equivalence has been established in statistical literature and made use of in popular neuroimaging software such as FMRIB Software Library (FSL) and Statistical Parametric Mapping (SPM). However, the merits of the SS approach have not been well recognised in neuroscience beyond that context. We conducted statistical simulations demonstrating equivalence of MM and SS approaches for analysing nested data and provide support for the utility of the conventional SS approach in nested experiments. We demonstrated that losses in power attributed to the use of the SS approach in previous

publications (Aarts, Verhage et al. 2014, Aarts, Dolan et al. 2015) are unsubstantiated and due to inappropriate use of the log-likelihood test in MM. This method increased study power at the cost of inflated type 1 error (Fig. 1), attributable to singular fit errors when intraclass correlation was low (ICC=.1) but independent of these errors when intraclass correlation was high (ICC=.5). Use of restricted maximum likelihood estimation, however, resulted in power and error rates comparable to the SS approach, indicating that the formerly mentioned benefits in power with MM are in fact a myth.

Figure 1. Type 1 errors associated with log-likelihood ratio test and SS approach; a) including all 10,000 simulations; b) excluding simulations that resulted in singular fit or convergence warnings.



Disclosures: C.B. McNabb: None. **K. Murayama:** None.

Digital Abstract Session

P387. Data Analysis and Statistics: Human Data

Program #/Poster #: P387.05

Topic: I.07. Data Analysis and Statistics

Support: Chuck Noll Foundation for Brain Injury Research
CMU BrainHub award
NIH (NEI GrantRO1EY027018)

Title: Neural silences can be localized rapidly using template head models

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Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Rationale: The diagnosis and treatment of many neurological disorders can benefit from non-invasive and rapid monitoring of abnormal neural activity. We recently proposed a

method, called SilenceMap, for the detection and localization of neural "silences" in the brain using scalp electroencephalography (EEG). "Silences" are regions in the brain with suppressed neural activity, which can model conditions such as hypoxic, necrotic, or lesioned tissue in brain injuries and stroke. In situations where there is limited or no access to advanced imaging techniques such as magnetic resonance imaging and computed tomography, having access to a fast-to-apply monitoring system for assessment of neural damage is vital. This paper explores the possibility of silence localization in the brain based on a template head model and scalp EEG recording. Methods and Results: In a recent study, we tested the performance of SilenceMap on three male pediatric patients with lobectomy, where the structural MRI scans of these patients were used to extract the real head models for lead-field matrix estimation. We used a standard EEG grid with 128 electrodes to record EEG signals during visual and rest tasks. SilenceMap successfully (maximum distance error of 13mm) localized the regions of silence using fewer than 3 minutes of EEG recording. In the current work, a template head model is extracted using an MRI scan of a healthy individual (intact brain) using an open source MRI dataset. Using this template head model, along with the scalp EEG recording, SilenceMap localizes the correct regions in the brain. However, slight performance reduction is observed in silence localization using a template head model (see the attached Figure). Conclusions: This study further validates the capability of SilenceMap in localizing and monitoring neural silences in the brain, even without having access to any structural information of the brain, since use of template head models results in only a slight performance reduction. This paves the way towards fast and reliable monitoring of neural silences in emergency situations.

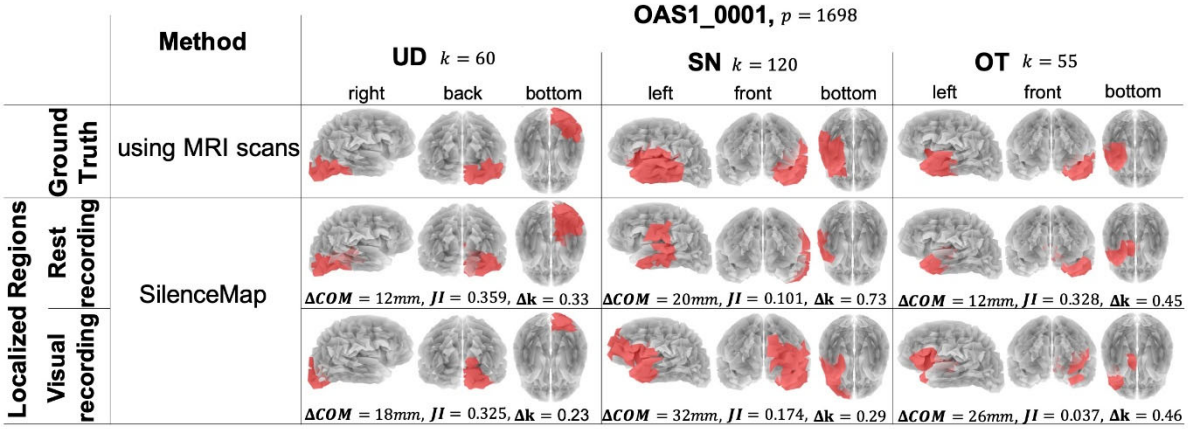

Fig. Performance of SilenceMap in both the rest and the visual recording conditions, using a template head model: the first row shows the extracted ground truth regions of silence (red regions) overlaid on the resected cortical region of three patients based on a symmetric brain model extracted from the structural MRI scan of a healthy individual in an open source dataset (OAS1_001 in OASIS1[1] dataset); the second and third rows show the performance of SilenceMap, based on the Rest and Visual recordings respectively, through both visual illustration (red regions) and using performance metrics of center-of-mass (COM) distance (ΔCOM), Jaccard Index (JI), and size error (Δk). p is the total number of sources in each brain model, and k is the size of ground truth region of silence. The results show only small reduction in the silence localization performance using a template head model in comparison with the results using the structural MRI scans of each patient (see Fig. 3 in Chamanzar, et al., 2020[2])

1. http://www.oasis-brains.org
2. https://doi.org/10.1101/2020.10.11.334987

Disclosures: **A. Chamanzar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision Neuroscopics, LLC. **M. Behrmann:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision Neuroscopics, LLC. **P. Grover:** E. Ownership Interest (stock, stock options, royalty, receipt of

intellectual property rights/patent holder, excluding diversified mutual funds); Precision Neuroscopics, LLC.

Digital Abstract Session

P387. Data Analysis and Statistics: Human Data

Program #/Poster #: P387.06

Topic: I.07. Data Analysis and Statistics

Support: NIH/NIBIB (P41-EB018783)
NIH/NIBIB (R01-EB026439)
NIH/NINDS (U24-NS109103)
NIH/NINDS (U01-NS108916)
NIH/NIMH (P50-MH109429)
Fondazione Neurone

Title: Implication of disregarding the fundamental characteristics of neural signals

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Abstract: The large scale electrophysiological signals recorded from the cortex or scalp encompass valuable information about neuronal activities and are thus widely used in brain research. The inherent complexity of these signals has urged neuroscientists to adopt sophisticated analysis tools and methods, mostly developed in other disciplines such as Digital Signal Processing and Economics. While applying such analysis approaches can be instrumental in processing neural data, lack of proper understanding about their underlying assumptions and treating them as 'black box' can be detrimental. The majority of methods and techniques in commonly used analysis pipelines are based on certain ideal assumptions about the input data, which are often incongruent with the nature of brain signals. Our research aims to address this profound issue and shed light on the ramification of lack of such consistency. To accomplish this, we investigated the fundamental characteristics of large-scale electrophysiological signals, their presence under different physiological conditions, and the optimum methods for their quantification. Our study hypothesized that fundamental signal characteristics play a critical role in choosing appropriate analysis approaches. To test this hypothesis, we applied widely used techniques to simulated and real neural data. Our results demonstrate that a lack of concordance between the premise of the processing methods and inherent signal attributes can be misleading and introduce dire analytical confounds in the context of different neuroscientific applications. We hope this study serves as a guideline for neuroscientists and encourages them to be cognizant of the essential attributes of neural data in each experiment and meticulous about choosing the proper analysis technique.

Disclosures: **A. Nourmohammadi:** None. **P. Brunner:** None.

Digital Abstract Session

P387. Data Analysis and Statistics: Human Data

Program #/Poster #: P387.07

Topic: I.07. Data Analysis and Statistics

Support: ERC CoG 647954

Title: A standardised framework for re-referencing strategies of human intracranial electrophysiological data.

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Abstract: In recent years human electrophysiological studies, particularly intracranial and human single neuron studies, have become increasingly popular as the field grows in funding and stature. It is important, therefore, that there is some form of standardisation that enables comparison between studies and findings. Such studies inherently rely on the analysis of electrical signals that are emitted by the combined neural circuitry of the brain. These electrical signals must be recorded in relation to some grounding signal, meaning that this reference signal will play a large role in the resultant waveforms that are ultimately recorded. At the point of recording, there is a general understanding that typical grounding sources will help to minimise surrounding electrical interference whilst not removing much of the original signal. For this reason, grounding references are often situated around the periphery of the head or skull of the subject being recorded from. However, neuroscientific studies often employ another step of re-referencing, whereby a single or amalgamation of electrodes or micro-wires are selected as grounding references. In theory, this is pursued to further increase the signal to noise ratio. However, it is often the case that a re-referencing strategy is chosen to remove signals that are undesirable for the hypothesis in question. Re-referencing might be achieved in any number of ways that are currently preferred by the field, whether it is the subtraction of an average set, an independent-component, an electrode situated near white matter or the skull, or a bi-polar subtraction of each electrode from its neighbours, amongst others. Preferred strategies are often employed on a case-by-case basis, rather than being prescribed from a standardisation of re-referencing methods. We feel that this can murky the underlying theory behind secondary re-referencing, potentially leading to disagreements and an inability to reproduce findings across studies. As such, we here explore a human episodic memory paradigm to assess the effect of a variety of popular re-referencing techniques on resultant oscillatory signals and spike-field coherence, using intracranial EEG data collected from 16 subjects. By comparatively analysing

time-frequency space and the degree to which spikes conform to oscillatory signals, we here hope to provide a standardised framework for human intracranial re-referencing methods. Our aim is to aid colleagues in selecting an appropriate strategy for their hypothesis, such that the field can engage in more transparent and comparable experimentation.

Disclosures: G. Parish: None. **F. Roux:** None. **L. Kolibius:** None. **R. Chelvarajah:** None. **D.T. Rollings:** None. **V. Sawlani:** None. **H. Hamer:** None. **S. Gollwitzer:** None. **G. Kreiselmeyer:** None. **M. Ter Wal:** None. **B. Staresina:** None. **M. Wimber:** None. **S. Hanslmayr:** None.

Digital Abstract Session

P387. Data Analysis and Statistics: Human Data

Program #/Poster #: P387.08

Topic: I.07. Data Analysis and Statistics

Title: Development of an application for real-time acquisition and synchronization of data from operative patient monitoring instruments

Authors: K. PUTHUVEETIL[1], ***A. VENKATESAN**[2], R. HANG[1], D. J. KRUSIENSKI[1]; [1]Biomed. Engin., [2]Biol., Virginia Commonwealth Univ., Richmond, VA

Abstract: Successful outcomes during surgery critically depend on a variety of instruments in theoperating room for monitoring and regulating patients' vitals. It is common to employ trainedclinicians or teams solely dedicated to specific monitoring modalities, such as cardiopulmonaryperfusion, neuromonitoring, or anesthesia, for instance. The surgeon intimately relies on theseclinicians to maintain patient vitals and to communicate any irregularities seen in their respectivemodalities. While an active dialog about the readings from the individual instruments ismaintained between the clinicians and surgeon during the case, the signals are often notsystematically synchronized or permanently recorded. Such synchronization and recording haveseveral advantages beyond creating an archival record, such as using the data to betterunderstand and model the impacts of the interventions during a case on the vitals and patientoutcomes. The ultimate objective of this research is to not only synchronize monitoring but alsoto enable offline analysis for improved automated event detection. An application that supportsrecording from a multichannel analog-to-digital converter or via serial ports was developed. Theapplication allows for a simple configuration of device communication parameters, enabling datacollection from any medical devices with an analog or RS232 output, like those for transcranialdoppler and perfusate flow/blood parameter monitoring modalities in our test case. Theapplication also allows clinicians to make time-stamped annotations about events of interest,which can then be used to identify trends in signal characteristics during proceduralabnormalities. The synchronized, annotated, multichannel data can be stored and used for laterinspection or analysis. In our test case in partnership with VCU Health, the recording applicationwill be used to track annotations and record signals from neuromonitoring

instruments, amongothers, during cardiac surgery. The collected data will be used to evaluate the efficacy of theneuromonitoring system used by the hospital in improving patient outcomes.

Disclosures: A. Venkatesan: None. **K. Puthuveetil:** None. **R. Hang:** None. **D.J. Krusienski:** None.

Digital Abstract Session

P387. Data Analysis and Statistics: Human Data

Program #/Poster #: P387.09

Topic: I.07. Data Analysis and Statistics

Support: BMBF CRCNS EB027586

Title: Phase-contrast micro computed tomography for vasculature imaging in post mortem human brain stem

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Abstract: Studying the vasculature in the human brain using classical histology can be challenging due to the necessity for high quality, stained tissue slices. Here, we propose to use phase-contrast micro computed tomography (microCT) for identifying the vasculature in the unstained, paraffin-embedded brain. With a phase contrast technique, which incorporates the phase shift of the X-rays, we could obtain edge enhanced images from the tectum of the midbrain. Human brain stems were provided by the University of Tübingen Body Donor program. Samples were fixed in formaldehyde for a minimum of 4 weeks and then were embedded in paraffin. Acquisitions were made at the SYRMEP beamline of the Elettra Synchrotron Facility in the “white-beam” configuration mode, illuminating the sample with a mean energy of 22 keV. The samples were measured with a rotation angle of 180° with a total of 3600 projections. We scanned 4 samples in 4.9µm pixel size (detector-to-sample-distance, DSD: 90cm) and 2 samples in 0.9µm pixel size (DSD: 20cm). We applied Paganin’s phase retrieval algorithm for signal-to-noise ratio improvement and filtered back projection for image reconstruction. Vessels were automatically segmented using; a median filter, non-local means denoising, a variance filter followed by thresholding and opening. Vessel volume within the superior colliculus was calculated using 0.9µm pixel size data. Figure 1 shows that the vessel structure segmented from microCT matches the structure revealed by Indian ink staining in a previous study (Durvernoy, H. M., 1978.). Within the superior colliculus, vessels project from the surface towards the cerebral aqueduct in a parallel manner. Within the superior colliculi, vessel volume made up a mean of 3.47 % (SD= 0.53%) of the tissue. These findings show that phase contrast microCT is suitable for investigating the vasculature of post mortem human brain.

This method can also be used for vessel tracing in three dimensional space and analyzing features such as length, diameter and branching.

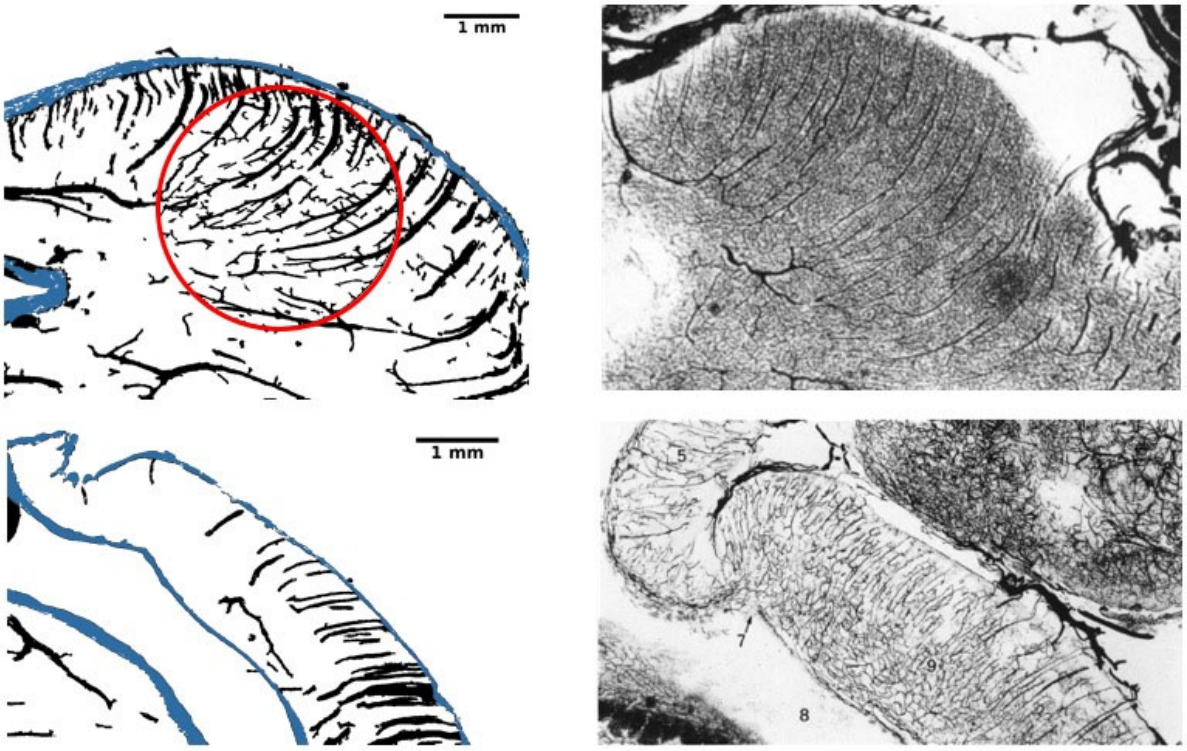

Figure 1. Top: Vessel structure in the superior colliculus from microCT (left) and Indian ink stain (right) in a transversal plane. In the left image, the vessel structure segmented from 0.9 micron resolution microCT is shown inserted in the red circle. Bottom: Sagittal plane of the vasculature in medial tectum from microCT (left) and Indian ink stain (right). (Indian ink stain images are adopted from Durvernoy, H. M., 1978.)

Disclosures: J. Lee: None. **A. Mack:** None. **T. Shiozawa-Bayer:** None. **K. Scheffler:** None. **F. Di Lillo:** None. **R. Longo:** None. **G. Tromba:** None. **G. Hagberg:** None.

Digital Abstract Session

P387. Data Analysis and Statistics: Human Data

Program #/Poster #: P387.10

Topic: I.07. Data Analysis and Statistics

Support: CONACyT 619683/330142

Title: Network-based r-statistics software for longitudinal (unbalanced) brain networks

Authors: *Z. GRACIA TABUENCA, S. ALCAUTER;
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Abstract: The brain can be modeled as a set of interacting elements, allowing its characterization from a systems perspective using network science. In particular, Zalesky et al. (2010) proposed Network-Based Statistics (NBS), a mathematical approach that applies statistical inferences at the (sub-)network level, with a trade-off between the control for false positives and higher statistical power than mass univariate approaches (e.g., FDR). Nevertheless, NBS relies on the general linear model (GLM) which limits its application to longitudinal samples, in particular, to unbalanced samples, that is, with a variable number of sessions per subject. We developed a publicly available software (an R-package), NBR (Network-Based R-statistics), that performs mixed-effects models (LME) in the NBS framework, allowing the exploration of unbalanced longitudinal samples. We then tested GLM- and LME-NBS in the SWU-SLIM database, which is publicly available at the International Neuroimaging Data-sharing Initiative (INDI). The dataset includes 333 participants (145 males; 17-28 years old.) with two (n=212) or three (n=121) sessions each. All sessions include a resting-state fMRI scan and psychometric data. State anxiety scores and brain connectivity matrices were used. GLM and LME tested the edgewise brain-behavior relationship for balanced (424 matrices) and unbalanced (787 matrices) samples, respectively. Significance was assessed based on permutation tests including 1000 permutations restricted to within-subject swapping.
The LME approach found a significant subnetwork, which includes the cingulum, the frontal, parietal and occipital lobes, and the cerebellum (pFWE = 0.001), while GLM found no significant results (pFWE = 0.355).
In summary, we developed an R package that implements LME for NBS. We showed that LME-NBS overpowers GLM-NBS when dealing with unbalanced longitudinal samples. This is relevant given that missing data is very common in longitudinal studies, and balanced testing could dramatically undermine statistical power. Besides, we were able to show a brain network related to anxiety symptoms that vary over time, which would not be identified using standard methods. Considering the growth of longitudinal studies in neuroscience, we anticipate this method being potentially useful in the field.

Disclosures: Z. Gracia Tabuenca: None. **S. Alcauter:** None.

Digital Abstract Session

P387. Data Analysis and Statistics: Human Data

Program #/Poster #: P387.11

Topic: I.07. Data Analysis and Statistics

Title: Shoulder Motion Evaluation in Patients with Cervical Spinal Cord Injury via Inertial Measurement Unit-based System

Authors: *R. BRAVI[1], S. CAPUTO[2], S. JAYOUSI[2], A. MARTINELLI[2], L. BIOTTI[2], I. NANNINI[3], E. J. COHEN[1], E. QUARTA[1], S. GRASSO[1], G. LUCCHESI[3], G. RIGHI[3], G. DEL POPOLO[3], L. MUCCHI[2], D. MINCIACCHI[1];
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Abstract: In an attempt to achieve functional independence of individuals with cervical spinal cord injury (CSCI), several rehabilitation programs or surgical interventions have been developed to restore shoulder range of motion (ROM) [1]. In clinical settings the gold standard for measuring joint ROM in patients with CSCI is the goniometer, a portable, inexpensive, quasi-objective tool [2,3]. However, the need for more objective methods when evaluating motor parameters appears to be of great importance. The aim of the study was to test the validity of a wearable Inertial Measurement Unit (IMU) sensor system by comparing the accuracy of the IMU system to goniometer method in measuring shoulder ROM [4]. Eight participants with CSCI completed four shoulder movements (forward flexion, abduction, and internal and external rotation) on dominant shoulder. Each movement was assessed with a goniometer and the IMU system by two testers independently. The extent of agreement between each tester's goniometer and IMU measurements was assessed with intra-class correlation coefficients (ICC) and Bland-Altman 95% limits of agreement (LOA). Additional analysis compared agreement between tester's goniometer or IMU measurements (inter-rater reliability) using ICC's and LOA. Preliminary results showed that goniometer and IMU measurements had excellent levels of agreement for all tested movements; however, LOAs were found high. Inter-rater reliability was found excellent between the IMUs measurements, while it was found lower between tester's goniometer. LOAs were found high also when agreement between tester's goniometer or IMU measurements were compared. The current study provides preliminary evidence of the concurrent validity of a wearable IMU sensor system for assessing shoulder movements in patients with CSCI. This can be a potential monitoring and clinical diagnostic tool to be widely used in clinical settings at the service of specialized personnel supporting spinal cord injured subjects.
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Disclosures: R. Bravi: None. **S. Caputo:** None. **S. Jayousi:** None. **A. Martinelli:** None. **L. Biotti:** None. **I. Nannini:** None. **E.J. Cohen:** None. **E. Quarta:** None. **S. Grasso:** None. **G. Lucchesi:** None. **G. Righi:** None. **G. Del Popolo:** None. **L. Mucchi:** None. **D. Minciacchi:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.01

Topic: I.06. Computation, Modeling, and Simulation

Support: JSPS KAKENHI 18H01414

Title: Mathematical Model of Motion Sickness: Capturing the Effect of the Prediction of Exogenous Motion

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Abstract: Computational models for estimating motion sickness build in prior studies do not capture the fact that the predictability of the exogenous motion patterns affects the sickness. This study presents a new computational model that includes the effect of the predictability of the dynamics of the exogenous motion stimulus on the motion sickness. The new model combines a conventional observer-theoretic sickness model with a sub-model representing human features of online learning of motion dynamics and the future prediction using a recursive Gaussian process regression. Using the proposed model, a simulation experiment was conducted to calculate the predicted motion sickness incidence under the condition that a human was moved horizontally for 900s, in which the motion was composed of repetitions of 9 m back-and-forth movement and a pause. As a motion factor, the direction and timing of the motion were changed as follows: predictable (M_P), the direction of the motion and duration of the pause (8 s) were fixed; direction unpredictable (M_dU): the pause duration was fixed as in (M_P), but the motion direction was randomly determined; and timing unpredictable (M_tU), the motion direction was fixed as in (M_P), but the pause duration was random between 4-12 s. The results demonstrated that the predicted MSI for (M_P) was smaller than those of (M_dU) and (M_tU). Results agreed with the actual sickness observed during an experimental study with human participants conducted under motion conditions similar to our simulation. No significant difference in the predicted MSI was found when using the conventional model. This demonstrated that the proposed model effectively captured the effect of human motion prediction on motion sickness.

Disclosures: T. Wada: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ritsumeikan University.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.02

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant R24EB029173

Title: Nih funded nitrc's triad of services: software, data, compute

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[3]The Paulson Venture, Huntington Beach, CA; [4]TCG, Inc, Washington, DC; [5]Preuss Enterprises Inc., Miami, FL

Abstract: Aim of Investigation: NeuroImaging Tools and Resources Collaboratory (NITRC) is a neuroinformatics knowledge environment for MR, PET/SPECT, CT, EEG/MEG, optical imaging, clinical neuroinformatics, computational neuroscience, and imaging genomics tools and resources.
Methods: Initiated in 2006 through the NIH Blueprint for Neuroscience Research, NITRC's mission is to foster a user-friendly knowledge environment for the neuroinformatics community. By continuing to identify existing software tools and resources valuable to this community, NITRC's goal is to support its researchers dedicated to enhancing, adopting, distributing, and contributing to the evolution of neuroinformatics analysis software, data, and compute resources.
Results: Located on the web at www.nitrc.org, the Resources Registry (NITRC-R) promotes software tools and resources, vocabularies, test data, and databases, thereby extending the impact of previously funded, neuroimaging informatics contributions to a broader community. All services freely downloadable, NITRC-R offers over 1,300 public resources; NITRC-Image Repository (NITRC-IR) offers 17 data projects, 11,559 subjects, and 13,282 imaging sessions, and NITRC Computational Environment (NITRC-CE) provides cloud-based computation services downloadable to local machines or via commercial cloud providers such as Amazon Web Services.
Conclusions: In summary, NITRC is an established knowledge environment for the neuroimaging community where tools and resources are presented in a coherent and synergistic environment. NITRC is a trusted source for the identification of resources in this global community. With over 9,790 citations on Google Scholar, NITRC has supported over 34,560 registered users, served up 11.4 million total, and of that, 9.9 million data downloads, to over 1.4 million users generating 3.1 million sessions. In addition to untold downloaded Virtual Machines, NITRC-CE currently supports over 250 subscriptions on AWS Marketplace running over 365,400 compute hours. We encourage the neuroinformatics community to continue providing valuable resources, design and content feedback and to utilize these resources in support of data sharing requirements, software dissemination and cost-effective computational performance.

Disclosures: D.N. Kennedy: None. **N. Preuss:** None. **C. Haselgrove:** None. **A. Crowley:** None. **A. Paulson:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.03

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH SPARC OT2 025340

Title: A Python-based pipeline for computational modeling of peripheral nerve electrical stimulation

Authors: *E. D. MUSSELMAN, J. E. CARIELLO, W. M. GRILL, N. A. PELOT;
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Abstract: Electrical stimulation of peripheral nerves is used to treat a variety of diseases and to restore function following injury. However, translation of therapies to the clinic can be unsuccessful despite promising preclinical data, particularly if differences in nerve morphology require distinct cuff designs and stimulation parameters to achieve target responses. Computational modeling of nerve stimulation enables robust quantification of the effects of stimulation parameters and optimization to design device geometries and stimulation parameters to achieve desired responses to stimulation.

We developed an open-source computational modeling infrastructure to automate the complex, multi-step simulations of the responses of fibers within individual-specific nerves. The pipeline uses Python, the COMSOL API for use with Java, and NEURON, and all model parameters are defined in JSON files. Model inputs, such as nerve morphology, coordinates of nerve fibers, and stimulation waveforms, are prepared in Python. In COMSOL, extracellular potentials are computed along the length of model fibers seeded in the three-dimensional representation of the nerve and cuff electrode. Extracellular potentials along each fiber are applied as a time-varying signal in NEURON, and Python is used to analyze and plot simulation outputs (e.g., thresholds). The pipeline increases the accessibility of model-based design for peripheral nerve interfaces and enhance reproducibility of modeling data. Researchers will be able to plan and interpret results from surgical interventions using our tools that are essential for achieving model accuracy: automated nerve geometry representation from images of segmented histology or user-defined nerve and fascicles sizes and locations, a library of parameterized cuff electrode parts for assembling custom nerve interfaces, integration of published biophysical nerve fiber models (e.g., MRG model of myelinated fibers, Tigerholm model of unmyelinated fibers), and parameterized stimulation waveforms.

Computational modeling is a critical tool to determine and control the input-output relationship between applied electrical stimulation and the complement of nerve fibers that are activated or blocked. This simulation pipeline enables large-scale simulations with a platform that is user-friendly, expandable, and thorough in its feature set.

Funding: NIH SPARC OT2 025340

Disclosures: **E.D. Musselman:** None. **J.E. Cariello:** None. **W.M. Grill:** None. **N.A. Pelot:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.04

Topic: I.06. Computation, Modeling, and Simulation

Title: Inferring forward and inverse dynamics using a NeuroBondGraph Network

Authors: *Y.-J. CHANG[1], Y.-I. CHEN[1], H.-C. YEH[1], J. M. CARMENA[3,4], S. R. SANTACRUZ[1,2];
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Abstract: Local field potentials (LFP) provide rich information about population dynamics across neural networks for neuroscientific studies and therapeutic applications. Yet, the invasiveness of LFP and incomplete cortical coverage limit the acceptability and usage of LFP-based brain-machine interface (BMI). Here, we introduce the NeuroBondGraph Network (NBGNet), a deep learning method to infer forward and inverse dynamics between neural activities in different modalities. When applied to the macaque cortical datasets, the bi-directional translation modeling accurately estimates LFP from brain signals recorded from intracranial macroelectrodes (ICME) and vice versa. To validate the predictive power and stability of NBGNet, we utilized multiple metrics: (1) scale-dependent measures such as root mean squared error to determine how close the predictions and the ground truth were, (2) cross-correlation to evaluate the similarities between them, and (3) phase synchrony to quantify the phase difference between them. The presented analysis could significantly improve the performance of LFP-based closed-loop neuroprosthetic applications without increasing the LFP channel count. Furthermore, our predictive models have future clinical utility by illuminating the brain computations in multi-scale networks to facilitate the development of clinical devices and rehabilitative therapies.

Disclosures: Y. Chang: None. **Y. Chen:** None. **H. Yeh:** None. **J.M. Carmena:** None. **S.R. Santacruz:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.05

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH OT2 OD025340

Title: Particle swarm optimization of ionic conductances identifies models of varied c-fiber subpopulations

Authors: *B. J. THIO, N. D. TITUS, N. A. PELOT, W. M. GRILL;
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Abstract: C-fibers constitute the vast majority of axons within peripheral nerves, but their small diameters prevent application of voltage clamp to characterize their membrane properties. Previous C-fiber models only replicated a subset of experimental conduction responses for

specific C-fiber subpopulations. We created a library of novel isoform-specific ion channel models and used particle swarm optimization to reverse engineer underlying C-fiber membrane properties of two C-fiber subpopulations. The resulting optimized models of autonomic and cutaneous C-fibers reproduced a broad range of experimentally measured electrophysiological properties including conduction velocity, chronaxie, and the dynamics of the paired pulse recovery cycle. As further validation, we simulated activity dependent slowing (ADS), a property not included in model optimization. The models exhibited ADS that closely matched published experimental data for autonomic (1.5-1.8% slowing in model vs. 1.9-2.2% slowing in experiment) and cutaneous fibers (12.7-16.1% slowing in model vs. 13.8-20.7% slowing in experiment). Finally, we simulated the fidelity of action potential activation of the C-fibers to pulse trains of extracellular stimulation at rates ranging from 2 to 10,000 pulses per second which is of interest in understanding the mechanisms of pain. Both fiber types exhibited conduction failure starting at 10-20 pulses per second and conduction was blocked by kilohertz rate stimulation. The resulting C-fiber models constitute an important tool for understanding the mechanisms of nerve fiber responses to electrical stimulation and designing novel neuromodulation interventions. Additionally, the novel reverse engineering approach of the particle swarm optimization framework can be applied to generate models of other neurons where electrophysiological data are available but voltage clamp data are not.

Disclosures: **B.J. Thio:** None. **N.D. Titus:** None. **N.A. Pelot:** None. **W.M. Grill:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.06

Topic: I.06. Computation, Modeling, and Simulation

Support: RGPIN 2020-0675

Title: Investigating the reliability of fast motor maps at varying motor threshold intensities

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Abstract: The organization of the primary motor cortex (M1) can be non-invasively assessed using transcranial magnetic stimulation (TMS) combined with frameless stereotaxic brain navigation. Traditionally, this technique requires stimulation of M1 in a grid-like pattern at predefined targets, delivering 3-5 stimulations per site with an interstimulus interval (ISI) of at least 5s. Various characteristics of these motor maps can be explored such as area to visualize the somatotopy of M1. However, the time required to produce these maps (~20 min per map) reduces the feasibility of this technique in more time sensitive protocols, such as fatigue studies or clinical brain tumor mapping. Van de Ruit (2015) recently developed a new way of collecting these maps, termed “fast motor maps”, that relies on delivering TMS pulses to pseudo-random locations within a 6x6 cm space overlaying M1. This technique improves map acquisition time

(~3 minutes per map) and is reliable within a session when performed at 120% resting motor threshold (RMT). No study however, has examined fast motor map reliability at more than one stimulus intensity. Higher TMS test intensities have been shown to decrease variability and improve reliability for MEP responses. The primary purpose of the present study was to investigate the reliability of fast motor maps as a function of TMS intensity using both relative and absolute reliability statistics. Intrasession reliability was assessed in 12 healthy participants for maps acquired at 110, 120, and 130% RMT in the right and left FDI muscles, before and after a 2-hour rest period. Relative reliability was assessed using the intraclass correlation coefficient (ICC) to quantify the degree to which individuals in the sample differed from one another and how well these differences could be distinguished by the measurement/test. Absolute reliability, measured with standard error of measurement (SEMeas), was used to estimate the distribution of repeated measures around the true score of the individual. Results illustrated that maps collected at 130% RMT exhibited the greatest level of absolute and relative reliability for area. Specifically, map area was moderately reliable for both RFDI (ICC 0.72, SEMeas 22%) and LFDI (ICC 0.73, SEMeas 15%) muscles at 130% RMT. Map area at 120% RMT was poor and moderately reliable for RFDI (ICC 0.42, SEMeas 34%) and LFDI (ICC 0.6, SEMeas 18%) respectively. These results demonstrate that higher stimulation intensities are required to elicit the most reliable motor map using the fast mapping technique.

Disclosures: S. Foglia: None. **C. Turco:** None. **A.J. Nelson:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.07

Topic: I.06. Computation, Modeling, and Simulation

Support: Independent. First author. For common good.

Title: A new neuromechanic semantic of turning using fuzzy spinors for quantum-like electric-charge relocation in intact brains: a non-classic, electromagnetically sensitive and delicate probabilistic process

Authors: *J. F. GOMEZ-MOLINA;
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Abstract: INTRODUCTION. Excitable tissues are more probabilistic but more organized, delicate and sensitive at the subcellular and sub-millisecond level than previously thought. They present new phenomena in relation to electroneutrality as well as capacitive and ephaptic effects that need to be revisited (Savtchenko et.al. 2017). The LFP, EEG and MRI-techniques cannot longer be understood in terms of deterministic equations for co-activated cells but in terms of open, activation and relocation probabilities of coactivated ion channels and electrically charged particles (Gomez 2003, 2016). Electrophysiological and inflammatory activation states need to be defined not only in terms of mitochondria, neurons, glia and vascular cells but also in terms of

free radicals and unpaired electrons in order to apply appropriately the equations (Gomez 2020 NIH Rehab). New circuit or single-particle models can be developed to describe possible forms of signaling with electronic unbalances (in charge, spin and pairing) associated to membranes and macromolecular networks of ion channels or the cytoskeleton. Here we present some elements to facilitate new models. -METHODS. Simplified toy-models based on fuzzy math and probabilistic approaches. Analysis of sensolocomotion and turning in ants, humans and robots during climbing against gravity. -RESULTS. Some elements of the Schrodinger's equation, Spinors and (de)fuzzification are used to revisit controversies, explore wave-like propagation of states, exotic and generalized forms of charge relocation and long-range electric signals. -DISCUSSION. Modern physical models are complex, too difficult and abstract (Gomez and Ricoy H-abstract SfN 2020). Tests with new non-invasive biophysical and neuromechanical experiments are needed. CONCLUSIONS. 1. The concept of spinors of Pauli-Dirac can be applied to charge relocation (discontinuous changes in vector position) in the brain. 2. To understand electromagnetism in general we need: i) new logic and probabilistic expressions ii) new kind of experiments in biophysics and psychophysics iii) an intuitive semantics with this new phenomenology. iv) more sensible techniques (QFT-type?) to model, measure and stimulate probabilistic molecular signals. 4.Recent finding showing high sensitive of neurons to electromagnetic fields, critically multiply the measurement problem in physics, making us to assume a more humble epistemological attitude. This has important consequences in the design of non-invasive research, from the subcellular (electron transfer signaling, quantum dots, nanosensors) to the system (NMR/EPR, neuroprothesis, EEG/MEG, TMS) level.

Disclosures:

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.08

Topic: I.06. Computation, Modeling, and Simulation

Title: Microstructural variation in the human striatum using non-negative matrix factorization

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Abstract: The striatum is a deep grey matter nucleus involved in various neurological and neuropsychiatric disorders. Although the functional role of the striatum has been broadened, its tissue microstructural organization and variation remain poorly known. Here, we developed a normative multimodal, data-driven microstructural parcellation of the striatum and explored how variation from the normative model relates to motor and cognitive performance as well as demographics. We used a subset of the multimodal MRI data along with behavioural/demographic data from the Human Connectome Project Young Adult dataset

(n=333, age: 22-35). We assessed microstructure using mean diffusivity (MD), fractional anisotropy (FA) and the ratio between T1- and T2-weighted structural MRI data (T1w/T2w; putative marker of myelin). We extracted spatially distinct components representing patterns of covariance in microstructure across subjects using non-negative matrix factorization (NMF). NMF decomposed a vertex-wise matrix with each subject's metric as columns and voxels as rows. Using the stability and accuracy of NMF clusters in a split-half analysis, we identified 5 striatum components. Then, we used partial least squares (PLS) to link inter-individual variation in the striatal components to selected behaviours and demographics. We found four significant latent variables (LV, $p<0.05$), two for each the left and right striatum. The first left LV (left LV1, 57% variance explained) was associated with young age, male sex and increased MD across all 5 components. Left LV2 (29%) correlated with young age, female sex, decreased FA in components 1, 3, 5 and decreased T1w/T2w in all components. Right LV1 (58%) correlated with young age, male sex and increased MD across all components. Right LV2 (31%) was associated with young age, increased FA in all components and increased T1w/T2w in components 1, 3. Our findings suggest distinct microstructural patterns in the human striatum that relate to demographics. Our work also highlights the gain in clusters' stability when using multimodal versus unimodal metrics.

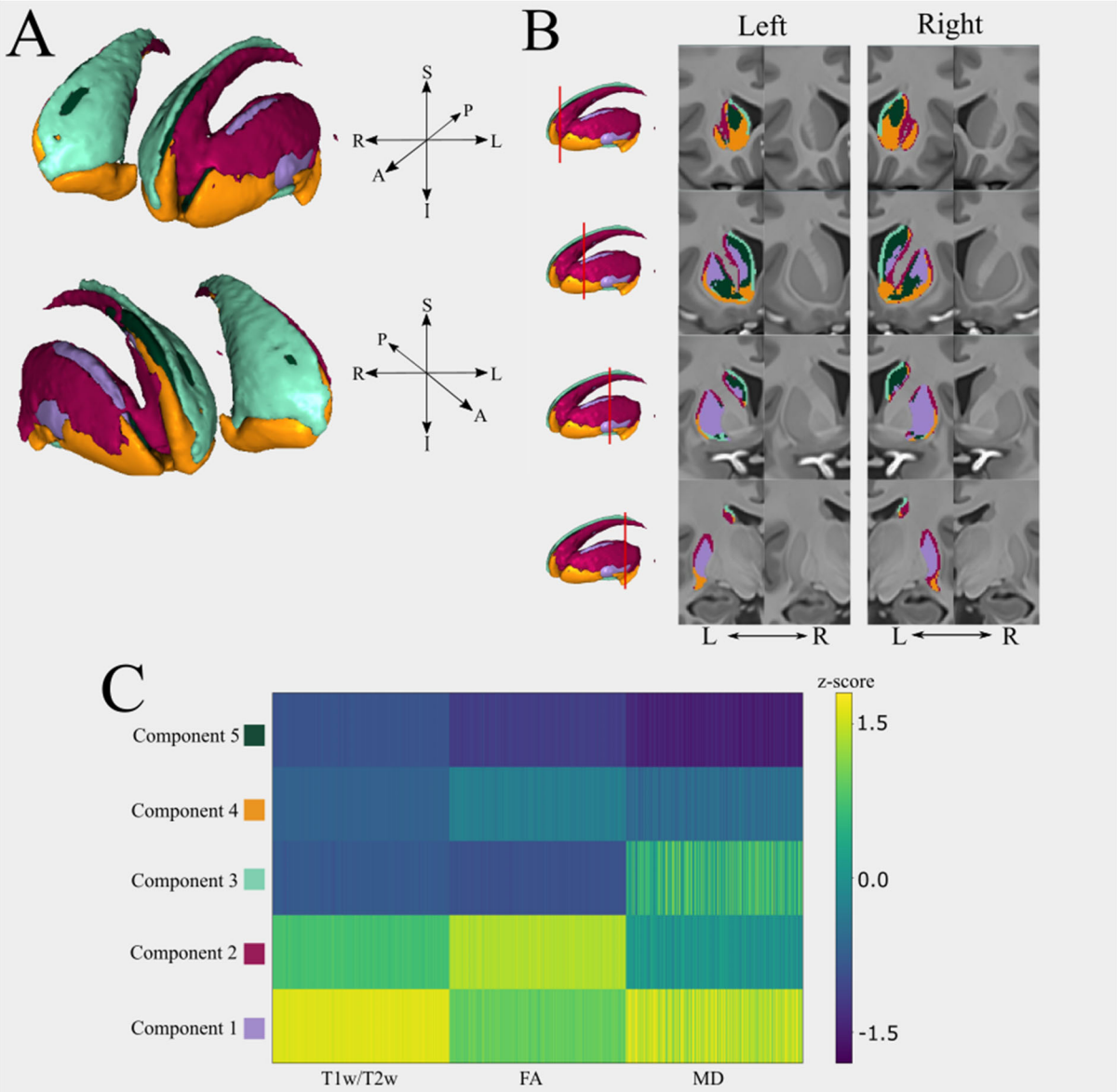

Figure 1: A) 3D rendering of the 5 components solution (A: anterior; P: posterior; S: superior; I: inferior; R: right; L: left). B) Coronal slices showing the labelled and unlabelled (side-by-side columns) of the left and right striatum. C) Weight matrix output from NMF of the left striatum, showing how the microstructural metrics weight into each component (the right weigth matrix is similar).

Disclosures: C. Robert: None. **R. Patel:** None. **N. Blostein:** None. **C. Steele:** None. **M. Chakravarty:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.09

Topic: I.06. Computation, Modeling, and Simulation

Support: Conacyt:712328

Title: Application of the novel object recognition test in Wistar rats by the use of the MOTUS software

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Abstract: The study of animal behavior can be carried out using different behavioral tests. The novel object recognition test (RON) is relevant because it assesses short-term (MCP) and long-term (MLP) memory. The use of software for behavioral tests has shown advantages by facilitating and presenting results in greater detail. This study describes the use of software (MOTUS) on animal behavior, analyzing its performance on the RON test, through the detailed exposition of the path that the rodent follows and the interaction with objects (Familiar Object and Novel Object). Route graphs, accumulated time in regions, speed and distance to each of the objects are obtained. In the present work, 6 male rats of the Wistar strain without any treatment were used at the age of P120. For the behavior test a 90x90x50 cm sand was used and there were three objects: the familiar object (pyramid) and two novel objects (cube and cylinder), which were selected and placed at a distance of 20 cm from the corner to the sand and 50-60 cm between them (Ordoñez, 2013; Aubele *et al*., 2008; Goulart *et al.,* 2010). The test was carried out in four sessions (habituation, training, MCP and MLP), each session lasted 5 min. The MOTUS program worked with X, Y coordinates which were previously obtained using the idTracker software. For the graphic representation of the results were indicated in the software: the specific coordinates of the objects in addition to the size of the sand. In the MCP session it was obtained an average percentage of 8.66% in the latency interaction and 58.84% on frequency interaction with the novel object while of the familiar object it was 4.59% and 41.15% respectively, the relevant objects distance graph was generated by this information. On the other hand, in the MLP session it was obtained a percentage of 11.30% by the latency interaction with the novel object and a 64.62% on frequency interaction while of the familiar object was 3.59% and 35.79% respectively. In addition, the software is freely accessible and has provided information on the average speed, distance traveled and acceleration, thereby prove to be an effective, practical and easy software for monitoring behavioral dynamics in the RON test and, potentially, with other behavioral test. https://www.uv.mx/ceicah/proyectos-academicos/desarrollos/motus/ (León *et al.,* 2020).

Disclosures: **M. Alvarado:** None. **P. González:** None. **V. Hernández:** None. **A. León:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.10

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant MH119735
NIH Grant NS088590
NIH Grant TR000448
NIH Grant MH104592
NIH Grant HD087011
NIH Grant MH104605

Title: Precision dynamical mapping using topological data analysis reveals a unique Transition State at rest in highly sampled individuals

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Abstract: Even in the absence of external stimuli, neural activity is both highly dynamic and organized across multiple spatiotemporal scales. The continuous evolution of brain activity patterns during rest is believed to help maintain a rich repertoire of possible functional configurations that relate to typical and atypical cognitive phenomena. Whether these transitions or "explorations" follow some underlying arrangement or instead lack a predictable ordered plan remains to be determined. Although innovative approaches have been previously developed to reveal the temporal structure underlying transitions in the brain at rest, several methodological and data quality issues precluded the development of a clear understanding of network dynamics at the individual level. Here, we overcame these limitations by examining the dynamic states during intrinsic brain activity using Topological Data Analysis (TDA) on a maximally denoised precision neuroscience dataset - The Midnight Scan Club (MSC). This dataset comprises of ten highly sampled individuals, with >5 hours of resting state fMRI data per individual and individualized parcellations of each brain. Without temporal averaging or sliding windows, our TDA-based approach mapped whole-brain resting state volumes onto a set of individually defined intrinsic dynamical manifolds or "state spaces". For reliability, all reported results were validated using split-half cross validation. Further, multivariate autoregressive and phase randomized null models were used to examine how topological properties of the revealed landscape were driven by linear versus non-linear properties of the data. Using our TDA-based approach, we observed a rich topographic landscape in which the transition of activity from one network to the next involved a large shared attractor-like basin, or "transition state", where all networks were represented equally prior to entering distinct network configurations. The intermediate transition state and traversal through it seemed to provide the underlying structure for the continuous evolution of brain activity patterns at rest. In addition, differences in the manifold architecture were more consistent within than between subjects, providing evidence that this approach contains potential utility for precision medicine approaches.

Disclosures: **M. Saggar:** None. **J.M. Shine:** None. **R. Liegeois:** None. **R. Raut:** None. **T.O. Laumann:** None. **A.Z. Snyder:** None. **N.U.F. Dosenbach:** None. **D.A. Fair:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.11

Topic: I.06. Computation, Modeling, and Simulation

Support: NRF-2017M3C7A1049051
NRF-2017H1D3A1A01053094

Title: A computational control scheme for self-adjustment brain system

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Abstract: Treatment of brain disease is difficult due to the complexity of the brain. Computational frameworks, which can simulate dynamics of the before and after treatment brains, have been developed to resolve this problem. However, in these frameworks, self-adjusting nature of brain system after treatments has not been fully considered and the brain system was only regarded as a passive responding system. Thus, an initial plan of treatment may not always the best solution. In the present study, we propose a computational framework for optimal control of the self-adjustment brain system.
To describe brain dynamics, we adopted dynamic causal model (DCM) [1] which can generate experimental observation signals from neural activities of the models. We modeled the plasticity of the effective connectivity of the neural system after the treatments. Two types of plasticity, activity-dependent plasticity and homeostatic plasticity, were implemented as the brain's self-adjustments, and neural system was updated by self-adjustments after each treatment.
The solutions for optimal control of the brain were explored with considerations of the brain self-adjustments. Considering various clinical treatments, we set several restrictions such as one treatable node which mimics transcranial magnetic stimulation (TMS), global treatment which mimics medications, and mixed treatments (simultaneous treatments of TMS and medications). In the present scheme, first, using neuroimaging data, the effective connectivity of the brain system was estimated by DCM. Then, applying the treatment and self-adjustment rules, we simulated observation signals of updated system, and explored the best solution that can generate similar observation signals of target brain system. To estimate system parameters of self-adjustments, we used signals that were obtained from previous treatment.
To test our scheme, we used hippocampus systems of mutant and wild-type mice that were analyzed in our previous study [2], where the effective connectivity of the rodent's hippocampus circuitry were estimated by using DCM of the voltage-sensitive dye imaging (VSDI).
Simulation results showed that optimal control of the abnormal mutant system successfully approached to the signals of the normal wild type under restrictions that mimic various clinical treatments. We believe that the proposed computational framework of the self-adjustment system could help optimal control of the dynamic brain after treatment.

[1] Friston, K. J., et al. *Neuroimage* 19(2003) 1273. [2] Kang, J.,et al., *NeuroImage* 213 (2020) 116755.

Disclosures: J. Kang: None. **H. Park:** None. **J. Eo:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.12

Topic: I.06. Computation, Modeling, and Simulation

Support: CONP/ Brain Canada and Djavad Mowafaghian Centre for Brain Health.

Title: Automated scaling and segmentation of mouse mesoscale cortical maps using machine learning.

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Abstract: Understanding the basis of brain function requires knowledge of cortical operations over wide spatial scales, and quantitative analysis of brain activity in well-defined brain regions. Matching an anatomical atlas to brain functional data has traditionally been a labor-intensive procedure that precludes high-throughput analysis. We have developed an automated machine learning-based registration and segmentation approach for quantitative analysis of mouse mesoscale cortical images. We fine-tuned a pre-trained deep learning model to identify nine cortical landmarks using a wide-field calcium imaging dataset from more than 400 GCaMP transgenic mice. A fully convolutional network was trained to delimit brain boundaries. We present the methodology to automatically register and align mesoscale cortical images as MesoNet, a robust and user-friendly analysis pipeline using pre-trained models to segment brain regions as defined in the Allen Mouse Brain Atlas. This Python-based toolbox can also be combined with existing analysis methods to cluster distinct cortical activity motifs.

Disclosures: D. Xiao: None. **B. Forys:** None. **M. Vanni:** None. **T. Murphy:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.13

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant OD030541

Title: The SPARC Data Structure: serving the peripheral nervous system research community funded under SPARC

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Abstract: The Stimulating Peripheral Activity to Relieve Conditions (SPARC) program is a NIH funded project to fund, collect, and curate various peripheral nervous system data for inclusion into an open data portal, https://sparc.science. 28 awards have been funded over 4 years to groups from 19 universities and 4 companies; it was necessary to develop a consistent structure to organize, label, and annotate data. The project is composed of investigators who generate data, 4 data handling cores (data storage, data curation, mapping, and simulation), as well as investigators who are building stimulators that will eventually be used to intervene with peripheral nervous system disorders.
The SPARC Data Structure, SDS (https://github.com/SciCrunch/sparc-curation/releases), is derived from the Brain Imaging Data Structure (BIDs). BIDs is a simple folder structure with predictably named metadata files that enables investigators to align data files and describe them in a consistent manner. The SDS is also a simple folder with metadata files, but it allows inclusion of data modalities such as RNASeq, immunohistochemistry, in addition to imaging. Like BIDs, the top level folder includes standardized metadata description files, containing specific information about subjects as well as general dataset details, and unlike BIDs samples, and finding specific information. All four of these files are created from standard templates designed by the curation team and approved by the SPARC data structure committee. Each folder includes data files, named in a consistent manner with the metadata files and includes a manifest describing additional details about the files.
As each dataset is submitted by investigators, it is checked by an automated tool (SDS Validator https://github.com/SciCrunch/sparc-curation), which produces a set of errors and warnings. An upload assistant, SODA (https://github.com/bvhpatel/SODA), is a desktop application designed to organize data automatically for the SPARC investigators. Curators work with dataset authors to eliminate the errors and reduce the warnings, ensuring that every dataset is properly structured, contains appropriate metadata elements, and is machine readable. The current SPARC portal contains 55 datasets, as of November 12, 2020, and an additional 186 datasets are in process. Each released dataset has a descriptive image, a structured abstract, curation notes, a set of machine readable data files aligned to SDS structure.
The SDS should improve the usability of SPARC data.

Disclosures: **A.E. Bandrowski:** A. Employment/Salary (full or part-time):; SciCrunch Inc. **A. Pilko:** None. **T. Gillespie:** None. **M. Surles-Zeigler:** None. **G. Pine:** None. **T. Sincomb:** None. **P.L. Pascual:** None. **D. Rawool:** None. **I.B. Ozyurt:** None. **J.K. Boline:** None. **J.S. Grethe:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SciCrunch Inc. **M.E. Martone:** A. Employment/Salary (full or part-time):; SciCrunch Inc.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.14

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant MH121119

Title: Waaves+ 2.0: a process for developing an innovative web-based automated usv scoring platform

Authors: *C. L. DUVAUCHELLE[1], R. SNYDER[3], J. SHEPHERD[3], C. CERVANTES[1], C. ASHLEY[3], N. MITTAL[4], S. M. FLEMING[5], J. BAILEY[2], M. NIEVERA[1], S. SOULEIMANOVA[1], B. NYAOGA[1], L. LICHTENFELD[1], W. MADDOX[6];
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Abstract: Ultrasonic vocalizations (USVs) are known to reflect emotional processing, brain neurochemistry, and brain function. USVs in the 22-28 kHz and 50-55 kHz frequency range are widely recognized forms of social and emotional expression in rodents. 22-28 kHz USVs reflect negative affect status and are initiated by regional activation along the ascending mesolimbic cholinergic (ACh) pathways. 50-55 kHz USVs reflect positive affect status and are initiated by regional activation along the mesolimbic dopaminergic (DA) pathway. Collecting and processing USV data is manual, time-intensive, and costly creating a significant bottleneck by limiting researchers' ability to employ fully effective, and nuanced experimental designs, and serving as a barrier to entry for other researchers. Duvauchelle et al (2013, 2014) addressed this bottleneck by developing WAAVES, an automated USV assessment program that utilizes a decision tree architecture. Unfortunately, WAAVES requires manual threshold calibration for each unique environmental context reducing its benefit to the wider research community. Last year at SFN we introduced a new automated USV scoring algorithm, WAAVES+ that is environment- and animal-agnostic. WAAVES+ uses supervised and unsupervised machine learning techniques to detect, isolate, parameterize, group, and classify USVs. In this poster, we present the detailed process that we utilized to further optimize WAAVES+ 2.0. First, we developed an Amazon Web Service (AWS) back-end framework for storage and automated analysis of USV files with an intuitive user interface using the React framework (Javascript). The main processing script can be run both on AWS (where a run is triggered by uploaded files) and via local resources providing the ability to gather data from many sources simultaneously without disrupting the development cycle. Program updates and new analytics can be deployed to all users directly and instantaneously via the website. We have also modified the call detection program (Python) for off-line batch processing of USV audio files that can provides the developer with a number of useful data metrics and statistics. Third, we hand scored several hundred minutes of new USV files from multiple recording environments into 17 rat and 9 mouse call categories. We are currently developing a machine learning approach that utilizes these data in an ongoing and

iterative fashion to improve call detection and call classification capabilities via reinforcement learning algorithms that continue to improve WAAVES+ 2.0 performance based on user feedback.

Disclosures: **C.L. Duvauchelle:** None. **R. Snyder:** None. **J. Shepherd:** None. **C. Cervantes:** None. **C. Ashley:** None. **N. Mittal:** None. **S.M. Fleming:** None. **J. Bailey:** None. **M. Nievera:** None. **S. Souleimanova:** None. **B. Nyaoga:** None. **L. Lichtenfeld:** None. **W. Maddox:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.15

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH R21 (NS108380)
ONR MURI (N00014-16-1-2832)

Title: Evaluating methods for estimating geometry of neural representations

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Abstract: There is growing recognition that the geometry of neural population representations place important constraints on cognitive function and behavior. Representational geometry refers to the relationships among the firing patterns of a population across its inputs. One key feature of the geometry is its dimensionality, defined as the number of unique axes required to fully specify the position of all the firing patterns of a population across all conditions of a task. Representational dimensionality has been shown to correlate with behavior in highly trained monkeys, but has not been systematically studied in humans. A major obstacle to bridging human and animal studies in this domain is that we presently lack techniques to estimate representational dimensionality non-invasively in humans, using methods like fMRI and EEG. Recently, Rigotti & Fusi (2016) proposed a method for leveraging repetition suppression (RS) effects in fMRI to estimate representational dimensionality. However, the impact of realistic hemodynamic effects would be on these estimates was not considered. In this study, we sought to address this gap and validate the method in realistic neural-to-BOLD simulations of repetition suppression effects using efficient trial sequences which could be feasibly given to human subjects. We employed a model of repetition suppression to simulate neural firing patterns of known dimensionality and used a standard gamma function to simulate hemodynamic responses to these firing patterns. We then employed a previously developed cross-validated singular value decomposition method to estimate the dimensionality of the recovered signal from the BOLD response, which was evaluated against the known underlying dimensionality of the neural firing patterns. Results from our simulations provide a validation of the method and systematically demonstrate that the method is useable, but the robustness of dimensionality estimations is affected by three main factors: neural and fMRI noise, correlated history effects of the BOLD

signal, and the shape of the input matrix used to test dimensionality. Each of these factors drives a bias toward under-estimation of the true representational dimensionality in the neural signal. We discuss ways to mitigate these effects and achieve better dimensionality estimations through careful study design.

Disclosures: **H. Keglovits:** None. **E. Chicklis:** None. **A. Bhandari:** None. **D. Badre:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.16

Topic: I.06. Computation, Modeling, and Simulation

Support: NIBIB U01 EB025830

Title: Meso-scale structure of population spiking dynamics as revealed by correlation and filter estimation in a computational model of rat hippocampus

Authors: ***G. J. YU**, J.-M. C. BOUTEILLER, T. W. BERGER;
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Abstract: Investigations into the meso-scale connectivity of hippocampus have revealed a spatial modularity that is dependent on axonal anatomy and may provide a means to identify higher-level functional units beyond the neuronal and microcircuit levels. These meso-scale functional units have yet to be fully explored. Two methods - spatio-temporal correlation and Laguerre-Legendre basis functions - were used to predict the spatial and dynamical properties of putative higher-level functional units using spiking data generated by a large-scale and multi-scale neuronal network mode of the rat hippocampal entorhinal-dentate-CA3 system. The network contained 112 000 entorhinal neurons, 120 000 dentate granule cells, and 25 000 CA3 pyramidal cells. The neuronal models were distributed along the septo-temporal and transverse axes to represent the full hippocampal volume which was necessary to accommodate the meso-scale connectivity. The connectivity of neuronal network was constrained using anatomical data that characterized the size and shape of projections and their topographical organization. The first method for identifying a meso-scale functional unit was the correlation map. The correlation map was constructed by computing pairwise temporal correlations which were then sorted based on the relative distance between the neuron pairs. The second method was to represent the transformation of neural spiking using Laguerre and Legendre basis functions which account for the temporal and spatial properties, respectively. Both the correlation map and Laguerre-Legendre filters used spiking activity for their analysis and characterized the functional similarity of neurons in space and time. Both methods captured the spatial size and shape within which neural spiking was related, thereby estimating the spatial size and shape of the functional unit. Finally, both methods contain a temporal aspect which captures the dynamics of the functional unit. Key differences are that correlation is acausal and linear, and Laguerre-Legendre is causal and nonlinear. The results suggest that neural modules are not discrete but change over a

continuum depending the subpopulation of neurons being observed and are dependent on the topography of the connectivity. The characterization of these meso-scale functional units offers a method to understand how information within a neural system is processed and distributed throughout a network.

Disclosures: G.J. Yu: None. **J.C. Bouteiller:** None. **T.W. Berger:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.17

Topic: I.06. Computation, Modeling, and Simulation

Title: Modularity measures of functional brain networks predict individual differences in delayed recognition memory

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Abstract: Long-term memory (LTM) is crucial to daily functioning, and individuals show a large variance in LTM capacity. In this study, we ask: to what extent does the functional organization of the brain explain individual differences in a widely studied form of LTM, delayed recognition memory (REC)? Recent work has adopted tools from graph theory to examine how modularity, the degree to which the brain can be divided into distinct modules, is related to cognition (Bertolero et al., 2018). Using fMRI data from n-back tasks and recognition memory data from the Human Connectome Project (n=708), we explored how different modularity properties of nodes in the brain may relate to individual differences in REC. More specifically, we focused on two properties that describe distinct aspects of network functioning: diversity (the extent to which a node has diverse connections with different modules; measured with participation coefficient [PC]) and locality (the extent to which a node has more connections within their own modules; measured with within-module degree [WMD]). To investigate if node diversity and locality are related to REC, we built a predictive model with these two measures and validated it in a leave-one-out cross-validation scheme. For PC, we first correlated the PCs of each node with REC across the subjects within the training sample and selected nodes whose PCs were correlated with REC. We then correlated each subject's PCs with the group-based PC-behavior correlation map of the selected nodes. This value represents how optimized this subject's PCs among the selected nodes are for REC performance. The same procedure was repeated for WMD. We then used linear regression to relate the PC and WMD optimality measures with REC performance in the training sample. This model was used to predict the REC score in the left-out test subject based on their modularity measures. This predictive model significantly predicted individual differences in REC (Pearson's $r = 0.26$, $p < .001$). In addition, we found that the node diversity of the Medial Frontal network contributed most to the prediction, while the node locality of the Frontoparietal, Visual I, and Visual Association networks contributed most to the prediction. Interestingly, for nodes in the Default

Mode network, both diversity and locality played important roles in the prediction of REC. Using recognition memory as an example, our findings extend previous work on how the modularity of brain is related to cognition and demonstrate the utility of using graph-theory-based measures to reveal how the modularity of brain networks is related to individual differences in LTM performance.

Disclosures: **M.B. Zhou:** None. **Q. Lin:** None. **M. Chun:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.18

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH BRAIN U01-NS108637

Title: Computational tools for rapidly visualizing large-scale activity with single-neuron, single-spike resolution in simple brains

Authors: J. W. BROWN, E. S. HILL, W. N. FROST;
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Abstract: Despite its unceasing activity and centrality in an animal's moment-to-moment existence, the brain operates invisibly. While functional imaging techniques like fMRI and a range of optical electrophysiological methods can provide both large-scale and granular insights and into neurophysiology, achieving simultaneous resolution of brain activity at both of these scales with high temporal resolution to yield a "complete" picture of real-time brain function remains a challenge. Simple nervous systems comprising relatively few, large, mostly superficial, and individually identifiable neurons enable electrophysiological investigations and analytical approaches that allow single-neuron, single-spike readouts over significant portions of those nervous systems. Here, we describe and illustrate several computational tools we have recently developed to visualize and animate optical data collected in the brains of gastropod mollusks, including that of *Berghia stephanieae*, a new model system and the subject of a current BRAIN Initiative U01 collaboration. Data are collected using a photodiode array imaging at 1,600 Hz and the fast, absorbance voltage-sensitive dyes RH155 and RH482. Following implementation of a previously developed method for blind-source separation of optical signals into individual neuronal activity traces using independent component analysis, we employ an automated algorithm to map individual neuronal signals onto the neurons that generated them in an image featuring a significant percentage of the imaged brain. Further code animates these maps to generate audiovisual "playbacks" of the networks encoding specific behaviors with high spatiotemporal resolution. Through the use of a previously developed community detection algorithm, we can furthermore highlight the specific activity patterns of functional neuronal ensembles over the course of a behavioral program. Together, these tools constitute an ultra-fast workflow that can produce same-day insights about single-cell activity and network organization

across large portions of a nervous system, enabling a range of subsequent analyses and allowing for analysis-driven follow-up electrophysiology in just-imaged preparations.

Disclosures: J.W. Brown: None. **E.S. Hill:** None. **W.N. Frost:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.19

Topic: I.06. Computation, Modeling, and Simulation

Title: Modeling of Neural Spike Pattern Transformation as Classification of Binary Images with a Convolutional Neural Network

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Identification of causal relationships of neural activity is an important problem in neuroscience and neural engineering. Past work has shown that cognitive processes such as memory formation rely on transformations of spatio-temporal patterns of spikes across different neural populations. Such transformations are nonlinear dynamical, and cannot be captured by linear models or generalized linear models. Shallow nonlinear models, including generalized functional additive models (GFAM) and multi-input multi-output models, have attempted to capture this nonlinearity with some success. Alternatively, Convolutional neural networks (CNN) can capture complex nonlinearity and abstract low-level and high-level features in natural images via the use of deep layers of nonlinear activation functions. It thus holds the promise of modeling complex neural spike transformations underlying cognitive processes.We treat spatio-temporal patterns of past input spikes as binary images and use a CNN to predict spiking in a single output neuron in a binary classification task. We generate synthetic data using a GFAM with multiple neurophysiologically inspired functions (kernels) and a probit link function. This creates a probability of spiking over time in the output neuron that is passed to a threshold to produce a binary neural time series representing spikes. A sliding window generates binary training images from this time series that are passed to a CNN with multiple layers of convolution with filters convolving over the time domain to capture temporal nonlinearity. Results show the CNN is able to achieve a high correlation between the predicted probability of spiking in the output neuron and the true probability of spiking in the output neuron for the data generated with a generalized functional additive model. Most importantly, the CNN is able to recover the ground truth kernels used to generate the probability of spiking in the output neuron during synthetic data generation. This shows that CNNs show promise in discovering the functions that determine neural spike transformations. Based on the CNN's validation via a GFAM, our current work includes validation of our approach with data generated with more complex nonlinear kernels as well as further validation with animal data.

Disclosures: B.J. Moore: None. **D. Song:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.20

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH NIBIB U01 EB025830

Title: Quantitative analysis of entorhinal cortical axonal arbors characteristics and its impact on axonal arbor growth using ROOTS algorithm

Authors: *S. FARZAD[1], T. MILLARD[1], C. BINGHAM[2], G. J. YU[1], J.-M. C. BOUTEILLER[1], G. LAZZI[1], T. W. BERGER[1];
[1]Biomed. Engin., USC, Los Angeles, CA; [2]Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: The advancement of computation and neuronal modeling in recent years has facilitated the study of neural tissue systems with increasing biological realism. Many model-based neuronal studies neglect axons and focus predominantly on dendrites and soma. However, because axons are often preferentially activated by extracellular electrical stimulation, relying on models that only focus on dendritic/somatic morphologies may result in overestimation of activation thresholds. Further, explicitly represented axonal arbors greatly facilitate spatiotemporal analysis of activity conducted through fiber systems and circuits. Ruled-Optimum Ordered Tree System (ROOTS) is a generative algorithm that yields unique and highly realistic axon terminal arbors constrained by user-specified morphometric parameters. ROOTS enables construction of population models for the analysis of the influence that microscale structures and branching patterns have on spatiotemporal patterns of activity in the presence of extracellular electric fields. However, the effects of the morphometric parameters on the final construction of increasingly complex axon arbors are unknown. Using a volume reconstruction of rat dentate gyrus and ~30 000 postsynaptic target locations in that volume, ROOTS was used to generate a three-dimensional entorhinal cortical axon arbor using multiple combinations of morphometric parameters. The resulting arbors were characterized using methods that have been applied for neural dendrites, e.g. total length, direct vs. path ratio, branch length, branching order and angle, and compared with experimental values. Correlation analyses reveal the relations between the various ROOTS morphometric parameters and the resulting emergent morphological properties of the arbors. The correlations discovered in this work can then be used to guide what morphometric parameters for axon arbors beyond the entorhinal cortex.

Disclosures: **S. Farzad:** None. **T. Millard:** None. **C. Bingham:** None. **G.J. Yu:** None. **J.C. Bouteiller:** None. **G. Lazzi:** None. **T.W. Berger:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.21

Topic: I.06. Computation, Modeling, and Simulation

Title: Creation of an automated registration and analysis pipeline for whole rat brain serial two photon tomography images

Authors: *A. D. AJAY[1,2], J. P. MEEKS[4,1,3,2], A. TAVAKKOLI[5], D. M. O. RAMIREZ[1,2]; [1]Dept. of Neurol., [2]Peter O'Donnell, Jr. Brain Inst., [3]Dept. of Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [4]Univ. of Rochester Sch. of Med. and Dent., Rochester, NY; [5]Dept. of Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Serial two-photon tomography (STPT) combines high throughput image acquisition and serial tissue sectioning to generate high resolution whole brain images. Such images have been widely used in studies involving cellular distribution and connectomics in mouse. We and others have earlier developed robust automated pipelines to quantify whole mouse brain volumetric image data acquired on the TissueCyte 1000 STPT system, but to date a similar pipeline did not exist for rat brain. Here, we report the development of a modified registration pipeline to allow for effective automated quantification of rat brain STPT datasets. The new pipeline incorporates a rat brain average template which was created using STPT images as well as annotations derived from the Waxholm Rat MRI atlas. Whole rat forebrain images were acquired using STPT at 0.875um/pixel lateral resolution and 100um axial resolution resulting in 150 serial sections per brain. In total, 15 rat brains were imaged. The MRI reference atlas template in the Waxholm coordinate space for the Sprague Dawley rat brain (WHS-SD MRI) was obtained from the Neuroimaging Tools and Research Collaboration website and the images were pre-processed in order to generate a STPT image based reference template to perform single modality image registration. First, the WHS-SD MRI reference atlas was scaled to match the STPT data size and all the input datasets were registered to this reference using SimpleElastix registration workflow for global and local registration. An initial average template was created from the 6 best registered samples and the workflow was performed iteratively in three steps until the majority of the input datasets registered accurately with the intermediate average template. The final template from the iterative process contained clearly defined anatomical features and resulted in more precise alignment of the individual brain samples. Next, the annotation atlas was rescaled to match the average template and was corrected for gaps due to resizing using ITK-snap software. Finally, we applied signal segmentation to the input fluorescent brain images using a the “Pixel Classification” applet in ilastik, generating probability maps that estimate signal density in registered brains. The adapted analysis pipeline utilizing the newly created rat brain average template and modified reference atlas will be a useful resource for large scale volumetric imaging projects in rats.

Disclosures: A.D. Ajay: None. **J.P. Meeks:** None. **A. Tavakkoli:** None. **D.M.O. Ramirez:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.23

Topic: I.06. Computation, Modeling, and Simulation

Title: The accurate localization method of the amygdala neural activities using magnetoencephalography and the advanced brain source imaging methods

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Abstract: The amygdala is a key subcortical brain structure in the appraisal of stimulus relevance, including those of emotional stimuli. The knowledge of the human amygdala comes from fMRI and PET studies, but they do not provide enough temporal resolutions required for analysis of temporal dynamics of the neural responses. Most amygdala neurons are pyramidal cells, the main sources of the magnetoencephalography (MEG) signal from the brain. Although the pyramidal cells in the amygdala nucleus do not show a laminar organization, it is possible that the activation of subpopulations of these cells may produce a non-zero net current dipole source that can be detected in the scalp-level MEG signals. Despite recent MEG studies of subcortical source imaging, the noninvasive MEG-based brain source imaging methods for the amygdala are still not well established due to limited accuracy in the conventional source estimation methods. We developed a MEG source localization pipeline that integrates anatomical structures obtained from T1-weighted individual MRIs and diffusion tensor imaging (DTI) tensor information, which can provide a more accurate estimation of inhomogeneous conductivity associated with white matter fiber structures. Given the subcortical structures that are located underneath of white matters, we hypothesized that the utilization of the DTI tensor information of white matter will substantially improve the source localization accuracy of the amygdala and other subcortical brain structures. We used the Minn-WashU Human Connectome Project (HCP) data, including10 individuals' T1, DTI, and 256 channel MEG data collected using 248 magnetometer sensors during resting state. We created synthetic MEG data by adding simulated neural activities of the amygdala and the primary visual cortex that varied in terms of the temporal width, amplitudes, and temporal overlaps between the subcortical and cortical brain activities to the original resting MEG signals that were preprocessed. We evaluated the source localization accuracy of the amygdala activities across the results with and without DTI-based inhomogeneous WM conductivity information as well as those obtained with the boundary element method (BEM) and finite element method (FEM) head models. We found substantially higher source localization accuracy of the amygdala with the DTI and FEM methods compared to those with the other methods. In particular, when we used both DTI and FEM methods, the amygdala source localization error was very low (<.5 cm). By combining the multimodal neuroimaging data and the advanced head modeling techniques, it is possible to obtain highly accurate MEG source imaging of the amygdala.

Disclosures: **S. Kang:** None. **M. Shim:** None. **C. Im:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.24

Topic: I.07. Data Analysis and Statistics

Support: National Natural Science Foundation of China, Grant number 82041023

Title: The risk of progression to dementia and cognitive characteristics in MCI participants: a cohort study using the NACC database

Authors: *F. ZHOU, V[1], X. ZHANG[2], X.-H. ZHOU[1,3];
[1]Dept. of Biostatistics, Sch. of Publ. Hlth., Peking Univ., Beijing, China; [2]Macau Univ. of Sci. and Technol., Macau, China; [3]Dept. of Epidemiology, Natl. Alzheimer's Coordinating Ctr., Univ. of Washington, Seattle, WA

Abstract: Objectives: We estimated the incident density and rate ratio of dementia in mild cognitive impairment (MCI) participants who were cognitively normal (CN) at the baseline and compared the cognitive characteristics of these MCI with different outcomes.**Methods**: Data were from the National Alzheimer's Coordinating Center (NACC), gathered from 32 Alzheimer's Disease Centers. A cohort of 2,180 participants with CN at baseline and progressed to MCI was established from 2005 to 2019, and follow-up their outcome. The Cox proportional hazards model was used to estimate risk factors associated with the progression of dementia.**Results**: Five hundred thirty-two participants progressed to dementia during the follow-up (108.5 per 1,000 person-years), 441 cases of them were Alzheimer's disease (AD, 89.9 per 1,000 person-years), another 305 participants converted from MCI to CN. Compared with 65-70 age group, rate ratio of dementia increase from 1.73 (95% CI: 1.11, 3.05) aged 70-75 to 4.25 (95% CI: 2.84, 7.39) aged more than 90, and AD rate ratio from 2.34 (95% CI: 1.37, 5.10) to 6.34 (95% CI: 3.91, 13.85). After adjusting for other confounding, a higher risk of progression to dementia was observed in elderly married women with a low level of education. Specifically, hazard ratios (HR) is 1.28 (P=0.0206) for female, 1.15 (P=0.0306) for education level (≤12, 13-16 vs. ≥17), 1.36 (P=0.0041) for married and 1.28 (P<0.001) for age (compared with 65-70 age group). Moreover, a significant risk factor for dementia was identified in participants with an APOE ε4 allele (HR=1.696, P<0.001). Although there was no significant difference between amnestic and non-amnestic MCI participants, the risk was reduced in participants with single-domain MCI (0.651, P<0.001). In addition, MCI participants progressed to dementia had a higher proportion of language and executive affect compared with participants converted from MCI to CN (21.24% vs. 14.43% and 42.48% vs. 33.11%). There is no significant difference in the effects of attention and visuospatial.**Conclusions**: This study providing information about the risk of progression to dementia in MCI individuals. Given these risk factors, this may provide helpful guidance to health-care providers on MCI prevention and screening.

Disclosures: **F. Zhou:** None. **X. Zhang:** None. **X. Zhou:** None.

Digital Abstract Session

P388. Tools For Analyses of Neural and Physiological Data

Program #/Poster #: P388.25

Topic: I.07. Data Analysis and Statistics

Support: Institute for Collaborative Biotechnologies
California NanoSystems Institute
Hellman Family Foundation

Title: Functional connectivity profiles predict trial-by-trial success in a navigation task

Authors: ***R. F. WOODRY**, E. R. CHRASTIL;
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Abstract: Functional connectivity between brain regions reveal network properties that reflect cognitive differences across tasks, individuals, and groups. Connectome-based Predictive Modeling (CPM) is increasingly used to predict how brain states fit these functional connectivity patterns, which provides novel insights into psychological traits. However, CPM has not yet been used to analyze task performance, especially at the trial level. Here, we tested the performance of four machine learning models and three task-based functional connectivity metrics to predict individual trial-by-trial behavioral performance during a navigation task. The first-person navigation task consisted of two phases: exploration and test. In the exploration phase the subject was instructed to explore and find all 9 objects within a maze. In the test phase, objects were masked and subjects were instructed to locate target objects on a trial-by-trial basis. The test trials lasted approximately 30 seconds each - much longer than a standard cognitive task - providing a rich timeframe to examine the dynamics of connectivity. Task-based fMRI time-series were extracted and binned into time windows bound by the start and end times for each individual trial. Trial connectomes were computed using correlation, partial correlation, covariance, and tangent space connectivity metrics. These connectomes were fed into a linear Support Vector Machine (SVM) classifier and fit to trial accuracy. For predicting accuracy during a trial, the SVM performed better than chance across all functional connectivity metrics. Notably, in line with previous connectome model studies, tangent space functional connectivity outperformed other functional connectivity metrics. We find that CPM is a promising tool for investigating trial-by-trial connectome contributions to task performance. These findings suggest that functional network communication during test can be used as a marker for success.

Disclosures: **R.F. Woodry:** None. **E.R. Chrastil:** None.

Digital Abstract Session

P389. New Approaches Toward Classification

Program #/Poster #: P389.01

Topic: I.07. Data Analysis and Statistics

Title: Fully-automatic Ultrasound-based Neuro-navigation Approach for Functional Ultrasound Imaging the vascular brain GPS

Authors: *M. NOUHOUM[1,2], J. FERRIER[2], B. OSMANSKI[2], S. PEZET[1], M. TANTER[1], T. DEFFIEUX[1];
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Abstract: Functional ultrasound (fUS) is a recent imaging technique capable of mapping cerebralvascular network but also to capture indirectly neuronal activity with very high sensitivity.One remaining hurdle is the expertise required to position the probe and identify brainstructures solely based on 2D vascular images, especially when it comes to oblique non-coronal and non-sagittal slice.Here we develop and validate a fully-automatic and integrated approach combiningacquisition of 3D multi-slice ultrasensitive Doppler imaging of the mouse brain and itsonline automatic registration to the reference 3D anatomical atlas from the Allen brainInstitute for neuro-navigation and for guided injection in deep brain.Using Iconeus One scanner driving a 15MHz probe mounted on a motorized setup,successive 2D transcranial ultrafast Doppler images were acquired in a male C57BL/6mouse anesthetized with 1.5% isoflurane. The resulting 3D vasculature can be alignedoffline on any atlas and then used online as a template for automatic non-rigid datasetsregistration within experiments using an iterative optimization algorithm based onintensity matching of big vessels.Despite a 400 µm spatial resolution in the elevation direction, Doppler images registrationhad an average accuracy (n=20 estimations for each marker) of 156±52 - 129±62 - 109±43- 103±20 µm for four vascular landmarks compared to ground-truth estimates fromdifferent days and acquisitions by an expert neuroanatomist. During the experiments, agood matching with the Allen reference atlas was obtained under 2 minutes. Combinedwith a 4-axis motorized system, the software was used to automatically position the probefor functional imaging to an oblique plane including both the primary visual and barrelfield cortex. Whisker and visual stimulations revealed strong activation of both corticalareas, validating the slice positioning.The proposed functional ultrasound “on the fly” neuro-navigation approach allowsautomatic brain navigation and positioning, ensuring standardized experiments andprotocols for non-experts.

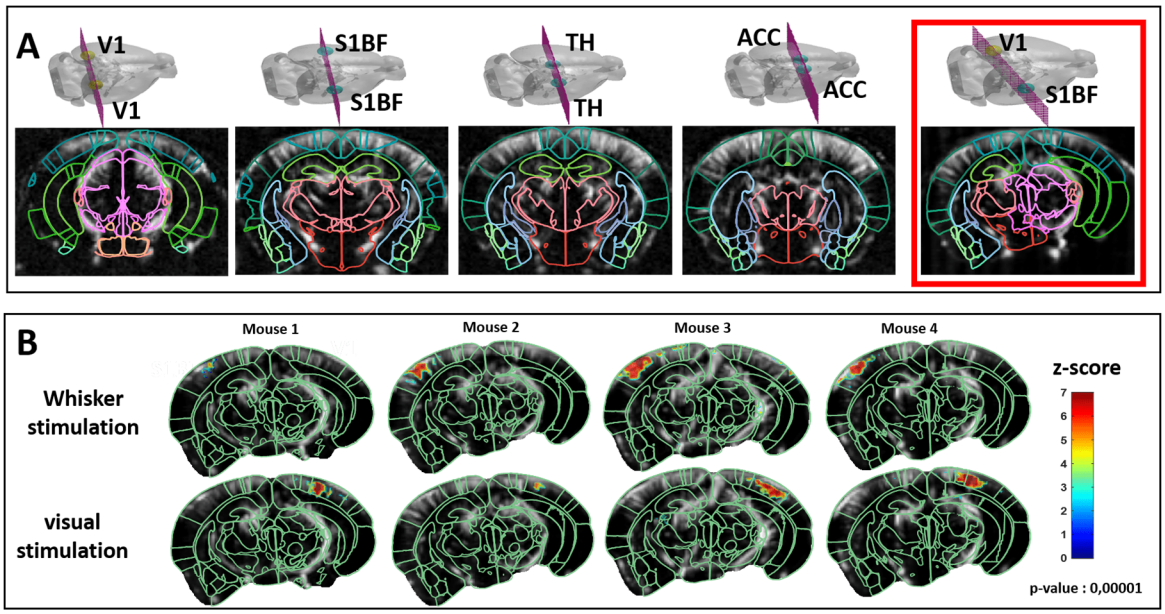

Fig6. **Transcranial functional imaging session. A.** Automatic online positioning guided by Allen brain Atlas. **B.** Functional imaging after automatic position on an oblique plane encompassing both V1 and S1BF. Activation map obtained by computing z-score based on generalized linear model with Bonferroni correction is superimposed on baseline image

Disclosures: M. Nouhoum: None. **T. Deffieux:** None. **M. Tanter:** None. **J. Ferrier:** None. **B. Osmanski:** None. **S. Pezet:** None.

Digital Abstract Session

P389. New Approaches Toward Classification

Program #/Poster #: P389.02

Topic: I.07. Data Analysis and Statistics

Support: HFSP Cross-disciplinary Postdoctoral Fellowship (LT000669/2020-C)
EuroTech postdoctoral fellowship (754462)
Mexican National Council for Science and Technology, CONACYT (709993)

Title: Liftpose3d, a deep learning-based approach for transforming 2D to 3D pose in laboratory animals

Authors: *A. GOSZTOLAI[1], S. GUNEL[1], M. P. ABRATE[1], D. MORALES[1], V. LOBATO RIOS[1], H. RHODIN[2], P. FUA[1], P. RAMDYA[1];
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Abstract: Markerless 3D pose estimation has become an indispensable tool for kinematic studies of laboratory animals. Most current methods compute 3D pose by multi-view triangulation of deep learning-based 2D pose estimates. However, triangulation requires multiple, synchronized cameras per keypoint and elaborate calibration protocols that hinder its widespread adoption in laboratory studies. In this talk, I will describe LiftPose3D, a deep learning-based method that overcomes these barriers by lifting 2D poses from a single camera view into 3D poses. Our method builds on a state-of-the-art deep neural network architecture designed to lift 2D human

poses without requiring temporal information, or a skeletal graph. I will demonstrate how specific data transformations and adaptations to network training enable accurate 2D-to-3D pose lifting with significantly less training data, across a wide range of animals, from different camera angles, and in stereotyped and non-stereotyped behaviors. These transformations also enable our method to work despite self-occlusions in freely behaving animals and weakens the effect of outliers in training data. I will illustrate this versatility by applying it to multiple experimental systems using flies, mice, and macaque monkeys and in circumstances where 3D triangulation is impractical. Additionally, LiftPose3D networks can be adapted to different experimental domains by matching pose statistics across datasets and by coarse-graining the network across image resolutions. In fruit flies, I will demonstrate how this allows one to use pre-trained networks to obtain 3D poses in experiments, where multi-camera triangulation was previously not possible. Thus, LiftPose3D permits high-quality 3D pose estimation in the absence of complex camera arrays, tedious calibration procedures, and despite occluded keypoints in freely behaving animals.

Disclosures: **A. Gosztolai:** None. **S. Gunel:** None. **M.P. Abrate:** None. **D. Morales:** None. **V. Lobato Rios:** None. **H. Rhodin:** None. **P. Fua:** None. **P. Ramdya:** None.

Digital Abstract Session

P389. New Approaches Toward Classification

Program #/Poster #: P389.03

Topic: I.07. Data Analysis and Statistics

Support: NIH R43 GM134789
NIH R01 DA033404
NIH R01 DA040965

Title: Pipsqueak AI: A standardized and automated method of biomarker quantification in digital histology using machine learning.

Authors: ***J. H. HARKNESS**[1,2,4], K. J. LAWTON[1,2], W. O'KEEFFE[1], G. WADE[1], P. N. BUSHANA[6], A. E. GONZALEZ[3,4], A. B. COFFIN[2], B. A. SORG[5,2];
[1]Rewire Neuro, Portland, OR; [2]Washington State Univ., Vancouver, WA; [3]Neurosci., Washington State Univ., Portland, OR; [5]Integrative Physiol. and Neurosci., [4]Legacy Res. Inst., Portland, OR; [6]Biomed. Sci., Washington State Univ. - Spokane, Spokane, WA

Abstract: Manual analysis of biomedical images by researchers and pathologists is time intensive, requires extensive training, and is prone to introduce bias and error. Unintentional bias and attentional limitations during analysis of biomarkers can underlie poor reproducibility of findings in biomedical research and potentially introduce errors to clinical diagnostics. These problems are significant barriers to delivering the most beneficial evidence-based medicine, developing effective medical treatments, and promoting public confidence in scientific inquiry. We developed a software program to improve automation and standardization of image analysis,

called “Pipsqueak,” which significantly increases data reliability between image raters and decreases the time required for analysis by more than 100-fold. However, suboptimal conditions, like high background staining, off-target structures, overlapping or clustered biomarkers, and atypical morphologies, can lead to artifacts and consequently to inaccurate results and erroneous conclusions when using the first generation of Pipsqueak. Here, we used machine learning to tune several convolutional neural networks to accurately detect the staining in a wide variety of representative cell types, including by *Wisteria floribunda* agglutinin, 8-oxo-dG, parvalbumin, cFos, DAPI, red blood cells, and human sperm. We demonstrate significantly increased inter- and intra-rater reliability of tissue sample analysis and decreased analysis time for multiplexed biomarkers. The integration of machine learning capabilities into our Pipsqueak AI technology produces an adaptive, near real-time, biomedical image analysis platform that quickly and accurately identifies biomarker targets. Pipsqueak AI is available now as an integration to ImageJ/FIJI (https://Pipsqueak.ai), and is capable of returning hundreds of accurate cellular target detections to the user within 300ms of image upload. Our end goal is for this software platform to accurately detect a wide range of novel biomarkers, beyond those tested here, by utilizing innovative transfer learning and adaptive modeling techniques.
Support: NIH R43GM 134789, WSU Gap Fund grant, WSU Postdoctoral Alcohol and Drug Abuse Research Program grant, NIH DA 033404, and NIH DA 040965.

Disclosures: **J.H. Harkness:** A. Employment/Salary (full or part-time):; Rewire Neuro, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rewire Neuro, Inc. **K.J. Lawton:** A. Employment/Salary (full or part-time):; Rewire Neuro, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rewire Neuro, Inc. **W. O'Keeffe:** A. Employment/Salary (full or part-time):; Rewire Neuro, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rewire Neuro, Inc. **G. Wade:** A. Employment/Salary (full or part-time):; Rewire Neuro, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rewire Neuro, Inc. **P.N. Bushana:** None. **A.E. Gonzalez:** None. **A.B. Coffin:** F. Consulting Fees (e.g., advisory boards); Rewire Neuro, Inc. **B.A. Sorg:** None.

Digital Abstract Session

P389. New Approaches Toward Classification

Program #/Poster #: P389.04

Topic: I.07. Data Analysis and Statistics

Support: ARCS Foundation Predoctoral Fellowship
NIMH T32MH073526
NIMH K01MH116264
NARSAD Young Investigator Award
Whitehall Foundation Research Grant

Brain Research Foundation Seed Grant

Title: BehaviorDEPOT: A novel tool for automated behavior classification and analysis in rodents

Authors: *C. J. GABRIEL, B. JIN, Z. ZEIDLER, L. A. DENARDO;
Physiol., UCLA, Los Angeles, CA

Abstract: Detailed classification of animal behavior is essential to understand the underlying neural substrates. Behavioral neuroscience relies on analysis of video recordings for behavioral classification, which is traditionally labor-intensive or dependent on expensive software packages with limited capabilities. Recent development of open-source tools has automated the extraction of animal poses from video recordings. However, automatically classifying animal poses into behavioral categories remains challenging, requiring both programming experience and skilled manual annotation for validation. To overcome these barriers, we developed BehaviorDEPOT (DEcoding behavior based on POsitional Tracking), a software package which imports animal pose data (from DeepLabCut or similar), to automatically identify behavioral bouts based on custom classifiers and analyze behavioral statistics in user-defined ways. We provide classifiers for freezing, rearing, jumping, and walking, yet BehaviorDEPOT is customizable for inclusion of additional classifiers. To accommodate a variety of experimental designs, BehaviorDEPOT optionally applies custom spatiotemporal filters which allow users to quantify behaviors occurring in a particular location, during a particular epoch, or during the intersection of the two. BehaviorDEPOT also features modules that support new rater training and measure inter-rater reliability. These features help users generate reliable 'ground truth' human labels, regardless of the behavior type, which is an essential first step for developing behavioral classifiers. The inter-rater reliability module will thus be a useful tool for scientists classifying and quantifying behavior irrespective of application. Finally, to reduce user reliance on expensive computer workstations, we also include pre-trained DeepLabCut networks for rodent pose tracking in standard behavioral arenas. BehaviorDEPOT provides a simple, flexible, automated pipeline to move from raw pose tracking to reliably quantifying task-relevant behaviors.

Disclosures: **C.J. Gabriel:** None. **B. Jin:** None. **Z. Zeidler:** None. **L.A. DeNardo:** None.

Digital Abstract Session

P389. New Approaches Toward Classification

Program #/Poster #: P389.05

Topic: I.07. Data Analysis and Statistics

Support: NIH R01 MH105330
NIH R01 MH10413
NIH R01 MH087332
DA052209 (to MK)

Title: An Accessible Automatic Cell Counting Methodology Using Trainable WEKA Segmentation in ImageJ Validated on Mouse Cortex Microglia

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Abstract: Counting cells is a cornerstone of tracking disease progression in neuroscience. A common approach for this process is having trained researchers individually select and count cells within an image, which is not only difficult to standardize but also very time-consuming. Digital images have become both easier to take and to store, allowing an automatic cell counting methodology to vastly reduce analysis time. We present an automatic cell counting method for microglia and validate it against trained expert's hand counts in mouse cerebral cortex. This method leverages the Trainable WEKA Segmentation (TWS) plugin available in the FIJI distribution of ImageJ (Agranda-Carerras 2017, Schindelin 2012, Schneider 2012). This program utilizes user-selected input and image filters in a machine learning context to automatically identify objects. All processing and analysis was performed in the free ImageJ toolbox to maximize accessibility. TWS's graphical user interface makes it accessible to operate and adjust, but to perform analysis on whole datasets requires further automation through the use of macros which we have constructed within ImageJ maintain this high level of accessibility. To validate our method, we compared our automatic counts to a published, hand counted dataset of Immunofluorescence stained mouse cortex microglia (Singh et al. 2020). For this analysis, we used two genotypes, a wild-type (WT) control and a transgenic mouse model for HIV-induced brain injury (HIVgp120), which expresses the gp120 envelope protein under a GFAP promoter. HIVgp120 mice showed behavioral deficits, and increase in microglia numbers presumably caused the gp120 protein. 10X images were collected in the sagittal plane from brain slices of three 11-14 month-old male mice for each genotype with a mean of 124 microglia per image. For an automatic counting methodology to be effective, it must adapt to changes in cell morphology such as microglial activation (Lynch 2009). The auto counting strategy with TWS replicated the increase in mean microglial number in images from the HIVgp120 mice (Manual p =0.0046; Auto p=0.0002). The auto count also correlates significantly with the manual counts in both genotypes (WT p= 2.297 e^{-07}, adj. R^2= 0.65; HIVgp120 p=2.591 e^{-04} adj. R^2=0.423). The main advantage of this strategy is the much shorter time required to apply the automatic counting strategy compared to a manual count, taking less than a fifth of time. Our study demonstrates the general applicability of this accessible technique to quickly explore large amounts of image data, leaving open the possibility to audit the results by manual counting a smaller, randomly selected subset of images.

Disclosures: T. Kataras: None. **H. Singh:** None. **J. Koury:** None. **M. Kaul:** None.

Digital Abstract Session

P389. New Approaches Toward Classification

Program #/Poster #: P389.06

Topic: I.07. Data Analysis and Statistics

Support: ERC-CoG GEOCOG 724836

Title: DeepMReye: MR-based camera-less eye tracking using deep neural networks

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Abstract: Viewing behavior provides a window into many central aspects of human cognition and health, and is an important variable of interest or confound in many fMRI studies. To make eye tracking freely and widely available for MRI research, we developed DeepMReye: a convolutional neural network that decodes gaze position from the MR-signal of the eyeballs. It performs camera-less eye tracking at sub-imaging temporal resolution in held-out participants with little training data and across a broad range of scanning protocols. Critically, it works even in existing datasets and when the eyes are closed. Decoded eye movements explain network-wide brain activity also in regions not associated with oculomotor function. This work emphasizes the importance of eye tracking for the interpretation of fMRI results and provides an open-source software solution that is widely applicable in research and clinical settings.

Disclosures: M. Nau*: None. **M. Frey*:** None. **C.F. Doeller:** None.

Digital Abstract Session

P389. New Approaches Toward Classification

Program #/Poster #: P389.07

Topic: I.07. Data Analysis and Statistics

Support: F32NS77840

Title: The ΔF/F's are too small! A computational method for removing light contamination in two-photon calcium imaging

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Abstract: Two-photon (2P) imaging of calcium indicators has enabled major advances in systems neuroscience. Many applications of this technique rely on accurate estimates of ΔF/F, the change in fluorescence ascribed to each neuron. One potential source of error in ΔF/F measurements is contamination by background light, which increases the denominator (resting fluorescence) without affecting the numerator (deviations from rest). To test whether current cell finding techniques are susceptible to background light contamination, we applied them to a simulation where ground truth was known (Charles et al. 2019, Biorxiv). Surprisingly, two popular methods greatly underestimated the true ΔF/F (CaImAn, median 0.02 of true ΔF/F; Suite2P, median 0.27). Moreover, the degree of underestimation varied widely across cells

(CaImAn quartile range 0.0082-0.031; Suite2P 0.21-0.32). Underestimation could introduce unexpected errors into spike inference algorithms or analyses that try to explain variance. Inconsistent errors across cells might also obscure activity patterns, such as functional cell classes that could otherwise be identified by the amplitude of their transients.To obtain more accurate measurements of ΔF/F, we developed a technique to remove background contamination using a post-hoc computational analysis. We constructed an estimator of cell brightness at rest (ΔF/F denominator) using a simple model of 2P fluorescence. When applied to simulated data, our method estimated ΔF/F nearly perfectly on average (median 1.10) and with a tighter distribution of ratios (quartile range 0.90-1.12). When applied to real data, where ground truth is not available, our method found much higher values of ΔF/F than popular methods (median ratio 7.9, quartile range 6.9-8.9), consistent with improved background compensation. This method could improve applications of 2P imaging that rely on correctly-estimated ΔF/F and also has the potential to correct published findings.

Disclosures: **G. Zipursky:** None. **B. Lau:** None. **J.L. Gauthier:** None.

Digital Abstract Session

P389. New Approaches Toward Classification

Program #/Poster #: P389.08

Topic: I.07. Data Analysis and Statistics

Support: NIH 1UF1-NS107678
NSF 3332147
1DP2-NS111505
Beckman Young Investigator Program
Sloan Fellowship
Vallee Young Investigator Program

Title: Fast neuron segmentation for two-photon imaging videos using light convolutional neural networks

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Abstract: Fluorescent genetically encoded calcium indicators and two-photon microscopy help understand brain function by generating large-scale *in vivo* recordings in multiple animal models. Automatic, fast, and accurate active neuron segmentation is critical when processing these videos. Existing methods that process an entire video have speed close to the video rate but leave little or no margins for extra processing. In this work, we developed and characterized a novel method to quickly and accurately segment active neurons from two-photon fluorescence imaging videos. We used pre-processing, including spatial filtering, temporal filtering, and whitening to maximize the signal-to-noise of active neurons; we used a series of light convolutional neural networks to quickly identify features of active neurons; we used post-processing to finalize the

spatial footprints of the consistently detected neurons. We also developed an online version, which employed nearly identical schemes as the batch version, but slightly differed in the pre- and post-processing steps. We evaluated our method using cross-validations on multiple public datasets, including Allen Brain Observatory, Neurofinder, and CaImAn dataset. The batch version was more accurate and faster than existing methods in all datasets. In addition, the accuracy of our method was equal to human accuracy, and the speed was an order of magnitude faster than the video rate when executed on a commercial desktop. The difference in accuracy between our method and existing methods was enlarged when these methods processed datasets with few manual marking ground truths. We compared the online version with another existing online method on the same datasets, and our method was also significantly better in both accuracy and speed. This automated, fast, and accurate active neuron segmentation method significantly improves state-of-the-art, and the online version can open a new regime of real-time feedback neuroscience experiments.

Disclosures: **Y. Bao:** None. **S. Soltanian-Zadeh:** None. **Y. Gong:** None. **S. Farsiu:** None.

Digital Abstract Session

P389. New Approaches Toward Classification

Program #/Poster #: P389.09

Topic: I.07. Data Analysis and Statistics

Support: ERC No 818179
ETH Zurich

Title: Sipec deep-learning based behavioral data analysis

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Abstract: Analysing the behavior of individuals or groups of animals in complex environments is an important, yet difficult computer vision task. Here we present a new deep learning architecture for classifying animal behavior and demonstrate how this end-to-end approach can significantly outperform pose estimation-based approaches, requiring no intervention after minimal training. Our behavioral classifier is embedded in a first-of-its-kind pipeline (SIPEC) which performs segmentation, identification, pose-estimation and classification of behavior all automatically. SIPEC successfully recognizes multiple behaviors of freely moving mice as well as socially interacting non- human primates in 3D, using data only from simple mono-vision cameras in home-cage setups.

Disclosures: **M. Marks:** None. **J. Qiuhan:** None. **O. Sturman:** None. **L. von Ziegler:** None. **S. Kollmorgen:** None. **V. Mante:** None. **W. von der Behrens:** None. **J. Bohacek:** None. **M. Yanik:** None.

Digital Abstract Session

P390. New Techniques in Electrophysiology

Program #/Poster #: P390.01

Topic: I.07. Data Analysis and Statistics

Support: NINDS Grant 1U01NS096767-01

Title: Independent evaluation of the Harvard Automatic Processing Pipeline using multi-site EEG data from children with fragile x syndrome

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Abstract: The Harvard Automatic Processing Pipeline for Electroencephalography (HAPPE) in conjunction with The Batch Electroencephalography Automatic Processing Platform (BEAPP) is a computerized EEG data processing pipeline specifically designed for multiple site analysis of populations with neurodevelopmental disorders. This pipeline has been validated in-house by the developers but external testing using real-world datasets strengthens support for its use. Using resting and auditory event related EEG data for 100 children ages 3-6 years with Fragile X Syndrome across 6 sites using 3 different EEG systems, the current data set represents an ideal test of the new processing pipelines. Therefore, we used this rich dataset to evaluate the software's noise reduction techniques, data standardization features, and data integration in comparison to traditional manualized methods of processing. Univariate results from a significant MANCOVA indicated that the HAPPE/BEAPP pipeline resulted in greater trials retained ($F(4,24) = 5.80$, $p = 0.02$), variance retained through ICA ($F(4,24) = 39.74$, $p < 0.01$), and smaller kurtosis ($F(4,24) = 4.29$, $p = 0.049$) than a manual pipeline for task-related data. No significant differences were found in signal-to-noise ratio (SNR) although there was a trend toward greater SNR in the manually processed data ($F(1,24) = 4.16$, $p = 0.052$). We did observe an overall reduction in signal amplitude in the HAPPE/BEAPP pipeline, which is supported by the decrease in kurtosis. To further explore this, we processed simulated data in both pipelines. The simulated data was composed of simulated brain, pink noise, and real artifact. Using a paired samples t-test we determined that the correlation between the pure signal and processed data was significantly higher for the manually processed data ($M = 0.96$, $SD = 0.03$) compared to the HAPPE processed data ($M = 0.29$, $SD = 0.03$); $t(55) = 105.87$, $p < 0.01$. In conclusion, data processed using HAPPE has many benefits including less active processing time and artifact reduction without removing segments, however users should be aware of potential over-cleaning of brain signal in certain circumstances, and some manual checking of the data post-HAPPE is recommended. Importantly, SNR in the real EEG data was not significantly different between manually processed data and HAPPE processed data, so the signal amplitude reduction may not negatively affect results depending on desired outcome measures. Recommended implementation of the HAPPE pipeline for neurodevelopmental populations depends on the goals and priorities of the research.

Disclosures: **E. Auger:** None. **L. Ethridge:** None. **E. Berry-Kravis:** None.

Digital Abstract Session

P390. New Techniques in Electrophysiology

Program #/Poster #: P390.02

Topic: I.07. Data Analysis and Statistics

Support: ERC Grant 759432
ERC Grant 647954

Title: Brain time toolbox: warping electrophysiological data to detect recurrence of active cognitive processes

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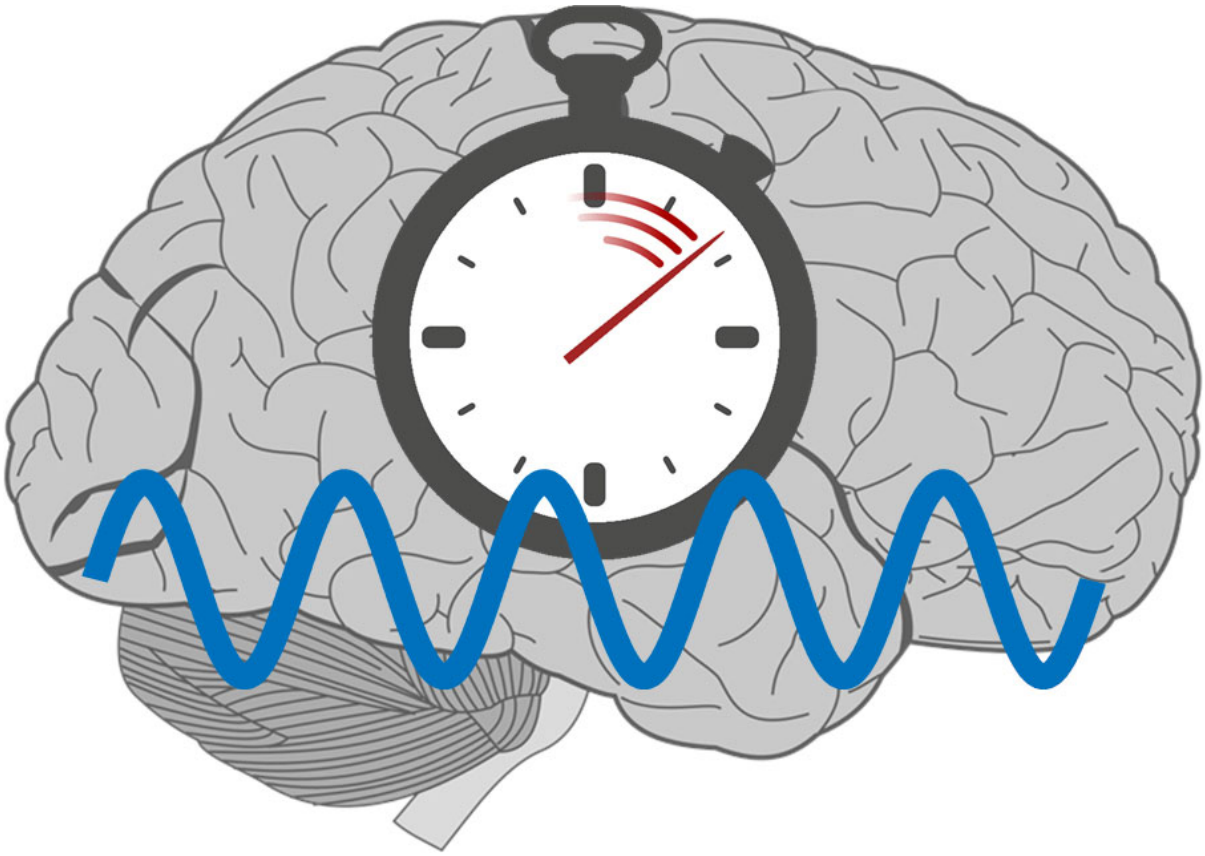
Abstract: Clock time is a human-invented measure that is entirely foreign to the brain. While cognitive processes may correlate with passed seconds, the brain organizes information according to its own internal dynamics: brain time. Neural oscillations clock windows of neural excitability and are thus a prominent brain time candidate. Crucially, oscillations are not stationary, and do not necessarily phase reset at the start of trials. This causes nonlinearities between clock and brain time. As a result, the dynamics of cognitive processes carried by oscillations are distorted in data organized by default clock time. Here, we present a toolbox that transforms electrophysiological data to be more faithful to brain time (inside-out approach), rather than projecting our notion of time onto the brain (outside-in approach). The goal of the toolbox is to uncover and analyse recurring patterns of cognitive processes clocked by oscillations. First, the toolbox performs independent component analysis on the data. Second, the user selects a component with oscillations at a frequency range (e.g. 4-8 Hz; theta) and topography relevant to the studied cognitive process (e.g. memory retrieval). Third, within the selected component, the phase of high-power oscillations is extracted. Fourth, the rest of the data is dynamically time warped based on the dynamics of the extracted phase ("brain time warping"). In effect, this transforms the time axis of the clock time data to be closer to brain time. Finally, the toolbox offers a range of analytical tools to investigate neural activity patterns relative to brain time. Temporal generalization matrices can be created which display recurring patterns of classification performance if cognitive processes operate with a similar neural code across time. The absolute amount and temporal evolution of this recurrence can be quantified and statistically evaluated. Using simulated data, we provide evidence that the toolbox can improve detection of recurrence. In addition, we present preliminary effects of brain time warping on empirical data using an attention paradigm.



Disclosures: **S. van Bree:** None. **M. Melcón:** None. **M. Wimber:** None. **S. Hanslmayr:** None.

Digital Abstract Session

P390. New Techniques in Electrophysiology

Program #/Poster #: P390.03

Topic: I.07. Data Analysis and Statistics

Support: NIH/NIBIB (P41-EB018783)
NIH/NIBIB (R01-EB026439)
NIH/NINDS (U24-NS109103)
NIH/NINDS (U01-NS108916)
NIH/NIMH (P50-MH109429)
Fondazione Neurone

Title: Overcoming heterogeneous hardware to facilitate dissemination and validation of electrophysiological experiments

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Abstract: The acquisition and processing of electrophysiological signals is an essential component of many scientific and clinical experiments. However, realization of these experiments is a daunting endeavor that requires extensive programming experience to integrate the underlying hardware and often leads to experiments that only work with the hardware and software environment present in the original laboratory. Given the heterogeneity of signal acquisition devices across laboratories, this approach impedes the dissemination of electrophysiological experiments and the validation of their results. To overcome this issue, we have developed a software abstraction layer that can accommodate a wide range of heterogeneous hardware. We demonstrate this using BCI2000, a general-purpose software for brain-computer interfacing (BCI). We investigated this concept by developing appropriate interfaces that realize a software abstraction layer for three different bio-signal acquisition devices (Nihon Kohden JE-120A; Neuralynx ATLAS; and Philips GTEN 200). These devices vary in their physical, technical, and conceptual complexity. For example, the acquired signals may be provided by the hardware using synchronous or asynchronous transfer methods. Each interface was implemented in C++ for high computational performance while using the high-level functionalities of the C++ standard libraries (STL). Within BCI2000, the acquired data is stored in a standardized format and made available in real-time for subsequent signal processing and translation algorithms. The experimental paradigm then uses the resulting control signals to provide appropriate user feedback. Abstracting the bio-signal acquisition device allows the signal processing and user applications to be agnostic to the underlying hardware. This abstraction enables the scientific and clinical experiments to be independent of hardware-specific parameters, making them suitable for dissemination and validation across laboratories that use a wide range of bio-signal acquisition devices. The abstraction layers are disseminated through a central online repository, allowing investigators to download the necessary abstraction layer to run experiments on their hardware. We expect that this software abstraction layer, implemented in widely used and well-maintained platforms such as BCI2000, will enable the widespread dissemination and validation of electrophysiological experiments.

Disclosures: **A. Belsten:** None. **M. Adamek:** None. **P. Brunner:** None.

Digital Abstract Session

P390. New Techniques in Electrophysiology

Program #/Poster #: P390.04

Topic: I.07. Data Analysis and Statistics

Support: NIH/NIBIB (P41-EB018783)
NIH/NIBIB (R01-EB026439)
NIH/NINDS (U24-NS109103)
NIH/NINDS (U01-NS108916)
NIH/NIMH (P50-MH109429)
Fondazione Neurone

Title: Vera - a versatile electrode localization framework for invasive electrophysiological research

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Abstract: Since the early 1900s, we've used electrophysiological recordings from electrodes placed on the brain's surface or within the brain to detect seizure foci, map functional areas prior to the resection of pathogenic tissue, or investigate brain function. These applications depend on knowing the precise anatomical locations of the implanted electrodes within the patient's brain. Determining these electrode locations is complicated by the inevitable variations in the imaging used across patients and institutions. While we can adjust the localization for each patient using appropriate methods, the imaging itself depends heavily on the institution. This is compounded by the inevitable variance in the parameters of the clinical imaging (e.g., postoperative MRI, interoperative photographs), the specifications of the implanted electrodes (material, diameter, thickness, arrangement), and the type of surgical procedure (e.g., craniotomy, minimally invasive procedure).
No software tool can currently generalize across the wide range of surgical techniques, imaging parameters, and implanted electrode types, thwarting efforts towards fully automated electrode localization, and reliable electrode projection for cohort analysis. Current software tools either only address a subset of the necessary steps for electrode localization or only work within a very narrow set of parameters. In this study, we attempt to overcome this limitation by developing a universal tool that can be customized to accommodate the variations across and within institutions while maintaining the ability to analyze data from these different institutions.For this purpose, we developed the software framework VERA, which allows customized electrode localization for a particular subject while maintaining a standardized data framework. Therefore, investigators can utilize the same tool independent of the underlying surgical techniques, imaging parameters, and implanted electrode configurations, streamlining the reconstruction process. VERA's central idea revolves around a pipeline definition file specifying individual components, each reflecting a step in the processing pipeline. With this approach, investigators can individualize VERA for their needs while ensuring compatibility across institutions. This inherent modularity encourages the development, validation, and distribution of new techniques (i.e., components) for electrode localization and promotes data sharing between institutions. With this, VERA is an invaluable tool for algorithm distribution, reproducibility, and collaborative research across multiple sites.

Disclosures: M. Adamek: None. **P. Brunner:** None.

Digital Abstract Session

P390. New Techniques in Electrophysiology

Program #/Poster #: P390.05

Topic: I.07. Data Analysis and Statistics

Support: Bridges to the Baccalaureate Program at NMSU NIHNIGMS-5R25GM048998-20
National Institute of General Medical Sciences of the National Institutes of Health
Grant #P20GM103451

Title: More efficient electrode localization in EEG: A method comparison between a traditional electromagnetic digitizer and a new photogrammetric method

Authors: *K. M. JENSEN[1], C. LEE[2], S. MAKEIG[2], J. K. KROGER[1];
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Abstract: While factors contributing to low spatial resolution in electroencephalography (EEG) are well-known, many researchers have derived valuable insight from attempts to associate areas of the cortex with recorded EEG signals. Inverse models of source localization rely on forward head models, the most accurate of which involve the co-registration of the actual electrode locations. When an MRI image of the head while wearing the electrodes is unavailable, electrode localization (or 'digitization') using electromagnetic position capturing wands has long been considered a gold standard. However, in recent years, many new methods for electrode digitization have appeared that may be faster, cheaper, and as or more accurate alternatives (Shirazi & Huang, 2019). Here we sought to compare the quality of electrode digitization obtained from a traditional electromagnetic position tracker with locations obtained using a new, relatively low cost photogrammetric method. Electrode digitization was obtained using a Polhemus PATRIOT from 23 participants fitted with a 128-channel BioSemi headcap prior to their participation in an EEG study. A 3D Structure Sensor (structure.io) attached to an Apple iPad was then used to capture a 3D head surface model of each participant. Later, two independent raters used the EEGLAB plug-in *get_chanlocs* to obtain electrode locations from the 3D head surface models (Lee & Makeig, 2019). Euclidean differences between the channel locations obtained from the Polhemus and the mean of the *get_chanlocs* raters' markings were calculated, returning a median difference of 2.85 mm (IQR = 2 - 3.88 mm) between methods. Median inter-rater differences between raters using *get_chanlocs* to mark electrode locations were 0.79 mm (IQR = 0.54 - 1.12 mm). While these results do not allow us to determine which method was the more accurate, they do reveal a strong similarity in the obtained electrode locations. The photogrammetric method that uses *get_chanlocs* should be considered for use as a more cost effective method for digitizing electrode locations, with accuracy comparable to that of the commonly used Polhemus PATRIOT. Further, while both methods require similar time investments from researchers, the photogrammetric method only requires the participant to sit patiently for a brief (< 5 min) scanning period. As a result, the photogrammetry method offers a ten to fifteen minute reduction in setup time for EEG studies, reducing the burden of participant fatigue, a common cause of unwanted artifacts, noise, and participant errors.

Disclosures: **K.M. Jensen:** None. **C. Lee:** None. **S. Makeig:** None. **J.K. Kroger:** None.

Digital Abstract Session

P390. New Techniques in Electrophysiology

Program #/Poster #: P390.06

Topic: I.07. Data Analysis and Statistics

Support: NIH/NIBIB (P41-EB018783)
NIH/NIBIB (R01-EB026439)
NIH/NINDS (U24-NS109103)
NIH/NINDS (U01-NS108916)
NIH/NIMH (P50-MH109429)
Fondazione Neurone

Title: Evaluating the Closed-Loop Performance of Clinical Electrophysiology Recording Systems using BCI2000

Authors: M. ADAMEK[1,2], A. BELSTEN[2], ***N. LUCZAK**[2], J. MELLINGER[2], K. REFAEY[3], P. BRUNNER[2,4];
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Abstract: Electrophysiological recordings from patient populations are invaluable for understanding disease progression and identifying possible biomarkers. Access to specific patient populations can be scarce and often necessitates collaboration across institutions. Fortunately, most clinical institutions already possess electrophysiological recording systems for clinical purposes that are suitable for research. However, there is currently no turnkey system that allows investigators to leverage the existing infrastructure without dedicating substantial time and resources. Our work addresses this issue by developing a BCI2000-based turnkey system that interfaces directly with a wide range of clinical neurophysiology systems (e.g., Natus, Nihon Kohden, Neuralynx, and Blackrock). This system allows investigators to create new paradigms and efficiently distribute them to multiple clinical sites. As part of this effort, we also verify system latencies critical to the underlying research. Specifically, we investigate the variability in closed-loop performance in various real-world scenarios. In this verification study, we tested our turnkey system combined with a wide range of clinical systems running a simple audio-visual paradigm implemented entirely in BCI2000. Next, we performed the same experiment implemented using Python code, leveraging BCPy2000, and finally, using BCI2000s ability to interface with PyschoPy. In each case, we sampled the signal of an external signal generator with the clinical system. We used this external clock to trigger the paradigm's audio and visual cues while recording them with the clinical setup. This approach allowed us to measure the full system latency independent of the clinical hardware capabilities. Furthermore, we compared the direct access to the clinical data stream in BCI2000 with indirect access through the commonly used Lab Streaming Layer. We compared the resulting system latencies from these clinical systems to those of dedicated research-grade devices. Our results highlight

the differences in performance between these systems. Evaluation of recording techniques is essential to ensure rigor and reproducibility in research. Therefore, validating and benchmarking clinical systems for research purposes provides investigators with the necessary information to ensure data quality.

Disclosures: **M. Adamek:** None. **A. Belsten:** None. **N. Luczak:** None. **J. Mellinger:** None. **K. Refaey:** None. **P. Brunner:** None.

Digital Abstract Session

P390. New Techniques in Electrophysiology

Program #/Poster #: P390.07

Topic: I.07. Data Analysis and Statistics

Support: NIH MH123192

Title: Deep Learning neural network for the detection of oscillatory bursts in intracranial EEG

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Abstract: Objective
Mechanisms of epilepsy are poorly understood and despite any available treatment approximately 30% of patients are refractory. Over the last two decades the evidence has been growing that in addition to epileptic spikes and discharges, transient oscillatory bursts (OB) of various frequencies are important indicators of seizure development and high frequency oscillations (HFOs, ripples and fast ripples) are good biomarkers of epileptogenic tissue. Those events represent a challenge to detect them due to their transient nature and, for HFOs, very small amplitude. Deep Learning (DL) neural networks provide additional tools for automated analysis of EEG. Here we present a Long Short-Term Memory (LSTM) neural network trained to detect OBs as well as spikes/sharp waves, ripple-on-oscillation (RonO) and ripple-on-spike (RonS).
Methods
We used intracranial EEG (iEEG; sampling frequency 500 Hz) from the open access database (ieeg.org; 7 patients). Spectral features (relative power within traditional frequency bands) were analyzed continuously across several one-hour iEEG records and the initial screening for event candidates was done with a novel thresholding algorithm. 1000 events of each class (theta, alpha, beta, gamma, ripples, spike/sharp wave and baseline) were visually verified and selected. A bidirectional LSTM layer network had hidden units varied from 50 to 200. Training was performed using random selections of 50-500 events (per class) while other 500 events (per class) were used for testing.
Results
The network was able to detect events of each class (not used in training) in each patient. The

average values for sensitivity/specificity were above 85-90% for all event classes. We also correlated the detected events with seizure onset zone (SOZ). Spikes (corr = 0.42, $p < 0.05$) and RonS (corr = 0.27, $p < 0.05$) showed significant correlation with SOZ in all patients while ripples correlated with SOZ in 4 out of 7 patients. The lower correlation of ripples with SOZ can be explained by the fact that clinical determination of SOZ is not based on ripples which are impossible to detect without spectral analysis.

Conclusions

The Deep Learning networks can be used for automated detection of oscillatory and epileptiform events within iEEG. Most importantly, the trained LSTM network shows generalizability detecting events with sensitivity and specificity higher than 85-90% in all patients tested. The DL networks can significantly accelerate the analysis of iEEG data and increase their diagnostic value which may improve surgical outcome in patients with localization-related refractory epilepsy.

Disclosures: A.V. Medvedev: None. **G.I. Agoureeva:** None.

Digital Abstract Session

P391. Electrical Stimulation: Approaches and Effects On Neuronal Function and Circuit Organization

Program #/Poster #: P391.01

Topic: I.08. Methods to Modulate Neural Activity

Support: Defense Advanced Research Projects Agency (DARPA) [BAA-16-24, W.N. & K.J.H]
Oak Ridge Institute for Science and Education (C.R.).

Title: Transcutaneous vagal nerve stimulation both activates and deactivates clusters of neurons in key brainstem nuclei: A coordinate-based meta-analysis of resting state fMRI studies

Authors: *C. RIDGEWELL, W. NEUMEIER, K. J. HEATON;
USARIEM, Natick, MA

Abstract: Transcutaneous vagal nerve stimulation (tVNS) is a non-invasive method of vagal neuromodulation. Application of tVNS has demonstrated cognitive performance enhancement in healthy individuals and clinical efficacy for conditions such as epilepsy and depression. Neuroimaging techniques, such as resting state functional magnetic resonance imaging (rs-fMRI), can provide insight into the effects of tVNS on underlying neural processes but research using these techniques with tVNS is, to date, limited. Therefore, the aim of this analysis was to conduct a meta-analytic review of available rs-fMRI studies to synthesize the current body of knowledge and identify gaps in this knowledge regarding the neurological mechanisms of tVNS. Using the search query "("transcutaneous" OR "noninvasive" OR "non-invasive") AND ("vagus" OR "vagal") AND ("stimulation" OR "neuromodulation") AND ("MRI" OR "fMRI" OR "imaging")," a review of existing literature following the Cochrane Collaboration's PRISMA

guidelines (Liberati et al., 2009) identified 20 articles. Of these, 7 articles reporting data from either 1.5 or 3 Tesla rs-fMRI, including a total of 194 unique foci across 146 participants, were included in the final sample after excluding those not reporting cluster coordinates. Stimulation sites were heterogeneous ($n_{cervical} = 1$, $n_{tragus} = 3$, $n_{cymba\ concha} = 3$). A coordinate-based meta-analysis using activation-likelihood estimation (ALE) was completed to identify relevant patterns of blood-oxygen-level-dependent (BOLD) activation during tVNS and rs-fMRI compared to sham stimulation. This analysis used a full-width half maximum Gaussian blur for each coordinate focus and a minimum cluster size of $15mm^3$. A total of 1000 thresholding permutations were calculated using an uncorrected p-threshold of $p < 0.001$ and a family-wise error threshold of $p < 0.05$. Compared to sham, active tVNS was associated with both greater and decreased activation in the locus coeruleus (LC) and dorsal raphe nucleus (DRN) and also decreased activation in the nucleus of the tractus solitarius (NTS). However, changes from pre- to post-stimulation were not associated with significant clusters of functional activity for either tVNS or sham conditions, which may be due to low statistical power. Overall, these findings suggest support of previous theories regarding the effects of tVNS on underlying neural function, particularly with respect to observations of increased norepinephrine circulation, as reflected by increased LC activity following tVNS relative to sham.

Disclosures: C. Ridgewell: None. **W. Neumeier:** None. **K.J. Heaton:** None.

Digital Abstract Session

P391. Electrical Stimulation: Approaches and Effects On Neuronal Function and Circuit Organization

Program #/Poster #: P391.02

Topic: I.08. Methods to Modulate Neural Activity

Support: TNT Grant N66001-17-2-4010

Title: Auricular Vagus Neuromodulation - A Systematic Review on Quality of Evidence and Clinical Effects

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Abstract: Background: The auricular branch of the vagus nerve runs superficial to the surface of the skin, making it a favorable target for non-invasive neuromodulation techniques. Given that auricular vagus nerve stimulation (aVNS) can be implemented non-invasively, there have been many early stage clinical trials on a diverse range of conditions and physiological outcomes, often with conflicting results.
Methods: We conducted a systematic review of 41 aVNS randomized controlled trials (RCTs) using the common Cochrane Risk of Bias tool as a framework. The Risk of Bias tool is intended

to identify deviations from an ideal RCT that may cause the effect of an intervention to be overestimated or underestimated. As is common for early stage studies, the majority of aVNS studies were assessed as having 'some' or 'high' risk of inadvertent bias, which makes interpreting their results in a broader context problematic.
Results: The reported outcomes were qualitatively synthesized across studies. There is evidence of a modest decrease in HR during higher levels of stimulation. Findings on heart rate variability conflicted between studies and were hindered by trial designs including inappropriate washout periods and multiple methods used to quantify HRV. There is early stage evidence to suggest aVNS may reduce circulating levels or endotoxin induced levels of inflammatory markers. Studies on epilepsy reached primary endpoint results similar to previous RCTs on implantable VNS, albeit with concerns over quality of blinding. aVNS showed preliminary evidence of ameliorating pathological pain but not induced pain.
Discussion: We show the need for direct measures of target engagement in aVNS. Firstly, for the determination of stimulation parameters and electrode design and placement. Secondly, direct measures of target engagement, along with consistent evaluation of the double blind, should be used to improve the design of controls in the long term - a major source of concern identified in the Cochrane analysis. Lastly, we list common improvements in reporting of results that can be addressed in the short term.
Conclusion: Direct measures of target engagement and evaluation of the double blind for the development of appropriate controls should be strived for in aVNS and is applicable to other paresthesia-inducing neuromodulation therapies, such as spinal cord stimulation.

Disclosures: J.D. Mudge: None. **M.S.E. Kasole:** None. **N. Verma:** None. **R.C.H. Chen:** None. **S.L. Blanz:** None. **J.K. Trevathan:** None. **E. Lovett:** A. Employment/Salary (full or part-time):; LivaNova. **K.A. Ludwig:** None.

Digital Abstract Session

P391. Electrical Stimulation: Approaches and Effects On Neuronal Function and Circuit Organization

Program #/Poster #: P391.03

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH SPARC Initiative (OT2 OD025340)
Natural Sciences and Engineering Research Council of Canada (PGS D3-437918-2013)
Duke University (University Scholars Program, Myra & William Waldo Boone Fellowship, and Pratt School of Engineering Faculty Discretionary Fund)

Title: Non-monotonic kilohertz frequency (KHF) block thresholds arise from charge imbalance not waveform asymmetry

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Abstract: Reversible block of nerve conduction using KHF electrical signals has substantial potential for treatment of disease. However, the ability to block nerve fibers selectively is limited by poor understanding of the relationship between waveform parameters and the nerve fibers that are blocked. Previous studies reported non-monotonic relationships between block signal frequency and block threshold that differed across fiber types, suggesting the potential for fiber-selective block. However, the origin of non-monotonic effects was unclear, and these findings were not replicated in a subsequent in vivo study. We hypothesized that non-monotonic threshold-frequency relationships emerge from frequency- and amplitude-dependent charge imbalances in KHF waveforms. We used high-fidelity computational models of human vagus and rat tibial nerves, as well as rat in vivo experiments, to quantify threshold-frequency relationships of rectangular waveforms with a range of asymmetries and charge imbalances. Across both models and in vivo experiments, non-monotonic threshold-frequency relationships only occurred with charge-imbalanced KHF block, and the charge imbalance was negatively correlated with changes in block threshold. In contrast, blocking with charge-balanced waveforms consistently produced monotonic threshold-frequency relationships, and the degree of asymmetry was positively correlated with changes in block threshold. Computational models of direct current equivalent nerve block revealed that non-monotonic block thresholds represented a transition from KHF block to direct current block. The models further identified complex interactions between KHF block mechanisms and direct current block mechanisms that could be understood through independent analysis of each block regime. These results suggest a mechanism for non-monotonic threshold-frequency relationships that reconciles previous contradictory studies. In addition, the analyses clarify the mechanisms of interaction between KHF and direct current block, and they demonstrate the potential for selective block of specific fiber types.

Disclosures: E. Peña: None. **N.A. Pelot:** None. **W.M. Grill:** None.

Digital Abstract Session

P391. Electrical Stimulation: Approaches and Effects On Neuronal Function and Circuit Organization

Program #/Poster #: P391.04

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH grants MH086400, DA026297, and EY017658
Defense Advanced Research Projects Agency (DARPA) under Cooperative Agreement Number W911NF-14-2-0045
NIH grants MH109722, NS100548, and MH111872
Tiny Blue Dot Foundation
NIH grant K24-NS088568

Title: Orientation relative to grey and white matter and brain region predictably shapes the response to intracranial direct electrical stimulation in the human cortex

Authors: *A. C. PAULK[1], R. ZELMANN[2], B. CROCKER[4], N. PELED[3], D. D. DOUGHERTY[1], A. S. WIDGE[5], E. N. ESKANDAR[6], D. WEISHOLTZ[7], J. LEE[7], R. M. RICHARDSON[1], G. R. COSGROVE[7], Z. WILLIAMS[8], S. S. CASH[1];
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Abstract: While electrical neuromodulation is employed to treat psychiatric and neurological disorders worldwide and is used to derive functional network connectivity in the human brain, the mechanisms of electrical stimulation remain poorly understood, manifesting a highly variable range of behavioral and neurophysiological effects. Few systematic studies address the physiological effects of human brain stimulation, in large part made complicated by the massive parameter space and numerous hypotheses as to why some stimulation parameters work and some do not. The considerable stimulation efficacy variability is likely a result of a complex interaction of multiple factors, including stimulation location, injected current density, the generated stimulation field, and stimulation frequency. We examined the relationship between single pulse and trains of evoked cortico-cortico evoked potentials (CCEPs) with current amplitude, location in the brain, electrode spacing with bipolar stimulation, and stimulation location with reference to the grey-white junction across a data set of patients (N=62) implanted with electrodes for intracranial monitoring for their clinical care. We measured peak and valley amplitudes, area under the curve, and energy of the responses while accounting for location, electrode spacing, current injected, and brain location. We found separable, nonlinear responses varying with stimulation location with respect to the cortical column / white matter and especially brain region. The peak CCEP amplitude and the distance between stimulating and recording site were negatively correlated for pulses delivered in the prefrontal cortex, dorsal anterior cingulate, and temporal lobe ($p<0.014$), but not for the rostral anterior cingulate ($p>0.297$), showing a regional specificity to stimulation spread (Wilcoxon signed rank test; N=49). In addition, stimulation sites with above-threshold (5 standard deviations above the baseline) responses across the brain were significantly closer to the grey-white matter boundary compared to sites with below-threshold responses with stimulation in the lateral prefrontal cortex ($p<0.001$) but, interestingly, not the cingulate, temporal lobe, or orbitofrontal cortex ($p>0.001$, Wilcoxon rank sum test; N=49). These results demonstrate that there are consistent location and stimulation frequency effects, and differences, on neural responses even in areas which are considered ‘similar’ in density, connectivity, neural morphology, and cortical architecture (e.g. rACC and dACC). These findings have significant implications for how stimulation is applied as a therapeutic tool.

Disclosures: A.C. Paulk: None. **R. Zelmann:** None. **B. Crocker:** None. **N. Peled:** None. **D.D. Dougherty:** None. **A.S. Widge:** None. **E.N. Eskandar:** None. **D. Weisholtz:** None. **J. Lee:** None. **R.M. Richardson:** None. **G.R. Cosgrove:** None. **Z. Williams:** None. **S.S. Cash:** None.

Digital Abstract Session

P391. Electrical Stimulation: Approaches and Effects On Neuronal Function and Circuit Organization

Program #/Poster #: P391.05

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH grant R01NS088674
NIH grant R01NS117405
NSF Graduate Research Fellowship DGF 1106401

Title: Multi-scale simulation of neuronal polarization by conventional and high-definition transcranial direct current stimulation (tDCS)

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Abstract: tDCS shows promise as an inexpensive, painless, and noninvasive technique for modulating brain activity by applying weak currents via scalp electrodes and has numerous potential clinical and research applications. However, it is unclear how specific electric field (E-field) distributions polarize different subcellular elements of the diverse range of cell types in cortex.
We used a multi-scale model of layer-specific, morphologically-realistic cortical neuron models (NEURON), embedded in a finite element method head model of the E-field (SimNIBS), to simulate steady-state polarization generated by conventional M1-SO tDCS (7x5 cm) and 4x1 "high-definition" HD tDCS. We quantified polarization in the somatic, axonal, and dendritic compartments of layer 1 neurogliaform cells, L2/3 pyramidal cells (PCs), L4 large basket cells (LBCs), L5 PCs, and L6 PCs in the M1 hand knob. We also used a uniform E-field approximation to estimate the tDCS-generated polarizations.
With matched peak E-field magnitude (0.5 V/m), 1.8 mA conventional tDCS (C) produced slightly stronger, more diffuse polarization, peaking in the central sulcus, while 2.0 mA HD tDCS produced peak polarization in the gyral crown, reflecting their respective E-field distributions. Both montages generated regions of depolarization and hyperpolarization beneath the M1 electrode and within each neuron. All neurons experienced higher peak axonal and dendritic polarizations than somatic polarization, with peak somatic polarization between cells in the L5 and L6 PCs (C: -0.057 - +0.082 mV, HD: -0.052 - +0.077 mV), peak axonal polarization in the tangentially oriented L2/3 PC axon terminals (C: -0.68 - +0.92 mV, HD: -0.78 - +0.82 mV), and peak dendritic polarization in the basal dendritic terminals of the LBCs (C: -0.17 - 0.21 mV, HD: -0.19 - 0.19 mV). These trends were consistent with the uniform E-field simulations. We used the polarizations from the uniform 1 V/m E-field to estimate the tDCS generated polarizations by matching the E-field direction to the somatic E-field vector for each neuron

position and scaling linearly by the amplitude, which yielded <6.83% median percent error and <57.8% mean percent error. Therefore, the uniform E-field approximation can capture broadly the polarization distribution, but may produce large errors in some regions.
These results suggest polarization of pre- and postsynaptic compartments of both excitatory and inhibitory cortical neurons may play a larger role in tDCS neuromodulation than somatic polarization, and these effects cannot be predicted by considering the E-field distribution alone.

Disclosures: **A.S. Aberra:** None. **A. Lopez:** None. **W.M. Grill:** None. **A.V. Peterchev:** None.

Digital Abstract Session

P391. Electrical Stimulation: Approaches and Effects On Neuronal Function and Circuit Organization

Program #/Poster #: P391.06

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 5R00NS097620
Center for Neural Science and Medicine, Cedars-Sinai

Title: Epidural cerebellar stimulation drives widespread neural synchrony in the intact and injured motor cortex

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Abstract: Introduction: Cerebellar electrical stimulation has shown promise in improving motor recovery post-stroke in both rodent and human studies. Past studies have used motor evoked potentials (MEPs) to evaluate how cerebellar stimulation modulates ongoing activity in the cortex, but our understanding of its effects remains limited. Here we used invasive electrophysiological recordings from the rodent primary motor cortex (M1) to assess how cerebellar stimulation modulates neural activity at the level of single neurons as well as at the level of mesoscale dynamics, both in intact and stroke-injured M1. **Methods:** We recorded single unit spiking and local field potentials (LFPs) in M1 or peri-infarct M1, contralateral to the stimulated cerebellum in adult Long Evans rats (10 intact and 4 stroke) under ketamine-xylazine anesthesia. In the injured group, stroke was induced via a photothrombotic model and the neural activity was recorded immediately anterior to the stroke site. **Results:** Our results show that post-stimulation, the firing rates of a majority of M1 neurons changed significantly with respect to their baseline rates. We observed this in both healthy and stroke-injured rats. These firing rate changes were diverse in character, as the firing rate of some neurons increased while others decreased. Furthermore, cross-correlation analysis showed a significant increase in coincident firing amongst neuronal pairs. Interestingly, this increase in synchrony was unrelated to the direction of firing rate change. We also found that neuronal ensembles derived through principal

component analysis were more active post-stimulation. Lastly, we found that cerebellar stimulation increased low-frequency power (< 4Hz) in M1 LFPs during the stimulation, but this increase did not persist post-stimulation. **Conclusion:** Thus, we conclude that cerebellar stimulation caused significant, long-lasting changes in the activity patterns of cortical neurons (in the intact or injured M1) by altering firing rates, boosting neural synchrony and increasing neuronal assemblies' activation strength. Our study provides evidence that cerebellar stimulation can directly modulate cortical dynamics in intact and stroke-injured rats.

Disclosures: **N. Danielsen:** None. **A. Abbasi:** None. **J. Leung:** None. **S. Patel:** None. **T. Gulati:** None.

Digital Abstract Session

P391. Electrical Stimulation: Approaches and Effects On Neuronal Function and Circuit Organization

Program #/Poster #: P391.07

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS109498

Title: Investigating early neural responses during transcranial magnetic stimulation in non-human primates

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Abstract: Transcranial Magnetic Stimulation (TMS) is a non-invasive neuromodulation technique commonly utilized in brain research and clinical applications such as major depression and ADHD. Despite its widespread use, the underlying neuronal mechanisms of TMS and how it modulates brain activity is obscure. It has been debated that some of the TMS evoked responses could be a result of peripheral effects rather than direct neural activation. To this end, we recorded brain activity from an anesthetized monkey with implanted electrodes projecting from visual area (V2) to frontal eye field (FEF), secondary auditory cortical (AUD) and temporal (TE) regions in the left hemisphere. The exact locations of electrode contacts were determined by co-registering post- and pre-surgery MR images. Biphasic single-pulse TMS was delivered over the left prefrontal cortex. A sham protocol was executed by tilting the coil away from the head to mimic the auditory response. Stimulation intensities were set to 70% and 90% of the maximum stimulator output. Concurrent electrical activity from cranial muscle depolarization was measured using needle electrodes. Data from muscle activity was processed by removing the TMS artifact and performing time locked averaging to verify the duration of muscle activity

immediately following TMS pulse. We preprocessed the neural data by removing channels contaminated with large noise through visual inspection, removing the TMS and the muscle artifacts (calculated from EMG data), interpolating, down-sampling to 1 kHz and bandpass filtering at 0.1-50 Hz. For analysis, we performed time-locked averaging across trials and time-frequency representation relative to the baseline prior to stimulus presentation. Preliminary time-frequency results indicate that under TMS condition, low frequency (4-7 Hz) activity is suppressed in frontal contacts of FEF electrode (located in caudate nucleus) in contrast to sham condition. This effect is not observed in frontal contacts in AUD electrode (located in auditory belt) or TE electrode. Furthermore, contacts of AUD electrode in auditory belt reveal consistent low frequency oscillations in both TMS and sham conditions, which is attributed to the auditory response to the 'click' sound. This provides preliminary evidence that the modulatory effects of TMS are localized and result mainly from direct neural activation with little or no peripheral stimulation. Future research directions include quantification of direct and indirect TMS effects and isolating early neural activity by blocking cranial muscle activity. This will enable us to distinguish between direct neural response and secondary stimulation effects.

Disclosures: N.D. Perera: None. **S. Shirinpour:** None. **I. Alekseichuk:** None. **G. Linn:** None. **C.E. Schroeder:** None. **A.Y. Falchier:** None. **A. Opitz:** None.

Digital Abstract Session

P391. Electrical Stimulation: Approaches and Effects On Neuronal Function and Circuit Organization

Program #/Poster #: P391.08

Topic: I.08. Methods to Modulate Neural Activity

Support: Weill Neurohub
NSF Grant EEC-1028725
University of Washington Royalty Research Fund
NSF Graduate Research Fellowship Program (GRFP)
Washington Research Foundation
NIH P51 OD010425

Title: Studying the mechanisms of plasticity following ischemic stroke and cortical stimulation in non-human primates

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Abstract: Brain plasticity has been widely observed during behavioral tasks and functional recovery after damage. As a result, brain stimulation protocols can be designed to take advantage of this natural plasticity and serve as a potential therapy for various neural disorders. In particular, ischemic stroke is a major cause of brain injury that results in lifelong disability. Although numerous work in rodents have demonstrated stimulation-induced plasticity following

stroke, few of these results have been replicated in humans due to the huge anatomical differences between rodent and human brains, and a limited understanding of the underlying plasticity mechanisms. Therefore, we combined electrophysiology and immunohistochemistry to study the mechanisms of neuroplasticity following cortical ischemic stroke and electrical stimulation in non-human primates (NHPs). To produce controlled focal lesions, we used the photothrombotic method on the cortical surface to cause targeted vasculature damage. In two adult rhesus macaques, we generated ischemic lesions in the sensorimotor cortex while collecting electrocorticography (ECoG) recordings. In a third animal, we followed the same lesioning procedures and applied repeated electrical stimulation via an ECoG electrode 8 mm away from the lesioned site. During 60 minutes of stimulation, we simultaneously recorded through the same ECoG array to monitor the network dynamics. We assessed the neural activity changes within the sensorimotor cortex of each macaque as quantified by the signal power. In addition, we performed histology analysis that consists of Nissl staining with thionin and immunostaining with antibodies against two common plasticity markers, postsynaptic density-95 (PSD-95) and growth-associated protein-43 (GAP-43). Our ECoG signals showed decreased signal power at each lesion site for the first two monkeys and reduced power at all sites post-stimulation for the third monkey. We also observed an elevation in both PSD-95 and GAP-43 markers around the lesion boundary, suggesting an increase in synaptic plasticity within the ischemic penumbra. We will compare these to the same markers found in the post-stimulation penumbra to study the level of stimulation-induced plasticity and the large-scale downregulation of activity we saw from electrophysiology after stimulation. These results will shed light on the mechanisms governing neuroplasticity following injury and subsequent stimulation in NHPs. With the similarity between NHP and human brains, it paves the path for developing effective stimulation-based therapy for stroke rehabilitation in clinical studies.

Disclosures: J. Zhou: None. **K. Khateeb:** None. **Z. Ip:** None. **A. Yazdan-Shahmorad:** None.

Digital Abstract Session

P391. Electrical Stimulation: Approaches and Effects On Neuronal Function and Circuit Organization

Program #/Poster #: P391.09

Topic: I.08. Methods to Modulate Neural Activity

Title: Controlling epileptic seizures using forced temporal spike-time stimulation

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Abstract: Epilepsy is typically characterized by highly synchronized episodes of neural activity. Currently, the existing stimulation therapies purely focus on suppressing the pathologically synchronized neuronal firing patterns during the ictal (seizure) period. While these strategies are

effective in suppressing the seizure when they occur, they fail to prevent the re-emergence of seizures once the stimulation is turned off. Experimental data from human patients and animal models show significant morphological and synaptic level changes in many brain regions after the episodes of epileptic seizures, which may be a possible reason for this re-emergence. Previously, we developed a novel neurostimulation motif, which we refer to as "Forced Temporal Spike-Time Stimulation" (FTSTS) based on a basic combination of out-of-phase biphasic pulsed stimulation, one for the excitatory neuronal population and one for the inhibitory neuronal population, that has shown remarkable promise in long-lasting desynchronization of excessively synchronized neuronal firing patterns by harnessing synaptic plasticity[1]. This FTSTS protocol can reliably and robustly desynchronize the excessively synchronized and rhythmic network response to a desired low level. The FTSTS protocol is also capable of the reverse, i.e., desynchronization to synchronization of the neural population can happen consistently using the exact reverse of these signals. We showed the capability of this selective FTSTS protocol in desynchronizing and synchronizing neuronal activity in a generic excitatory-inhibitory (E-I) network model consisting of up to 10,000 neurons. Building upon this prior work, we have recently investigated the FTSTS capability in desynchronizing the pathologically synchronized neuronal firing patterns during epileptic seizures using a recently published computational model of neocortical-onset seizures[2], validated with human epilepsy data. Our simulation results show that the FTSTS protocol can effectively desynchronize the pathologically synchronized neuronal firing patterns during the ictal period. Moreover, the FTSTS protocol can potentially reduce the chances of future episodes of seizure-like activity by decreasing the average long-term synaptic strength of the network.

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[2] Liou, J. Y., Smith, E. H., Bateman, L. M., Bruce, S. L., McKhann, G. M., Goodman, R. R., ... & Abbott, L. F. (2020). A model for focal seizure onset, propagation, evolution, and progression. Elife, 9, e50927.

Disclosures: J. Schmalz: None. **G. Kumar:** None. **M.V. Kothare:** None.

Digital Abstract Session

P391. Electrical Stimulation: Approaches and Effects On Neuronal Function and Circuit Organization

Program #/Poster #: P391.10

Topic: I.08. Methods to Modulate Neural Activity

Title: Modulation of motor sequence learning through targeted transcranial direct current stimulation

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Abstract: This is an exploratory study to observe whether high-definition transcranial direct current stimulation (HD-tDCS) with elevated intensity of 4 mA can 1. be applied without severe discomfort and 2. produce reliable behavioral effects on motor sequence learning during a finger tapping task (FTT). Functional magnetic resonance imaging (fMRI) of healthy participants (N = 10) performing sequential finger movements, combined with a transcranial electrical stimulation (tES) current flow model, was used to determine a generalized 4x4 focal HD-tDCS electrode montage to target the hand knob on the primary motor cortex (M1). In a single-blinded design, 50 healthy right-handed adult participants received either anodal (N = 25) or cathodal (N = 25) HD-tDCS while they performed a sequential FTT using their non-dominant hand for 12 minutes. After a 1-hour break, a follow-up session measured retention of task performance and included two control tasks with unfamiliar sequences on both the contralateral and ipsilateral hands. Discomfort at various time points during the procedure was measured using a visual analog scale (VAS). In both groups the mean VAS values did not exceed moderate levels of discomfort. Two-sample t-tests of the mean tapping speeds, calculated using the time intervals between key presses in all correctly completed sequences, indicate a significant difference in performance between the anodal and cathodal groups in the trained sequence, but not in the control sequences. These results suggest that high intensity HD-tDCS targeted to M1 has a specific effect on motor sequence learning. Furthermore, 4mA HD-tDCS is well tolerated.

Disclosures: G. Hsu: None. **A. Shereen:** None. **L. Parra:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lucas Parra is inventor on patents owned by the City University of New York related to HD-tDCS..

Digital Abstract Session

P391. Electrical Stimulation: Approaches and Effects On Neuronal Function and Circuit Organization

Program #/Poster #: P391.11

Topic: I.08. Methods to Modulate Neural Activity

Title: Assessment of Baseline Offsets and Charge Injection Limits of Neural Stimulation Electrodes using Voltage Transient Measurements: The Importance of Measurement Instrumentation

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Abstract: The development of safe and efficacious neural stimulation protocols critically depends on the accurate assessment of charge injection limits that do not result in toxic byproducts of stimulation or evolution of oxygen/hydrogen at neural stimulation electrodes. Since the introduction of voltage transient or voltage excursion methods by Roblee et al. in 1990, this method has become a standard used by pre-clinical researchers for understanding the charge injection limits of different materials or electrode geometries. It has also become an important

part of safety testing for clinical devices and common requirement by FDA reviewers in assessing device safety. Although voltage transient measurements have been commonly applied across neural engineering, assessment of charge injection limits in the presence of baseline voltage offsets at the stimulation electrode has been inconsistent across the literature. Additionally, voltage transient measurements have provided results for safe charge injection limits that are inconsistent with charge injection capacities measured using slow scan cyclic voltammetry as well as with in vitro and in vivo testing. These inconsistencies highlight the need for developing well validated methods for accurate assessment of charge injection limits across studies.
In this work, we assessed the effects of stimulation and measurement instrumentation on baseline offsets and charge injection limits calculated from voltage transients at polished 1mm diameter platinum disk electrodes. We compared baseline offsets collected with a conventional pre-clinical stimulator with two methods for minimizing DC leakage current and charge imbalance between stimulation phases. The first being capacitively coupling of the stimulator output with 1μF capacitors, and the second with shorting of the stimulator outputs between current pulses. Additionally, we compare baseline offsets and charge injection capacities observed with 1MΩ and 10MΩ input impedance measurement devices, that matches oscilloscopes conventionally used for recording voltage transients, with a high impedance >10GΩ input impedance measurement setup. Our data suggests that commonly cited charge injection limits may in fact be underestimating the charge injection limits for oxygen and hydrogen evolution at neural stimulation electrodes and underreporting baseline offsets occurring at platinum electrodes. These results highlight the need for consistent and accurate assessment of voltage transients across studies as well as a need for better understanding how benchtop measurements of charge injection limits translate to *in vivo* safety and efficacy.

Disclosures: J.K. Trevathan: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff. **S. Averbeck:** None. **K. Ludwig:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Neuronoff, LivaNova. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff, NueroOne. F. Consulting Fees (e.g., advisory boards); Galvani Bioelectronics/GlaxoSmithKline, Neuronoff, NeuroOne, Cala Health, Boston Scientific, Battelle, BlackFynn, Abbott.

Digital Abstract Session

P392. History of Neuroscience

Program #/Poster #: P392.01

Topic: J.01. History of Neuroscience

Title: Franz Nissl - more than just a stain

Authors: *B. W. BAKKUM;
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Abstract: Franz Alexander Nissl (1860-1919) was arguably the foremost neuropathologist of his time. He was also a respected psychiatrist who worked with other greats, including Aloysius Alzheimer (he was best man at Alzheimer's wedding), Korbinian Brodmann, and Emil Kraepelin. Besides those accomplishments, Dr. Nissl popularized the use of lumbar puncture, which had been introduced in 1891 by Heinrich Irenaeus Quincke. He actually became known as "Punctator Maximus". Dr. Nissl is best remembered for introducing a staining procedure that he devised while he was still in medical school in 1884. This method allowed the characterization of neurons and revealed several of their previously unknown components. For this he was honored with the eponym: Nissl stain. His original process was fixing the tissue with alcohol and staining it with methylene blue. Later cresyl violet has been commonly substituted for methylene blue. One of the main components of neurons that were made visible with this process was what he originally called: Chromatinkörper (colored bodies), but later seemed to use the term: Körperchen (little bodies). These came to be known as Nissl substance (or bodies). Later these large clumps of material in the soma and proximal regions of the dendrites, but not the axon nor axon hillock, were identified as mostly rough endoplasmic reticulum and polyribosomes. By the time that Nissl devised his staining procedure, Augustus Waller had already described the degeneration of the distal segment of a severed peripheral nerve, which became known as wallerian degeneration. Using his new staining technique, Nissl observed that there were also morphological changes that occurred in the cell bodies of the facial nucleus and spinal motor neurons. He called these changes: Methode der primäre Reizung (method of primary irritation). Therefore, he was the first to describe the process that is now known as the axon reaction. This included the "Zerfall" (disintegration) of the colored bodies or what is now termed chromatolysis, as coined by George Marinesco in 1896. This should be contrasted by an earlier use of the term: chromatolysis by Walter Fleming in 1885, who used it to describe a type of cell death that is now understood to be apoptosis. Nissl also described many of the other changes in the soma that are associated with the axon reaction including swelling of the perikaryon and movement of the nucleus to the periphery of the cell body. During World War I, Dr. Nissl was assigned administrative duties at a large military hospital. Overwhelmed by these along with his academic responsibilities and severe kidney disease meant that he died in 1919 leaving many of his scientific projects unfinished.

Disclosures:

Digital Abstract Session

P392. History of Neuroscience

Program #/Poster #: P392.02

Topic: J.01. History of Neuroscience

Title: The struggles and successes of T. S. Kanaka (1932-2018): lessons of life from Asia's first female neurological surgeon-scientist

Authors: *A. OZAIR[1], **V. BHAT**[2], A. FARUQI[1], A. BAJAJ[1], A. A. SONKAR[1];
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Abstract: Female surgeon-scientists have been a rarity worldwide, and significantly so in neurosurgery-neuroscience. This is especially true of South Asia, considering the added social and cultural expectations for women in the region. Yet it was in India, with its difficult history of gender relations in both the personal and the professional domains, that Asia's first fully qualified female neurosurgeon and the first female surgeon-scientist, Dr. T. S. Kanaka (1932-2018), took roots and flourished. As part of the Madras Institute of Neurology, she played an integral role in one of the earliest teams worldwide that pioneered stereotactic and functional neurosurgery.
While a few biographical accounts of her exist, we utilize many hitherto unused sources to highlight the several lessons that may be learnt from her illustrious career for aspiring neurosurgeons, neurosurgical trainees, and even fresh neurosurgeons of today. Many of the virtues that ensured her extraordinary victories are attributes that continue to be critical for obtaining success as a surgeon-scientist even today. Specifically, we illustrate, through previously unused sources, (A) how persistence and resilience were integral to her success, (B) how her mentors opened many doors for her, (C) how her personal sacrifices took her from 'good' to 'great', (D) how her pathbreaking research and innovation cemented her status, (F) how her selfless work to promote science-based treatment magnified her impact, and finally, (E) how a mindset of lifelong teaching and learning became a lasting legacy. Adoption of some of these lessons by female scientists and trainees will be especially valuable considering that in 2017, India only had 73 female neurosurgeons.
We also shed light on the circumstances that helped Kanaka succeed, as a lesson in promoting the same when working to enhance diversity and inclusion today. Finally, we describe her interests, sacrifices, and life choices, which are hitherto little explored but highly relevant considerations for women who wish to pursue a career as a surgeon-scientist.

Disclosures: A. Ozair: None. **V. Bhat:** None. **A. Faruqi:** None. **A. Bajaj:** None. **A.A. Sonkar:** None.

Digital Abstract Session

P392. History of Neuroscience

Program #/Poster #: P392.03

Topic: J.01. History of Neuroscience

Title: The Peter J. Morgane Research Collection on the Cetacean Brain

Authors: *D. J. MOKLER;
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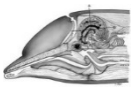
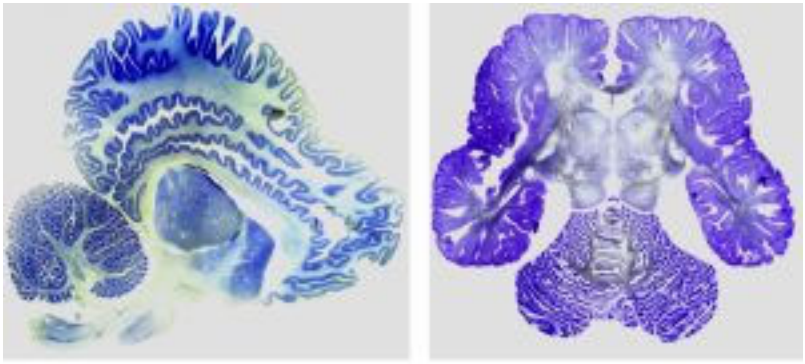
Abstract: Peter J. Morgane (1927 - 2010) has contributed significantly to the neuroanatomy of the cetacean brain. He published in the field of neuroscience from 1957 until the present

(colleagues are still publishing work that he contributed to). He published in the area of the neuroanatomy of cetacean brain from 1962 until 2004. His published work in neuroscience is found in over 200 papers and in the neuroanatomy of the cetacean brain is covered in 33 papers. The University of New England has established a special collection of his work. The material is on loan from Dr. Mokler. The material consists of photographs and slides of histological specimens used in publications. In addition, there are notes and correspondence from his research over 50 years. Researchers can access the collection by contacting David Mokler at dmokler@une.edu. More information on the collection can be accessed at https://www.library.une.edu/special-collections.In addition, the histological collection of cetacean brains are maintained in the lab of Patrick Hof at the Icahn School of Medicine at Mount Sinai.





Megaptera novaeangliae *Tursiops truncatus*

Disclosures:

Digital Abstract Session

P392. History of Neuroscience

Program #/Poster #: P392.04

Topic: J.01. History of Neuroscience

Title: Consideration of Balint's syndrome as the second disconnection syndrome and application of this as a paradigm to study the "what" and "where" visual pathways

Authors: *E. L. ALTSCHULER[1], B. M. ALTSCHULER[2];
[1]Physical Med. and Rehabil., Metropolitan Hosp., New York, NY; [2]Col., Havard, Cambridge, MA

Abstract: In1965 Geschwind called alexia without agraphia (AwoA), originally described by Dejerine in 1892, the first disconnection syndrome: left visual cortex lesion combined with a

lesion of the splenium of the corpus callosum disconnects intact vision in the right cortical hemisphere from the language centers in the left brain rendering the patient unable to read. Writing is intact as the left-brain language centers—Wernicke, Broca's areas—are intact as are the white matter tracts connecting them. I have noted that studying patients with AwoA may give an unprecedented opportunity to assess the capacity of the right brain for reasoning and logic: Recently we described a method, using question stimuli presented either as pictures or words, e.g., does a bone go with a dog, cat (or do not know the answer), to assess whether or not Wernicke's aphasia patients understand that they do not understand language (Hartman et al., & EL Altschuler, 2017). This method can be used with the picture stimuli—processed by the brain—as the test stimuli and the same questions asked verbally which should be able to be answered normally by the left brain as (positive) control stimuli. We here suggest that Bálint's syndrome (BS) should be recognized as the second disconnection syndrome and study of patients with the syndrome a most useful approach to elucidate the functions of the "what" vs. "where" visual pathways. In 1909 Hungarian neurologist Rezső Bálint described a patient with (1) a spatial disorder of attention—the inability to perceive several things in a visual scene at one time despite normal visual acuity—simultanagnosia (Wolpert, 1924), (2) "psychic paralysis of gaze" (ocular motor apraxia), (3) optic ataxia. Brzis et al. & Lee (1988) found that patients with BS and others with simultanagnosia could not name the (large) number on Ishihara color (vision testing) plates despite normal color vision and in particular normal naming of the colors of the individual (small) dots making up the number. This suggests that BS patients may have a frank disconnection of the "what" (ventral stream) and "where" (dorsal stream) pathways. Bálint's patient had bilateral parietal lesions from a series of strokes, and other early patients of Holmes and others were war veterans with brain injuries damaging the parietal lobes bilaterally. But we (Williams, Patira & Altschuler, 2016) and others have seen BS in patients with a single large (unilateral) stroke affecting the left poster cerebral artery (PCA). Thus, BS may be more common than typically appreciated and simple, non-invasive testing and assessment of patients with BS may provide significant insight into the functions of the "what" and "where" visual pathways.

Disclosures: E.L. Altschuler: None. **B.M. Altschuler:** None.

Digital Abstract Session

P392. History of Neuroscience

Program #/Poster #: P392.05

Topic: J.01. History of Neuroscience

Support: JSPS KAKENHI 18K03182

Title: William James's theory of emotion as a pioneer of affective neuroscience: Use of an introspection method in early psychology and knowledge of neurological findings prior to the early 1880s to develop a testable hypothesis assuming a body-mind interaction

Authors: *T. SATO;
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Abstract: William James published “What is an Emotion?” in 1884, in which he wrote the famous statement, “We feel sorry because we cry.” A major contribution of the paper to affective neuroscience was the making of hypotheses verified by neurological methods to explain the brain mechanisms of emotion, based both on previous neurological findings and on the introspection method (careful observation of his own subjective experience, a major research method in early psychology). This presentation focuses on the context in which James proposed his theory of emotion, rather than on the accuracy of the brain mechanisms he proposed. His hypothesis was called the “peripheral theory of emotion,” likely because it required the perception of a bodily state or change to indicate the occurrence of an emotion. Several physiologists have strongly criticized James’s hypothesis. Two such critics, Walter Cannon and Phillip Bard, proposed another theory as an alternative viewpoint of the brain mechanism of emotion. The so-called “Cannon-Bard theory” or “central theory of emotion” points to the role of the diencephalon. Putting aside consideration about validity of their criticism and their original theory, most differences between the two theories are not fully explained by a dichotomized examination of the peripheral and central origins of emotions. This is, in part, because James considered the emotional process to be a neural process in the brain, suggesting that the most important difference between the theories is the brain mechanism in which emotion occurs. James’s research method was limited to introspection, and he characterized emotions as being associated with bodily manifestations, such as fear and anger, as the “standard emotions.” Based on recent findings of affective neuroscience, in an earlier stage of standard emotions, bodily responses may occur based on neural activity in the amygdala stimulated by a direct route from a sensory organ; the neural pathway, called the “low road,” was defined by LeDoux. We then perceive these responses vividly, and these perceptions become central to our consciousness if the responses are sufficiently strong. At a later stage of feeling, we may subjectively experience emotions that probably depend on some cortical route or a “high road” of emotional processing in the brain. Thus, through careful observation of our subjective experiences, we can experience the subjective perception of bodily changes, followed by subjective emotional feelings, which truly correspond to James’s introspection results. His hypothesis appears to have been developed by both his careful introspection and his extensive knowledge of previous neurological findings.

Disclosures: T. Sato: None.

Digital Abstract Session

P392. History of Neuroscience

Program #/Poster #: P392.06

Topic: J.01. History of Neuroscience

Title: Olfactory bulbectomy animal model in depression through history

Authors: *C. GARCIA-OLVERA[1], I. CESAR ARTEAGA[2], D. JUÁREZ SERRANO[3], P. AGUILAR-ALONSO[4];
[1]Benemérita Univ. Autónoma De Puebla, Puebla, Mexico; [2]Lab. de Investigaciones Quimico Clinicas, Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; [3]Biochemistry-Molecular Biol., BENEMERITA UNIVERSIDAD AUTONOMA DE PUEBLA, Tlaxcala, Mexico; [4]BUAP, Puebla, Mexico

Abstract: At the beginning of the past century it was observed the first relationship between removal of olfactory bulbs in rats and changes in its behavior thanks to the research of Psychologist John B. Watson, who described that bulbectomized rats were showed aggressive and irritable than usual. Although, it was not until 70's decade when was confirmed by specific tests that bilateral ablation of olfactory bulbs in rats generate behavioral modifications as irritability. On the other hand, research for new pharmacological treatment on mood disorders, especially for depression after monoamine theory was hypothesized, allow the development of depressive animal models. However, there were many disadvantages among animal models, the major disadvantage was that the animal's recovery took place with an acute administration of the novel used antidepressant, while in clinical patients it was observed a longer time, even several weeks of treatment to recover. The introduction of olfactory bulbectomy in rodents as a new animal model of depression was proposed by van Riezen and colleagues in the middle 70's and later it was applied by Cairncross and colleagues with the aim to identify drugs with a potential antidepressant long-term activity. Bilateral olfactory bulbectomy (OBX) rodent model satisfy the criteria to consider it as an animal model of depression. It includes (1) to develop a depressive-like behavior similar to symptoms observed in patients with depression, (2) the behavioral changes can be measured and (3) reversed by antidepressant treatment. It had been considered diverse procedures for OBX, but nowadays there are little differences between them. The principal alterations observed in bulbectomized rats are variations of circadian rhythmicity, abnormalities in immune cells, high levels of ACTH and corticosterone, decreased serotonin and noradrenalin levels in brain, hyperactivity, modification of social behavior and cognitive function. Despite the fact that this animal model has been used in the last decades there are still discrepancies in results obtained, which must be analyzed carefully. Furthermore, due to complexity of surgery and time delay for recovery, OBX model is not the first option for *in vivo* studies. However, this model remains suitable for depressive-like behavior research.

Disclosures: C. Garcia-Olvera: None. **I. Cesar Arteaga:** None. **D. Juárez Serrano:** None. **P. Aguilar-Alonso:** None.

Digital Abstract Session

P392. History of Neuroscience

Program #/Poster #: P392.07

Topic: J.01. History of Neuroscience

Title: A brief history of EEG electrode localization

Authors: *K. M. JENSEN, J. K. KROGER;
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Abstract: In 1949, Herbert Jasper presented a system before the General Assembly of the International Federation in Paris, which would standardize the placement of electrodes on the scalp in electroencephalography (EEG). The purpose for this system was to make it easier for results to be reported, interpreted, and compared between labs. It was also hoped that by standardizing electrode placement, researchers could better estimate how activity recorded at the scalp correlates with underlying brain areas. These positions, based on proportional distance from landmarks on the skull, would be known as the 10-20 electrode system of the International Federation, and they would become a standard in EEG methods still used today (Jasper, 1958). Traditionally, this was done with the use of a tape measure. However, in the 1990's, new methods arose which utilized elastic caps and electromagnetic position trackers for marking electrode locations and saving them as 3D coordinates in a computer. This greatly reduced the time it took to prepare for data collection and allowed for the practical use of additional electrode sites beyond the 19 electrode locations used in the 10-20 system (Gevins et al., 1990). While manufacturers of elastic caps typically provide template 3D arrays for electrode positions when the cap is placed based on fixed points such as the Nasion, Inion, and preauricular points, researchers have achieved greater accuracy in source localization with methods which record the electrodes' actual position in 3D space and subsequently co-register them with a template MRI of the head (Akalin Acar & Makeig, 2013). For this reason, electromagnetic digitizers have been a popular method of electrode localization over the last few decades (Clausner et al., 2017). In recent years, many new methods have arisen which offer even greater opportunities for reduced experimental set up time, lower costs of equipment, and increased accuracy while recording electrode locations in EEG studies (Shirazi & Huang, 2019). One notable method is a photogrammetric approach which involves creating 3D head models of participants with the use of a 3D camera attached to a tablet. Electrode locations can later be marked in software, such as EEGLAB, with the *get_chanlocs* plug-in (Lee & Makeig, 2019). New methods have promising implications, using less expensive equipment and requiring less time from EEG study participants than methods involving electromagnetic digitizers, making electrode localization a more practical step in EEG research than ever before.

Disclosures: K.M. Jensen: None. **J.K. Kroger:** None.

Digital Abstract Session

P392. History of Neuroscience

Program #/Poster #: P392.08

Topic: J.01. History of Neuroscience

Title: Neuroscience in Peru, origins in ancient

Authors: *L. E. BAQUEDANO SANTANA;
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Abstract: Is Neuroscience in Peru a science that has just started? Peru is a country with numerous cultures of the prehispanic time. Many of them did science without knowing it. The curiosity of man about his origins is seen in all cultures who elaborate interpretations, mythical or rational about the origin of man and that can be answered through three different ways: with myths, speculatively or with methods typical of science. In ancient Peru, cranial trepanation was a technique with empirical origins but with a scientific evolution over time that occurred throughout ancient Peru. It developed in Europe and the South Pacific in the 7th century B.C. and it was developed in Peru in the 14th to 16th century A.D. It had generational significance that passed from the Paracas to Incas culture and consisted of a surgical practice in which an artificial hole was made in the cranial vault of a living person. There was a knowledge of anatomy, diagnosis and systematic therapeutic strategy that allowed a high survival and growing development of the conditions in the context of those times. Studying these ancient practices allows us to understand and educate the scientific community, in addition to recovering our identity and value in science from remote origins.

Disclosures: L.E. Baquedano Santana: None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.01

Topic: J.02. Teaching of Neuroscience

Support: UBC UILO Start-up award

Title: A Month in Neurodegenerative Disease Research (AMiNDR): An Open-Access Podcast Series for Researchers

Authors: *E. T. KOCH[1], S. LOUADI[2], E. M. ROWE[3], N. KUHLMANN[4], A. KAMESH[5]; [1]Psychiatry, [2]Med., [3]Pathology and Lab. Med., Univ. of British Columbia, Vancouver, BC, Canada; [4]Sch. of Physical and Occup. Therapy, [5]McGill Univ., Montreal, QC, Canada

Abstract: Background: With the ever-growing list of responsibilities for researchers, the vital task of staying up to date with the constant flow of new publications often gets pushed to the back burner. Given the rate at which new research is published — an average of 250 papers each week in the field of Alzheimer disease (AD) research — this can seem like an insurmountable task, and can result in wasted time and resources when researchers are not fully informed. In fact, in a survey of neuroscience researchers across the world, we confirmed that graduate students struggle to keep on top of the literature, and that a tool to facilitate this is urgently required.
Objective: To bridge this gap between the mound of new literature and the researchers who need to know about it, we recently developed and launched a new tool: A Month in Neurodegenerative Disease Research (AMiNDR). This is a student-run podcast that categorizes and summarizes new publications in AD research each month, allowing researchers to get an

overview of the literature while minimizing screen time.
Methods: Each month, we search PubMed for new publications using the key word “Alzheimer”, restricting the search to peer-reviewed original research articles. After downloading the resulting list of citations and abstracts, we filter out papers from predatory journals, reviews, or those which do not fit our inclusion criteria. We then manually sort the remaining abstracts into four overarching themes (risk factors, disease mechanisms, diagnostic tools, treatment development), containing sub themes for each podcast episode (ex. Fluid biomarkers). Our team then summarizes the abstracts to extract the key information, and scripts it into a listener-friendly format. The scripts are subsequently recorded by a podcast host, and new episodes are edited and released bi-weekly.
Results: AMiNDR is live as of June 2020, with 25-30 episodes covering a total of 500-600 papers each month. Each episode is also accompanied by a numbered bibliography to enable researchers to find papers that are covered in the podcast. Since our launch, we have acquired a global listenership, with an average of 30 downloads per episode, and over 1500 total downloads to date.
Conclusions: Based on feedback received so far, we believe our podcast is useful and accessible to researchers, enabling them to stay on top of the literature. We continue to refine and improve our methods with each month’s iteration, and hope to expand our listener base in the coming months.

Disclosures: **E.T. Koch:** None. **S. Louadi:** None. **E.M. Rowe:** None. **N. Kuhlmann:** None. **A. Kamesh:** None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.02

Topic: J.02. Teaching of Neuroscience

Title: Neuro at home: adapting experiential learning from the laboratory to the virtual learning environment with DIY brain mounting kits

Authors: *J. A. HONEYCUTT, A. FORCHE;
Bowdoin Col., Brunswick, ME

Abstract: The rapid switch from in-person to remote teaching at the onset of the pandemic significantly disrupted adherence to conventional educational pedagogies, particularly with respect to experiential learning in undergraduate higher education. Laboratory-based courses have taken a disproportionate hit with the pivot to online learning, with instructors struggling to adapt hands-on lab skills and experiments to an online platform in ways that are both cost effective and safe for home use. In an effort to ensure continuity in experiential learning outcomes across various instructional modalities, we have implemented a series of curated at-home lab experiences to enhance online instruction of a Behavioral Neuroscience Lab course. One such lab-based experience involved mailing DIY kits with pre-sliced rat brain tissue to

students' homes, paired with freely available online instructional tools (Zoom and Slack) to provide a guided experience with real-time feedback for skill building. Students utilized the supplies in their lab kits, in addition to the open-source Swanson rat brain atlas, to meet three primary learning goals: 1) identify and arrange free-floating rat brain sections in neuroanatomical order; 2) hone fine motor skills necessary for mounting delicate biological samples onto slides for microscopy; and 3) perform a modified on-slide structural stain to visualize major brain regions. Lab courses such as this are essential for training the next generation of neuroscientists on core methodological skills, thereby providing them the experiential learning necessary to compete and succeed at the graduate and industry levels post-graduation. Indeed, having hands-on experience with brain slices is the only way to learn this skill effectively, and provides crucial practice in the identification of key brain regions. Here, we provide the pedagogical framework for planning and implementing a successful brain tissue mounting skill-building lab remotely in your own neuroscience laboratory courses as we navigate online laboratory learning in our new normal.

Disclosures: J.A. Honeycutt: None. **A. Forche:** None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.03

Topic: J.02. Teaching of Neuroscience

Support: Cornell Center for Teaching Innovation
Hobart-William Smith Colleges

Title: Building your own neuroscience equipment; a precision micromanipulator and an epi-fluorescence microscope for calcium imaging

Authors: J. RYAN[1], ***B. R. JOHNSON**[2], D. DEITCHER[2];
[1]Biol., Hobart-William Smith Colleges, Geneva, NY; [2]Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstract: A faculty member's ability to develop meaningful research-oriented laboratories in neuroscience is often hampered by the rapid pace of new technologies and the increasing cost of equipment. To help undergraduate neuroscience faculty meet these challenges, we introduce two Do-It-Yourself (DIY) devices we designed and built as teaching/research tools. The first is a micromanipulator for fine probe placement. Our DIY manipulator costs about $40 in parts. It can be used to record intracellular potentials from muscles and neurons with similar stability and precision found in much more expensive commercial micromanipulators. Our second DIY device is a newly designed epi-fluorescence microscope with 3D printed components. Commercial fluorescence imaging devices often cost over $20,000. Our imaging microscope can be constructed with off the shelf commerical and 3D printed parts for about $1200. The imaging microscope uses interchangeable LED light sources and filter sets to image static fluorescence in

prepared slides and calcium imaging of neuronal activity in living Drosophila brains. This later technique uses transgenic flies with a genetically encoded calcium indicator, GCaMP, linked to green fluorescent protein. During an action potential, calcium ions enter neurons and are observed *in vivo* as an increase in fluorescence intensity from a series of video images. These neuronal firing patterns can be assessed quantitatively to understand neural circuits leading to specific behaviors. We are developing written and video guides for the construction and use of the manipulator, microscope, and associated lab modules to guide student inquiry-driven research. We plan an interdisciplinary classroom where students learn a cutting-edge neuroscience technique, engineering design and construction, including 3D printing, soldering, fine scale assembly, troubleshooting, optics and how to customize software code for image capture and data analysis. We believe this new toolbox will facilitate neuroscience teaching, learning and research for students and faculty, especially at national and foreign learning/research institutions with limited financial resources.

Disclosures: J. Ryan: None. **B.R. Johnson:** None. **D. Deitcher:** None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.04

Topic: J.02. Teaching of Neuroscience

Support: Mathworks Microgrant

Title: A user-friendly, smartphone-deployable, interactive musculoskeletal model for experiential learning

Authors: M. BADADHE, **J. MANCZUROWSKY**, K. SHUMOCK, H. CHEERMAN, *C. J. HASSON;
Northeastern Univ., Boston, MA

Abstract: Background: Musculoskeletal models, traditionally used to simulate human movement, represent dynamic relationships between bone, muscle, and tendinous tissue. Interactive musculoskeletal models (IMMs) can be controlled with electromyography (EMG) and are used in research to test sensorimotor control hypotheses. Their value in education, however, has been limited; IMMs typically require specialized technical knowledge and software to use and modify. This project's purpose was to develop an IMM for utilization: 1) in education across many disciplines, from physical therapists to engineers; 2) outside the laboratory with widely available, inexpensive technology. **Methods:** An upper-limb IMM was programmed in Simulink (Mathworks, Natick MA), a MATLAB-based graphical programming language. The rationale was that Simulink's block diagrams may be easier for naive users to understand compared to traditional scripting programming languages. The IMM can be controlled by on-screen muscular excitation buttons or by EMG measured with low-cost Arduino-powered myosensors; it can be run by student users in the Simulink environment (on a desktop/projected

screen) or deployed as a smartphone application. These flexible modes of operation allow students to experience sensorimotor control concepts critical to understanding human movement. In a classroom, for example, each student can control their smartphone-based IMM and perform goal-directed tasks individually or as a group. The students can explore musculoskeletal properties, such as changing muscle strength and stiffness to simulate and experience the effects of musculoskeletal disorders. Neurological deficits, such as spasticity, can also be programmed for educational purposes. An advantage of a smartphone application is that it affords more opportunities to interact with the model outside the classroom. This heightened flexibility-of-use could also be valuable for research, as motor learning studies are typically limited in the duration of practice (however, the trade-off is reduced experimental control). **Conclusion:** IMMs can help students further their understanding of how the nervous system organizes and adapts to changes in musculoskeletal dynamics to influence sensorimotor learning. A user-friendly IMM promotes high quality educational experiences that are important for students to transfer knowledge into research and professional practice. A smartphone-deployable IMM supports learning outside the classroom and increases accessibility for a wide range of students in rehabilitation, neuroscience, and engineering.

Disclosures: **M. Badadhe:** A. Employment/Salary (full or part-time):; Mathworks. **J. Manczurowsky:** None. **K. Shumock:** None. **H. Cheerman:** None. **C.J. Hasson:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Mathworks.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.05

Topic: J.02. Teaching of Neuroscience

Title: Using the Allen Brain Institute Database for Final Semester Projects in an Undergraduate Neurophysiology Lab Course During the COVID-19 Pandemic

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Abstract: The interruption of a neurophysiology lab class in the Spring 2020 by the COVID-19 pandemic created a challenge for hands-on and inquiry driven exploration of neuronal excitability. Before leaving campus, students understood and had proficiency with intercellular and extracellular recording techniques to explore neuronal excitability. This would normally be followed by student-designed experiments to examine underlying mechanisms of neuronal activity as a final semester project. Without student access to electrophysiological rigs, we redesigned the final project to focus on data analysis, interpretation and presentation skills. To acquire data, we used the online Allen Brain Institute (ABI) Cell Type Database. This open database contains whole cell patch clamp recordings to characterize electrophysiological

properties of brain neurons from mice and humans. We suggested choices for neuronal firing comparisons including: mouse neurons with different dendrite types, from different cortical areas or using different transmitters, and comparisons of human neurons with different pathologies such as epilepsy and tumors. Students also designed their own comparison set. The Allen software development kit (allenSDK) provides a free and simple gateway to analyze data using simple python programming. Students first measured action potential parameters (peak amplitude, trough, and width) and passive membrane properties (time constant, input resistance and rheobase) from single neurons with an interactive cursor (python function "mplcursors.cursor( )"). They compared these results with parameters calculated automatically by the ABI provided code and by the allenSDK "EphysSweepFeatureExtractor" module. Finally, students compared firing and membrane properties of different neuronal populations through statistical tests and by visualizing the distribution of data with Python code adapted from Ashley Juavinett of the University of California, San Diego. This final project introduced students to powerful online open data resources and python programming. They were able to consolidate and apply the electrophysiology concepts learned in the class. Students were challenged to explain the ionic mechanisms underlying excitability differences, and how this contributed to a neuron's function. They presented their work in a Journal of Neuroscience article format. This version of a final semester project allowed students to ask real-world medical and scientific questions through a virtual taste of a "start to end" research project.

Disclosures: Y. Ho: None. **A. Roeser:** None. **G. Law:** None. **B.R. Johnson:** None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.06

Topic: J.02. Teaching of Neuroscience

Support: Independent. First author. For common good.

Title: Teaching the strange and unsolved mathematical issues of electromagnetism and quantum fields in the brain with mechanics, toy models, history⋯and a humble philosophical perspective

Authors: *J. F. GOMEZ-MOLINA[1], U. M. RICOY[2], A. L. GOMEZ-MOLINA[3];
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Abstract: INTRODUCTION. In order to explore the brain, magnetic resonance and optical techniques have been successfully tested many times but we still do not have a consensus of what a field, a wave function, an electric charge, a magnetic spin or a (virtual) photon is. The interpretations of quantum phenomena are multiple and R. Penrose has said that quantum mechanics is not only incomplete but inconsistent. The truth is that anyone can check that the surprising spatial features of the magnetic, gravitational and electric fields (e.g. "action at a

distance") are an unsolved issue for logic or mathematical reasoning based on the idea of locality or "action by direct contact" (proximity). A humble and honest attitude to recognize these problems with the students is important. -METHODS. Mathematical-toy models. Mechanical models. -RESULTS. 1. Maxwell's equations (e.g. Gauss's law) can be explained with tubes and tanks. 2. The (quantum) harmonic oscillator can be explained with pendulums. 3. For motivated students, advanced concepts of geometry of rotations (e.g. SO(.), SO(.,.) groups), spin-spin relativistic interactions and spinors can be taught using nested torsion pendulums. -CONCLUSIONS. 1. Simple mechanical systems or our own bodies (during locomotion, turning and ball exchange) can illustrate the surprising effects of the electromagnetic fields and photon exchange by analogy with the gravitational field and the momentum of a ball. 2. Using tanks, tubes, pendulums, elastic cords, batteries and magnets we can construct (harmonic) oscillators and explain membrane biophysics, ephaptic phenomena, EEG waves and MRI-relaxation times. 3. Exotic concepts of probability, fuzzy logic, and almost-instantaneous-ephaptic-resonance can be also explored intuitively with students. 4. This problem can not be solved without teaching philosophical and historical perspectives. -REFERENCES. 1. Gomez JF 2018. Experiments with pendulums to teach and think about neuroscience: interactions between resonant states and subthreshold rhythmogenic signals. April 14, Citizen science day. Stadium, Metro station, Medellin, Colombia. 2. Gomez-Molina JF 2020. Electromagnetic recording and stimulation: biophysical equations of cell and mitochondrial membranes depend on activation state. Public discussion of submitted abstract (participation). NIH Rehabilitation research Oct. 15-16. Contact: Director Theresa Hayes Cruz PhD NICHD. 3. Gomez. SfN-S-Abstract. 2020. A new neuromechanic semantic of turning using fuzzy spinners for quantum-like electric-charge relocation in the intact brain: a non-classic, electromagnetically sensitive and delicate probabilistic process.

Disclosures: J.F. Gomez-Molina: None. **U.M. Ricoy:** None. **A.L. Gomez-Molina:** None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.08

Topic: J.02. Teaching of Neuroscience

Support: The Miami Project to Cure Paralysis

Title: Pivoting to a Remote Summer Undergraduate Program during the COVID-19 Pandemic: Neurotrauma Research at The Miami Project to Cure Paralysis

Authors: M. I. CHAGOYEN, ***N. DATTA**, D. C. CILIEN, K. L. GANT;
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Abstract: The sudden unexpected onslaught of COVID-19 in March threatened a much-anticipated tradition among undergraduate students; that of Summer internships, long considered to be a touchstone for those wanting to pursue higher education. The Miami Project to Cure

Paralysis, having hosted a Summer Internship Program for Undergraduates since 2013, determined to make the best of circumstances and successfully shifted to an online platform to provide students from across the US an introduction to neurotrauma research through The Remote Summer Undergraduate Program. The online format made it possible to offer 4 times the number of participants the opportunity to engage with our researchers and faculty from the comfort of their own homes. The event aimed to familiarize students to neurotrauma research through lectures and professional development workshops. This 8-week program consisted of a well-rounded curriculum of thrice-weekly zoom sessions introducing our 48 participants to concepts ranging from neuroinflammation and neuropathic pain in Traumatic Brain Injury to complications of Spinal Cord Injury. In addition, the program incorporated career development discussions emphasizing good scientific communication, CV writing, entrepreneurship in research, and cultivating skills required to succeed both academically and professionally. Further, The Journal Club encouraged students to exchange ideas and learn from each other. An online format has allowed us to reach a wider audience. Students from as far as Washington were able to participate in this event without incurring any cost. The Miami Project has endeavored to enhance participation of students from different backgrounds and ethnic groups by reaching out to over 150 departments of diversity and inclusivity across the US to promote this event. The number of female applicants has grown by 20 % from 2013 to 2020. At its conclusion, students shared their thoughts and experiences, expounding on their own experiences with injuries related to the brain and spinal cord and how they were able to connect with neurotrauma research and were inspired towards their futures. Highlighting one of the responses, “Looking back at these amazing 8 weeks, I realize that a key part of this program is it was designed for us, the students. It portrayed different vital aspects of the job as a researcher, bad and good, and the extraordinarily captivating and essential work that the researchers do in the lab. Overall, I now know I want to keep studying "brain stuff" and maybe follow the steps of the fantastic scientists I was introduced to.” Presently, these video lectures will be made available online for students to peruse at their convenience.

Disclosures: **M.I. Chagoyen:** None. **N. Datta:** None. **D.C. Cilien:** None. **K.L. Gant:** None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.09

Topic:

Support: NIH 2R25NS080687-11

Title: A multidimensional and virtual summer research training program in the Neurosciences for undergraduate students from underrepresented backgrounds at a Hispanic Serving Institution.

Authors: *C. S. MALDONADO-VLAAR[1], J. E. GARCIA-ARRARAS[1], E. CALDERÓN-CRUZ[2], J. RAMIREZ-LEITON[2];

[1]Biol., Univ. Puerto Rico, San Juan, PR; [2]Cooperativa de servicios de evalaución e investigación, San Juan, PR

Abstract: On March 11, 2020, the World Health Organization (WHO) declared a pandemic for the COVID-19 virus. In response to the public health crisis, Puerto Rico established strict lockdown measures. Since March 2020, the University of Puerto Rico-Rio Piedras Campus (UPRRP) moved all academic and research endeavors to online platforms. This institutional action significantly impacted the academic and research training programs sponsored by the National Institute of Health (NIH), among them the ENDURE Program -NeuroID. The main goal of the program is to increase diversity in the neurosciences by establishing a cohort of interested undergraduate students. Before the pandemic, an on-site summer intense 9-weeks research experience at UPRRP was offered. Resolute to move forward with the 2020 summer research experience, the program directors developed a multidimensional virtual program using digital platforms and objective-driven transformative activities. The summer training was adapted to the virtual stage and divided into seven initiatives: (a) a comprehensive integration to the research experience (b) peer-lead basic Neuroscience lectures (c) scholarly meetings with UPR neuroscientists (d) BP-ENDURE summer virtual seminar series (e) Neuroscience and society interactive lectures (f) workshop on Software Carpentry (g) workshops on the development of a professional skill set in the Neurosciences and (h) discussion forum on issues of racism, inequity, and discrimination in the sciences. Ten NeuroID fellows from primarily undergraduate institutions participated. Evaluation data collected examined the students' overall satisfaction with the virtual summer program. Findings from the external evaluation revealed a highly significant level of satisfaction from the participants with the use of the online platform to conduct the proposed activities. Most initiatives had a positive impact in engaging the students in learning about the neurosciences, strengthening their computational skills, improving their communication skills in science, designing and conducting a research project during the summer, developing a mentor-mentee relationship, improving networking abilities within the research setting, improving their scientific identity, understanding the importance of neuroscience research and how it impacts society and becoming advocates of diversity and inclusion. Several recommendations were offered to improve the quality of the virtual experience. Overall, the data collected strongly supports the effectiveness of the present multidimensional and virtual summer research training program in the neurosciences.

Disclosures: C.S. Maldonado-Vlaar: None. **J.E. Garcia-Arraras:** None. **E. Calderón-Cruz:** None. **J. Ramirez-Leiton:** None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.10

Topic: J.02. Teaching of Neuroscience

Support: NSF 1832338
NSF 1832345.

Title: Partnerships for Diversifying the STEM Workforce - The NSF HSI National STEM Resource Hub

Authors: *E. E. SERRANO;
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Abstract: Over 500 American colleges and universities currently meet federal criteria that define a Hispanic Serving Institution (HSI) as an accredited, degree-granting, public or private nonprofit institution with 25% or more total undergraduate Hispanic full-time equivalent (FTE) students, a high enrollment of needy students, and low expenditures per undergraduate FTE student. In the next decade, another 300 institutions are expected to achieve this federal designation. As compared to all other ethnic groups, Hispanics report the lowest percentage of educational achievement from high school (67%) to advanced (5%) degrees (Ryan and Bauman; US Census Bureau Report P20-578; 2016). In 2018, HSIs educated about two-thirds of the 3.8 million Hispanic college students and enrolled more Black students (~480,000) than all HBCUs, more indigenous students (~25,000) than all Tribal Colleges, and over 40% (~470,000) of all Asian Americans. HSIs are disproportionately underfunded and receive on average 68 cents for every federal dollar received by all other institutions of higher education. To address this gap, and in response to the American Innovation and Competitiveness Act and the Consolidated Appropriations Act of 2017, the National Science Foundation established the HSI Program. This initiative aims to increase degree achievement by students pursuing STEM associate's or baccalaureate degrees at HSIs, to enhance the quality of HSI undergraduate STEM education, and to build capacity at HSIs that receive little to no NSF funding. The NSF HSI National STEM Resource Hub, led by faculty from NMSU, CSUN, and DACC, serves as the first and sole Hub for the NSF HSI Program. The Hub is building a national community of HSIs and their allies by providing free and open access to tools for grantsmanship, capacity building, and STEM pedagogy through certifications, webinars, workshops, teleconferences, minigrants, and curated online resources. Here we show how neuroscience faculty and staff can access the Hub’s resources and work with the HSI community to strengthen STEM education offerings and grant submissions, and to leverage cross institutional partnerships to support the success of students from diverse backgrounds.

Disclosures: E.E. Serrano: None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.11

Topic: J.02. Teaching of Neuroscience

Title: School readiness in the Covid-19 pandemic preparing for online neurobiology

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Abstract: Learning readiness refers to, how likely a person is to seek out knowledge and participate in behaviour change. Further anything that affects physical or psychological comfort : pain, fatigue, anxiety, or fear can affect a person's ability and motivation to learn. School readiness prepares students for success in school and later in life. This includes knowledge application and socio emotional skills. So, before a semester starts there is a flurry of activity to prepare such a scene but what happens when a world-wide event occurs that changes the scenario totally. In March with the shutdown of our institution we went for online classes rather than physical interactions to allow the students to continue working towards their degree and course in neurobiology. In preparation we conducted two surveys from our faculty and students to understand the basic competency levels in the learning management system and availability of devices and internet connectivity. The survey was answered by 25 faculty and 77 students (Post-graduate). Most of the students had a smart phone or laptop and used mobile data to access the LMS classes. Though most of faculty were satisfied with the LMS it was a steep learning curve and had issues with assessments. The students were satisfied but wished for more interactive sessions. Thus along with planning lessons it is essential to prepare and master technology and motivate students to accept change.

Disclosures: Z. Huma: None. **N. Baseer:** None. **B. Zubair:** None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.12

Topic: J.02. Teaching of Neuroscience

Title: Converting a large, face-to-face Neurobiology course to online, remote teaching: Replacing attendance with metacognition, course feedback, and job readiness

Authors: *A. L. HAWTHORNE;
Univ. of Central Florida.

Abstract: For the fall 2020 semester, I converted ZOO3744-0M01 Neurobiology from face-to-face teaching to online, remote instruction. The class had 275 students and met remotely for the semester via Zoom. This course only has biology as a prerequisite, so the course is diverse, containing freshmen and first semester transfer students all the way through to seniors. All of the exams had to move online, and I chose Honorlock for the exam platform. I also had to decide what to do with the 5% attendance points. Due to COVID-19, I wanted to provide flexibility to students and also help them prepare for their future. I flipped the classroom, providing pre-recorded Zoom lectures and held question-based live Zoom sessions during class time. Instead of attendance, I created 3 reflection assignments spread throughout the course. The first assignment

focused on effective study techniques. I shared tables adapted from Dunlosky et al. (2013) and information from the Summer 2020 UCF Faculty Conference. After reviewing the tables, students reported what study strategies they originally planned on using for that exam and the utility of those techniques (low/moderate/high). Referencing the tables, they had to state what they could change about their study habits to enhance their preparation for the exam and why they choose those techniques. They then had to design a study schedule for the next exam. The next assignment was a course climate survey to gain feedback on how the class was doing and if there was anything they needed. This assignment was due about the mid-point in the class to check in with students that might not be participating in the live Zoom sessions and to give students a chance to voice concerns or report things that might help them. The last assignment was to find an internship and write a cover letter for that application. I provided information on the National Association of Colleges and Employers (NACE) skills that employers are looking for. Students had to explain the reasons they selected this program and what NACE skill(s) they would gain from the program. I received quite a few emails from excited students that are going to apply for summer internships. Many of the students did not know about these opportunities before this assignment. In the presentation, I will discuss course outcomes and feedback from students via an end of semester extra credit survey. The reflection assignments enhanced the course by providing metacognition, suppling students with effective study skills and tools for their future, and giving students an opportunity to provide feedback.

Disclosures:

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.13

Topic: J.02. Teaching of Neuroscience

Title: Hybrid course delivery and team-based learning approaches: lessons learned from teaching the principles of behavioral neuroscience to undergraduate psychology students

Authors: *M. GIL[1], C. BOTELLO[2], I. PEREZ[3];
[1]Psychological Sci. and SOM Neurosci., [2]Psychological Sci., Univ. of Texas Rio Grande Valley, Brownsville, TX; [3]Psychological Sci., The Univ. of Texas Rio Grande Valley, Brownsville, TX

Abstract: A growing trend in higher education is the integration of team-based learning with hybrid approaches to course content delivery, in an effort to improve mastery of student learning objectives. The goal of this presentation is to provide information about the potential and challenges of using team-based approaches, with a research focus, in an upper-level, undergraduate course, using both standard face-to-face (F2F) and hybrid course delivery methods. We tested the hypothesis that a combined hybrid and team-based approach improves student performance and engagement compared to a standard F2F (lecture) format. Although the hybrid approach appeared to change the final letter grade distribution, with fewer students receiving an F, there was no significant difference in the grade distribution between groups

(χ^2(4,N=155)= 3.227, n.s.). On the other hand, students in the hybrid course group earned significantly higher scores on their final research projects compared to students in the F2F group (Z= -2.171, P<0.05). Finally, we observed a strong relationship between high student engagement (i.e., number of in-person sessions attended) and final course grades (F= 10.458, P< 0.01). We will also share our experiences teaching behavioral neuroscience concepts to undergraduate students. At the completion of this presentation, the attendee is expected to learn: 1. How to design a hybrid course that involves a significant amount of team-based work, 2. How students generally feel about hybrid courses and team-based work, and 3. About integrating behavioral neuroscience research into an undergraduate course.

Disclosures: M. Gil: None. **C. Botello:** None. **I. Perez:** None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.14

Topic: J.02. Teaching of Neuroscience

Title: A crash course in computational neuroscience for undergraduate students

Authors: E. NOISIN, E. ZAKI, D. C. SUGATAPALA, *C. GUNAY;
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Abstract: Computational neuroscience is an advanced topic that is usually chosen to be taught and studied at post-graduate level in most textbooks. This choice is justified because understanding the topic requires knowledge of advanced concepts in multiple disciplines, such as biology, physics, mathematics, and computer science. However, it is desirable for students to be involved in research earlier, specifically at the undergraduate level. There are many opportunities for undergraduate research at teaching-focused colleges. To enable undergraduate-level research in computational neuroscience, we aim to develop open educational resources (OER) for an introductory course, providing the necessary background in fundamental topics with easy-to-understand diagrams. These materials are still under development, but we intend to publish them as freely available and open source. In this digital poster presentation, we will present our course materials that aim to explain the fundamentals of computational neuroscience to enable simulating computer models of neurons that include realistic morphologies, active Hodgkin-Huxley type ion channels, and synaptic components. We will review concepts, including Ohm's Law, synaptic transmission, membrane conductance, and action potentials. We will explain each concept separately, then show how they are interlinked and work together. The training course was originally intended to train undergraduate students specifically for working on a project simulating neuronal models of the *Drosophila* motor networks in the central nervous system.

Disclosures: E. Noisin: None. **E. Zaki:** None. **D.C. Sugatapala:** None. **C. Gunay:** None.

Digital Abstract Session

P393. Innovative Tools For Teaching and Disseminating Neuroscience

Program #/Poster #: P393.15

Topic: J.03. Public Awareness of Neuroscience

Title: Dissemination of Neurotechnology through The BRAIN Initiative[R]

Authors: *S. L. WHITE[1], K. B. DUPRE[1], K. ASHMONT[1], M. C. GRABB[2], K. M. RAMOS[1], M. OLENICK[1], N. HSU[1], N. T. LICHTENBERG[1], P. PRAKASH[1], S. BELL[1], A. ADAMS[1], J. A. GORDON[2], W. J. KOROSHETZ[1], J. NGAI[1];
[1]NIH NINDS, Bethesda, MD; [2]NIH NIMH, Bethesda, MD

Abstract: Launched in 2013, *The Brain Research Through Advancing Innovative Neurotechnologies*[R] (**BRAIN**) Initiative is revealing how the brain works, by developing and applying novel tools to precisely map and observe brain circuits in action. To date, the NIH BRAIN Initiative has awarded over 900 grants to hundreds of investigators, totaling nearly $1.8B in investment and resulting in over 1550 publications. These investments, in collaboration with other federal and non-federal groups - via the U.S. BRAIN Initiative Alliance (BIA), have accelerated the pace of tool, technological, and theoretical development in neuroscience and encouraged cross-disciplinary collaborations that enrich the community's scientific expertise and technological capabilities. A rapidly expanding array of cutting-edge resources is now becoming available to the entire research community. A core principle of the BRAIN Initiative, re-affirmed by teams of external neuroscience and neuroethics experts, is to develop and democratize novel technologies, tools, methods, theories, and other resources to advance the understanding of healthy and diseased brain states. The NIH supports research resource grants in cross-lab technology integration and works with the BIA to help distribute these innovative resources to the broader neuroscience community. Dissemination methods, encompassing communication platforms and funding mechanisms, include: 1. NIH BRAIN website (www.braininitiative.nih.gov), which includes Program pages, a Resources page, and list of Publications; 2. BIA website (www.braininitiative.org), which includes an updated Resources section with details about products developed by BRAIN toolmakers and a new BIA Toolmakers Newsletter; 3. assorted funding opportunities supporting the distribution, training, access, and adaptation of BRAIN technology for the larger scientific community; 4. NIH BRAIN Blog (https://brainupdate.nih.gov/), which has over 800 subscribers; 5. social media, including Facebook (@usBRAINInitiative) with over 6300 followers and Twitter (@USBrainAlliance) with over 2900 followers; 6. scientific outreach and educational activities to share discoveries with high school students; 7. keynotes, symposia, scientific panels, workshops, the annual BIA SfN satellite event (https://www.braininitiative.org/events/sfn-social/), and the annual BRAIN Initiative Investigators meeting (https://www.braininitiative.org/events/pimeeting/). This poster describes the current, numerous methods of BRAIN tool and technology dissemination and highlights a select number of BRAIN scientific resources.

Disclosures: **S.L. White:** None. **K.B. Dupre:** None. **K. Ashmont:** None. **M.C. Grabb:** None. **K.M. Ramos:** None. **M. Olenick:** None. **N. Hsu:** None. **N.T. Lichtenberg:** None. **P. Prakash:** None. **S. Bell:** None. **A. Adams:** None. **J.A. Gordon:** None. **W.J. Koroshetz:** None. **J. Ngai:** None.

Digital Abstract Session

P394. Outreach Activities

Program #/Poster #: P394.01

Topic: J.02. Teaching of Neuroscience

Support: Society for Neuroscience
Dana Foundation
American Psychological Association
International Brain Research Organization
Federation of European Neuroscience Societies
University of Maryland, Baltimore

Title: The International Brain Bee

Authors: *N. R. MYSLINSKI;
Univ. of Maryland Dent. Sch..

Abstract: The Brain Bee is alive and well. It is the preeminent neuroscience competition for teenage students. Worldwide there are about 175 chapter competitions, each one involving many schools, in 50 countries. Each year more than 20,000 teenagers compete in their chapter competitions. The Chapter winners then compete in their respective National Championships to earn the right to compete in the World Championship. In 2019, future neuroscientists from 28 countries met in Daegu, South Korea, to compete in the 21th World Championship. The event was hosted by the International Brain Research Organization. The winner was Yidou Weng from China. Second Place went to Natralia Koc of Poland, and Third Place went to Kamand Souflabadi of Iran. The Organizing Chairs were Julianne McCall and Yoo-hun Suh. The partners were the Korea Brain Research Institute, Korea Brain Education Society, and Leadership Initiatives: Youth. In 2020, the World Championship was planned for Washington, DC hosted by the American Psychological Association but it was canceled because of COVID19. However, a number of 2020 National Championships were held. The 2021 World Championship was initially planned for San Diego but it will now be in virtual format hosted by American Psychological Association during the second week of August, 2021. National Champions from both 2020 and 2021 will be invited to compete. The 2022 World Championship is planned for Paris, France, hosted by the Federation of European Neuroscience Societies. The Brain Bee is a Non-Profit Foundation with a Board of Directors from the American Psychological Association, Society for Neuroscience, Dana Alliance for Brain Initiatives, International Brain Research Organization, and Federation of European Neurosciences Societies. According to the founder, its purpose is to motivate young men and women to learn about the human brain, and to apply that

knowledge to their daily lives; and to inspire them to enter careers in the basic and clinical brain sciences to help treat and find cures for brain disorders. More than 100 newspapers, radio, television stations and web sites cover the Brain Bee. Presidents, Ambassadors and other public officials have recognized the IBB. Many former competitors are now working in neuroscience, neurology, psychology and related fields. The Brain Bee motto is: Building Better Brains to Fight Brain Disorders.

Disclosures:

Digital Abstract Session

P394. Outreach Activities

Program #/Poster #: P394.02

Topic: J.03. Public Awareness of Neuroscience

Support: DRGI, UV

Title: Virtual Brain Awareness Week: A pandemic challenge

Authors: *R. C. ZEPEDA[1], C. J. JUÁREZ-PORTILLA[2], J. CUETO-ESCOBEDO[3], G. GUILLÉN-RUIZ[4], A. CORTES-SOL[5], T. MEZA-MENCHACA[6], I. MARTÍNEZ-SERRANO[5], T. MOLINA-JIMÉNEZ[7];
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Abstract: This year has been particularly difficult in all aspects. The entire world shared, for the first time in a century, the same problem, and this was not different in science. The world stopped just before the brain awareness week appointment. Therefore, our group decided to postpone all the activities and events we had planned for BAW 2020, and canceled some days later. However, while the weeks of general closure advanced, we felt the necessity to keep sharing with the community some aspects of our neuroscience work. Therefore, the Biomedical Research Center, and Institutes of Neuroethology and Health Sciences organized the 1st Virtual Brain Awareness Week 2020 at the Universidad Veracruzana; in which five neuroscience researchers were considered. The first challenge we faced was the election of the virtual platform to reach the largest number of viewers. Following a deep analysis of the options, we decided transmit the lectures on Facebook live page @semanadelcerebroxalapa, thought Zoom. The lectures were about “Psychiatric diseases in Covid’s time”, “Epilepsy”, “Maternal stress and intra uterine growth restriction”, “New immediate effect antidepressive drugs” and “The enigmatic brain of dolphins”. Some international speakers were included as well as others from our own university. Even though Facebook event rise about 1,565 people; we just had 502 live

viewers, which show a rich interaction with speakers through the chat, where people could write their questions, and the speaker kindly answer them. The Virtual Brain Awareness week 2020 was a very positive experience that motives us to include it in future events to reach more people outside our city. This represents new knowledge for our group, trying to be adapted to our new normality. We are encouraged to keep working in pro to society.

Disclosures: R.C. Zepeda: None. **C.J. Juárez-Portilla:** None. **J. Cueto-Escobedo:** None. **G. Guillén-Ruiz:** None. **A. Cortes-Sol:** None. **T. Meza-Menchaca:** None. **T. Molina-Jiménez:** None. **I. Martínez-Serrano:** None.

Digital Abstract Session

P394. Outreach Activities

Program #/Poster #: P394.03

Topic: J.03. Public Awareness of Neuroscience

Support: Yale Pathways to Science Funding
Donations from Yale medical school departments

Title: Brain Education Day at Yale

Authors: *K. ZHANG, A. GUMASTE, H. ORTEGA;
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Abstract: Every year during the Dana Foundation's Brain Awareness Week, graduate students from the Interdepartmental Neuroscience Program at Yale organize Brain Education Day (BED). Over the past ten years, the in-person event brings 80-100 local 6th-12th grade students to Yale's medical campus for a full day of learning about neuroscience from faculty, graduate students, postbacs, and postdocs. Attendees hear from a talk from a faculty member about their science and their pathway to get there, and then rotate through four stations to learn about sensory physiology, how we image human brains, brain-machine interfaces and programming, and brain anatomy through a sheep brain dissection. Last year, we moved the event online and students heard graduate student presentations about their journey to science and their current research. This year, we are planning a series of virtual events where students will engage in hands-on sensory physiology activities using kits we send home to the students, hear graduate student presentations about their journeys to science, and participate in virtual real-time "experiments" to learn about human cognition including topics such as memory and executive function.

Disclosures: K. Zhang: None. **A. Gumaste:** None. **H. Ortega:** None.

Digital Abstract Session

P394. Outreach Activities

Program #/Poster #: P394.04

Topic: J.03. Public Awareness of Neuroscience

Support: NIH P60 AA011605

Title: Science keeps going: Outreach activity adaptations amid difficult times.

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Abstract: 2020 has been a challenging year. Almost all activities have been impacted by COVID-19, and the consequences of the pandemic are still unknown. Science holds the promise of possible solutions, offering the potential to overcome the pandemic through the development of vaccines and treatments for COVID-19. However, science is complex, and most non-scientists require support to understand it better. In that sense, outreach activities are more relevant now than ever, and part of the responsibility that scientists have with the public is to make science accessible for everyone. As a group of scientists involved with brain science outreach in the community, we have started to adapt all brain activities to a virtual format, not only to reach our local community and schools, but also beyond. We recruited volunteers for a "transition committee" from our outreach listserv and met via Zoom and on Slack to organize tasks. Here, we detail our steps to achieve this transition.1.Redesigned our website to support online materials and resources. Our first step was to build a website that would serve as a portal for all our online activities. 2."Meet the Scientist" videos. To replace some of our community interactions in which we talked about our research, the life of a scientist, or our career paths, we recorded short video segments on these topics. 3.Electronic versions of hands-on activities. While detailed instructions for our outreach activities were already online, not all were adapted for classroom or home use. As the original versions were adapted, they were replaced on the website. 4.Classroom and afterschool visits. Real-time interactions with schools moved to Zoom formats. We advertised our availability for classroom visits to schools and school districts. Classes could also view "meet the scientist" videos or conduct hands-on activities prior to scheduled interactive sessions.5.Neuroscience blog posts. We solicited blog posts of 300-500 words from our scientists.6.Online gross anatomy. While we previously guided students through brain dissections in person, we have now done it virtually via online resources (www.neuroanatomy.ca) and Zoom. 7.Increase accessibility. To increase accessibility of these materials to the community, we are translating materials into other languages and including closed-captioning videos. We are also recording/writing some materials in Spanish. While these efforts are still in progress, the shift from in-person to virtual outreach has this silver lining: we are building an education and engagement resource that will be more accessible and potentially reach more people than previously possible.

Disclosures: A. Gómez-A: None. **S. Faccidomo:** None. **C. Dannenhofer:** None. **L.C. Ornelas:** None. **J. Besheer:** None. **D.L. Robinson:** None.

Digital Abstract Session

P394. Outreach Activities

Program #/Poster #: P394.05

Topic: J.03. Public Awareness of Neuroscience

Support: University of Connecticut
Quinnipiac University

Title: The 33rd northeast undergraduate and graduate research organization for neuroscience (NEURON) conference held at Quinnipiac University's Center for Medicine, Nursing and Health Sciences in North Haven, CT

Authors: R. A. ROTOLO[1], G. R. TANNER[2], D. M. SMALL[3], V. FRANCONE[4], C. A. FRYE[6], A. C. BASU[7], J. G. TRAPANI[8], M. L. LINDEN[9], R. E. PRESBY[1], J.-H. YANG[3], D. C. LEE[2], A. BATTISON[2], J. L. HAIGHT[5], T. AHERN[5], *A. J. BETZ[5];
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Abstract: The 33rd NEURON conference was held on February 23rd, 2020, at Quinnipiac University's Center for Medicine, Nursing and Health Sciences. Quinnipiac University hosts the website for the NEURON conferences, which includes links to registration, abstract submission, archives of previous talks, and image galleries (www.quinnipiac.edu/neuron). The 2020 keynote speaker was Dr. Dana Small, Professor of Psychiatry and Professor of Psychology, Department of Psychiatry, Yale University School of Medicine. Her talk was titled Rethinking Food Reward: Integration of Mind and Metabolism. Dr. Small's laboratory, the Modern Diet and Physiology Research Lab, focuses on the mechanisms of taste, flavor and feeding in humans using lesion, neuroimaging, neuropsychology, metabolic, genetic, and psychophysics methodologies. Dr. Small's group has studied the interaction between sensory and metabolic signals in determining food reinforcement, demonstrating that post-ingestive signals regulate neural circuits in the dopaminergic meso-striato-prefrontal system that could influence reinforcement learning and food valuation. In addition, Dr. Small's lab has studied ingestive behavior, identifying a gut to brain pathway that can be targeted to rescue striatal circuits and functions impaired by alcohol abuse. At the conference, students and faculty participated in four sessions, including: Careers in Science Panel; Data/Technique Blitz; Detectives of Undiagnosed Disease: Utilizing the Undiagnosed Disease Network and Bioinformatic Tools; and Surgical Neurophysiology: An Exciting Frontier in Clinical Neuroscience. The Tieman and Frye awards were given to undergraduate and graduate students to honor the quality of their work and poster presentations. For the fifth year, NEURON partnered with Nu Rho Psi, the national neuroscience honor society, which offered a fourth student poster award. NEURON grew this past year, representing over 50 different institutions and 9 states. With continued local and regional support from faculty dedicated to student outreach and mentorship, and co-sponsorship from Quinnipiac University

and the University of Connecticut, NEURON has continued to expand beyond its original Boston locations to include greater representation from the northeast region and beyond.

Disclosures: **R.A. Rotolo:** None. **G.R. Tanner:** None. **D.M. Small:** None. **V. Francone:** None. **J.L. Haight:** None. **T. Ahern:** None. **A.J. Betz:** None. **R.E. Presby:** None. **C.A. Frye:** None. **M.L. Linden:** None. **A. Battison:** None. **J. Yang:** None. **A.C. Basu:** None. **J.G. Trapani:** None. **D.C. Lee:** None.

Digital Abstract Session

P394. Outreach Activities

Program #/Poster #: P394.06

Topic: J.03. Public Awareness of Neuroscience

Title: Update on a Community Open Source Bioprinter Project

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Abstract: Bioprinting is a process that can be used to create constructs composed of cell infused gels. The process is rooted in both inkjet and 3d printing. The potential for creating organs suitable for transplant early on generated excitement which was tempered as challenges became better understood. There has been renewed interest in the ability of bioprinters to make replicable environments for studying cells in 3 dimensions. This may have clinical applications in the neurobiology, as the printed cells can in concept come from patients. A community lab has made a printer and formulated a hydrogel that that can be used to print cubes, pyramids, cylinders, and organic shapes. We report here on preliminary results of tests of the viability of bacterial and invertebrate cells within the gel matrix.

Disclosures: **D. Foster:** None. **B. Teng:** None. **D. Wahlquist:** None. **R. Cruz:** None. **S. Loewner:** None.

Digital Abstract Session

P395. Ethics and Policy

Program #/Poster #: P395.01

Topic: J.04. Ethical and Policy Issues in Neuroscience

Title: Science communication: a multi-purpose tool for neuroscience advocates

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Abstract: Neuroscience advocates, educators, researchers, entrepreneurs, and professionals possess unique combinations of interdisciplinary expertise and experience. From exciting the next generation of scientists to engaging legislators on Capitol Hill, neuroscientists can use science communication as a multi-purpose tool to spread awareness of the societal impact of ongoing research and breakthroughs in neuroscience. Each year, the Society for Neuroscience invites advocates from across the country to communicate science to members of the House and Senate through meetings on Capitol Hill in Washington, DC. Effective science communication is grounded in a subset of skills, some of which include relationship building, storytelling, and understanding your audience. This presentation covers these skills and their applications in communicating science to members of Congress. These skills are transferrable across in-person and virtual meetings, as well as through other modes of communication. When sharing a message with policymakers, the ability to be succinct, focus the conversation on one or two issues, and appropriately limit the use of jargon and terminology can keep the message on track. When approaching policymakers with legislative "asks", keep in mind the legislators' priorities, the needs of their constituents, and the appropriations process. Continue the conversation by offering resources and opportunities to learn more, such as through a lab tour.

Disclosures: N.C. Catanzarite: None. **A. Bankston:** None. **Z. Guttman:** None.

Digital Abstract Session

P395. Ethics and Policy

Program #/Poster #: P395.02

Topic: J.04. Ethical and Policy Issues in Neuroscience

Support: R01MH114854
Supplement on R01MH114854
Brain & Behavior Research Foundation (NARSAD)

Title: Researchers' Views on perceived risks of device removal following brain implant research

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Abstract: Background: Clinical research with brain implant devices such as adaptive deep brain stimulation (aDBS) has led to ethical discussions about device removal, including who should cover the financial costs of removal upon study conclusion (Sierra-Mercado & Zuk et al., 2019). A related question about device removal is how to assess the risks involved, including physical risks such as hemorrhage and infection (Patel et al., 2015, Chen et al., 2017). Approach: Using in-depth, semi-structured interviews, we examined aDBS researcher (n=23) perspectives

regarding post-trial device removal. Results: Researchers largely believed that the physical risks of removing the neural components of the device outweigh the potential benefits of removal, citing the broad risks associated with neurosurgery. For participants who do not receive desired level of benefit, leaving the neural components of the device implanted but deactivated is typically recommended over removal. However, some researchers said that this strategy carries opportunity costs, such as potentially preventing participants from undergoing MRI, and that leaving a device implanted but deactivated may itself involve physical risks of device erosion, infection, or other unknown long-term risks. Other researchers pointed to the potential risk of continued device presence influencing psychological distress. Thus, here, a few researchers suggested that removal count as medically indicated rather than as elective. Finally, researchers highlighted financial risks posed by aDBS and similar devices associated with medically necessary or elective removal. Some research grants cover the cost of removal, even at times in the case of elective removal. But researchers said that this is often only during the grant period, after which researchers have fewer resources with which to facilitate device removal. Combined with uncertainty about whether insurance will pay for removal, financial issues were thus seen as a non-medical risk participants faced. Conclusion: There was broad agreement that removing neural components of the device carried risks associated with neurosurgery. However, researchers expressed various views on the acceptability of taking these risks. Researchers also appealed to several distinct categories of risk relevant to device removal: 1) physical, 2) financial, 3) opportunity costs, 4) psychological, and 5) risks of long-term dormant device. Overall, researchers expressed a commitment to honoring participants' informed preferences for device removal. Further analysis of risks associated with device removal will help to ensure that participants' preferences are fully informed.

Disclosures: D. Sierra-Mercado: None. **P. Zuk:** None. **K. Kostick:** None. **L. Torgerson:** None. **R. Hsu:** None. **J. Oliver Robinson:** None. **K.A. Muñoz:** None. **C. Sánchez:** None. **L. Kalwani:** None. **B. Koenig:** None. **S. Pereira:** None. **A. McGuire:** None. **G. Lázaro-Muñoz:** None. **S. Outram:** None.

Digital Abstract Session

P395. Ethics and Policy

Program #/Poster #: P395.03

Topic: J.04. Ethical and Policy Issues in Neuroscience

Title: Neuroethics a guide for bridging cross-sectoral neuroscience

Authors: A. U. MOSS, Z. LI, ***K. S. ROMMELFANGER**;
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Abstract: Neuroscience and its findings have deep personal and cultural meaning, so the implications of brain science raise new flavors of ethical issues not covered by traditional bioethics. The field of neuroethics bridges this gap, addressing and responding to the ethical,

legal, and social issues intimately related to the evolving landscape of neuroscience. Neuroethical concerns have registered at the highest levels of government. In 2018, an interdisciplinary global neuroethics group working with leading scientists from the International Brain Initiative published "Neuroethics Questions to Guide Ethical Research in the International Brain Initiatives". The document provides guiding questions to consider throughout the lifecycle of neuroscience research. These questions tackle issues such as identity, morality, cross-cultural differences, privacy, and potential stakeholder involvement in ethical decision-making. In our work with the International Brain Initiative, a consortium of 7 large-scale national-level brain research projects around the globe, we noted the important role that the private sector will play in translating and scaling neuroscience for society. We also noticed a gap in communication and collaboration between government, academia and the private sector. These guiding questions were largely co-created with policy makers and academics, so it was unclear how these issues might be received by neuro-entrepreneurs and neuro-industry. We hoped to identify not only common concerns, but also a common language for discussing neuroethical issues with stakeholders outside of government and academia. We used empirical ethics methods to assess the perceived value and attitudes of neuro-innovators toward neuroethical issues and whether or not these issues align with the process of neuro-innovation. We conducted one-on-one structured interviews with 21 neuro-innovators in the private sector and used two independent reviewers to analyze for themes. Themes were derived through an iterative process and mapped onto our theoretical framework. From this preliminary research, we identified key neuroethical themes and processural pain points of neurotech entrepreneurs throughout the innovation process. We will provide a preliminary neuroethics needs assessment for neuro-industry and suggest avenues through which neuroethicists can work with neurotech leadership to build an ethically aligned future. Overall, we hope to raise awareness and provide actionable steps toward advancing and accelerating societally impactful neuroscience.

Disclosures: **A.U. Moss:** None. **Z. Li:** None. **K.S. Rommelfanger:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; The Kavli Foundation; BrainMind.

Digital Abstract Session

P395. Ethics and Policy

Program #/Poster #: P395.04

Topic: J.04. Ethical and Policy Issues in Neuroscience

Title: Neuroethics: An essential partner to enhance the NIH BRAIN Initiative

Authors: K. M. RAMOS[1], J. D. CHURCHILL[2], S. HENDRIKS[1,3], *K. B. DUPRE[1], N. S. HSU[1], S. BELL[1], S. L. WHITE[1], A. ADAMS[1], J. A. GORDON[2], W. J. KOROSHETZ[1], J. J. NGAI[1];
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Abstract: The ***B**rain **R**esearch Through **A**dvancing **I**nnovative **N**eurotechnologies*® (**BRAIN**) Initiative aims to revolutionize neuroscience by accelerating the development and application of innovative neurotechnologies. This research and these novel tools have tremendous scientific promise, but also raise important ethical questions. Neuroethics – the study of the ethical, legal, and societal implications of neuroscience – can aid scientists in anticipating and addressing ethical questions raised by neuroscience research. The NIH BRAIN Initiative views neuroethics as a key partner for neuroscience research.

NIH uses a multi-faceted approach to achieve proactive, ongoing assessment and management of the neuroethical implications associated with the development and application of BRAIN-funded tools and neurotechnologies. Two primary goals are to identify and navigate ethical questions in BRAIN-funded research and to facilitate the collaboration of neuroethics and neuroscience. The NIH's strategy for accomplishing these goals includes managing an external Neuroethics Working Group that provides input on neuroethics issues; funding neuroethics research that is complementary to discoveries supported by the Initiative; organizing workshops on key neuroethics topics; and aiding coordination of global neuroethics efforts.

To identify ethical questions within the BRAIN portfolio, recent BRAIN-funded research was screened for potential ethical challenges. Broadly, these ethical challenges could be grouped into two categories: (1) research ethics-related questions and (2) the potential ethical and societal implications of new tools and neurotechnologies. This screening aided in identifying and prioritizing ethical questions relevant to the NIH BRAIN Initiative.

To increase collaboration between neuroethics and neuroscience, the NIH has facilitated partnerships between researchers and neuroethicists. Within the BRAIN Initiative, NIH supports both empirical neuroethics research and embedding neuroethicists into neuroscience research. Empirical neuroethics research, which involves collecting data relevant to ethical challenges in neuroscience research, can help determine how these challenges can be resolved. Embedded neuroethicists help researchers anticipate and mitigate ethical dilemmas in research or in the communication of results.

This poster highlights how neuroethics is embedded in BRAIN research, neuroethics research opportunities and funding in BRAIN, and summarizes the recent Neuroethics BRAIN Portfolio analysis. More details can be found at: https://www.braininitiative.nih.gov/brain-programs/neuroethics.

Disclosures: K.M. Ramos: None. **J.D. Churchill:** None. **S. Hendriks:** None. **K.B. Dupre:** None. **N.S. Hsu:** None. **S. Bell:** None. **S.L. White:** None. **A. Adams:** None. **J.A. Gordon:** None. **W.J. Koroshetz:** None. **J.J. Ngai:** None.

Digital Abstract Session

P395. Ethics and Policy

Program #/Poster #: P395.05

Topic: J.02. Teaching of Neuroscience

Title: Teaching neuroethics in a time of crisis

Authors: *G. E. HUE[1], A. E. FINK[2];
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Abstract: Teaching neuroethics provides value in times of crisis. The COVID-19 pandemic presents neuroscientists with responsibilities requiring consideration of individual and social values. In December 2019, COVID-19 caused illness and death in China before spreading across the world. The WHO urged countries to “take urgent and aggressive action” and many entered quarantine arrangements to reduce disease transmission. In the USA, public buildings shuttered; employers and educational institutions made dramatic shifts to remote work and learning. Reasonable accommodations and policies previously considered impossible or unfair arrangements for disabled people, were immediately and seemingly effortlessly implemented. This pandemic has starkly illuminated a capacity yet unwillingness to respond nimbly to accessibility demands, and the failure of eviscerated public health systems and the nation's patchwork for-profit, predatory health care structure. Long-standing economic and health inequities, layered onto structural racism, resulted in disproportionate illness and death in marginalized communities. This, accompanied by racially motivated murders and police brutality against Black Americans, sparked a revitalized movement for racial justice. This movement demands the restructuring of systems of living, working, and care, not only to repudiate white supremacy but also to promote the flourishing of historically oppressed populations. Neuroethics education at this time is important. First, the historical misuse of scientific authority has paved the way for disproportionate illness in marginalized communities. A neuroethics perspective recognizes the deeply social nature of scientific inquiry and encourages critical analysis. Students ask questions about power and participation in the sciences, who sets research priorities, and how distinct cultural priorities may allow different lenses on neuroscience and health. Students learn to examine multiple perspectives on the interpretation of scientific data, and question how neuroscientific knowledge and technologies may be used to help or harm. Such skills allow students to explore the chains of causality and responsibility leading to a public health crisis and the politicized and nuanced responses to said crises. They consider how a pandemic inevitably occurs via the strengths, weaknesses and inequities built into a society. An ethics view allows for novel conceptualizations of accountability and justice. We discuss how neuroethics relates to the multiple concurrent crises unfolding in the USA and how critical pedagogical approaches yield insight as a basis for transformative action.

Disclosures: G.E. Hue: None. **A.E. Fink:** None.

Digital Abstract Session

P395. Ethics and Policy

Program #/Poster #: P395.06

Topic: J.04. Ethical and Policy Issues in Neuroscience

Title: What women & minorities are afraid to speak up about

Authors: *D. M. TAYLOR[1,2,3], E. CASTAGNOLA[4];
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Abstract: Before the COVID-19 pandemic, we were invited to host the 'Power Hour™' at a scheduled Gordon Conference on Neuroelectronic Interfaces in March 2020. The Power Hour™ is a discussion/workshop with the goal of promoting women and underrepresented minorities (URMs) in our field. Since many people don't feel comfortable speaking up at these workshops, prior to the conference, we set up a website to collect anonymous anecdotes about the challenges women and URMs face as well as suggestions for improvement as a way to jumpstart the discussion (https://www.inequalitystoriesinstem.org/). The web link was shared with all the registered conference attendees by the organizers and forwarded to the greater Neural Engineering research community by an NIH program officer associated with the conference. Recipients were encouraged to forward the link to additional colleagues worldwide of any gender. To date, we have received ~140 anonymous anecdotes, and the results have been both *enlightening and heartbreaking!* Although the website did not specifically ask for anecdotes about sexual misconduct or sexual assaults in the workplace, we received an alarming number of anecdotes on this topic most ending in the statement, 'I never told anyone about this before'. Reasons given typically included, 'I didn't think I would be believed', 'speaking up would put my career in jeopardy', or 'the stress/embarrassment associated with speaking up was too much'. Another situation where problems were often undetected/unreported is when researchers do a good job of hiring a mix of genders and URMs in their lab but fail to recognize that some of their personnel may have strong gender/racial/religious prejudices. Too many stories described vicious hate speech, bullying and intentional career destruction by fellow lab members with the principal investigators oblivious to what was happening under their noses. Some of the most vulnerable to both sexual abuse and bullying/intentional harm are people with the most to lose by speaking up—specifically international scientists who could lose their visa status and get deported if fired, those financially dependent on their job often with a family to support and who don't have alternative support options, and people with no local support network or who are not fluent in the local language. These under-reported problems urgently need to be addressed. Unfortunately, due to the pandemic, the Gordon Conference is now rescheduled for 2022. Therefore, we are initiating these important discussions via other venues like this one. Please join our discussion at this meeting and/or submit suggestions via the website https://www.inequalitystoriesinstem.org/.

Disclosures: D.M. Taylor: None. **E. Castagnola:** None.

Digital Abstract Session

P395. Ethics and Policy

Program #/Poster #: P395.07

Topic: J.02. Teaching of Neuroscience

Title: Teaching awareness of prescribing privileges in Psychology undergraduate students

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Abstract: Neuropsychiatric disorders are often treated with psychopharmacological interventions that are mainly monitored and managed by psychiatrists and nurses. However, there is an untapped benefit to allowing limited prescribing privileges to trained psychologists. Currently, in the United States, psychologists with postdoctoral degrees in psychopharmacology have prescribing privileges in five states: Iowa, Idaho, Illinois, New Mexico, and Louisiana. Worldwide, this possibility is largely unknown and unexplored. For this reason, we set out to bring awareness of prescribing privileges in Psychology undergraduate students by having them critically evaluate the pros and cons of allowing psychologists with advanced training to prescribe limited medications. Our sample was comprised of 36 final-year undergraduate students who enrolled in a Psychology degree program at a European university. Students' perceptions were initially assessed with an ad-hoc questionnaire asking for their level of agreement on practices surrounding psychologists' involvement in pharmacotherapy. After generating a pros-and-cons list of prescribing psychologists, students rated their agreement with the statement that clinical psychologists with appropriate training may prescribe medications for mental health. Our results indicate that although the majority of students favored a high involvement of psychologists as collaborators in medication management, they were about evenly split with respect to the pursuit of prescriptive authority for psychologists. Further studies about the implications of these preliminary findings on the awareness of Psychology undergraduate students are warranted.

Disclosures:

Digital Abstract Session

P395. Ethics and Policy

Program #/Poster #: P395.08

Topic: J.02. Teaching of Neuroscience

Title: Isaac Newton was not implacably opposed to the scientific hypothesis, no matter what he said: lessons for today's neuroscientist.

Authors: *B. E. ALGER;
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Abstract: Although the scientific hypothesis is a bedrock principle of the scientific method, critics have recently created confusion by arguing that neither concept, especially the hypothesis, has a place in science today[1]. Perhaps partly for this reason, even neuroscience papers that test a hypothesis often do not mention it, which hampers communication. I found that, of 210 neuroscience papers (taken sequentially) in *J Neurosci*, *Nat Neurosci*, *Neuron*, and *Science*, 113 tested a scientific hypothesis (or "model," a synonym ") (excluding "statistical" hypothesis, an

entirely different concept). Only 47/113 (42%) explicitly stated their hypothesis[2,3] (in the rest the hypothesis was implied but not stated). Critics cite Isaac Newton's remark, "I frame no hypotheses," to support their anti-hypothesis arguments. But centuries ago, "hypothesis" referred to an empty, data-free intellectual construct that was posited like a mathematical axiom to serve as the basis for deductive reasoning about nature. This was what Newton rejected. He could not *explain* the force of gravity and refused to invent a meaningless fiction to do so. Years later Newton advanced, "An Hypothesis Explaining the Properties of Light Discoursed on in My Several Papers," where he hypothesized that light consisted of tiny particles. He said that this hypothesis was valuable in helping to understand phenomena as it potentially explained them and made his ideas "more intelligible." His change reflected a more nuanced interpretation of "hypothesis;" it was beneficial when it expressed a concrete, testable, explanation. Newton also stated many other hypotheses and predictions in his *Queries*. in which he placed a question mark after a statement of a hypothesis, presumably to show its provisional nature[4]. Modern critics of the hypothesis fail to acknowledge Newton's realization that having a hypothesis facilitates scientific thinking, practice, and communication. Today's neuroscientists should be encouraged to state the hypotheses in their own work for the same reasons. 1. Alger, BE (2019) *Defense of the Scientific Hypothesis: From Reproducibility Crisis to Big Data* (New York; Oxford University Press), Chp. 10. 2. ibid, p. 230. 3. Alger, BE (2020) Scientific-Hypothesis Testing Strengthens Neuroscience Research. *eNeuro* Jul 23;7(4):ENEURO.0357-19. 4. Wilczek, F (2015) *A Beautiful Question: Finding Nature's Deep Design* (New York; Penguin Press), pp. 280-6.

Disclosures: B.E. Alger: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oxford University Press.

Digital Abstract Session

P395. Ethics and Policy

Program #/Poster #: P395.09

Topic: J.03. Public Awareness of Neuroscience

Support: SFN
FENS

Title: How public acceptance and understanding of the use of animals in scientific research can be improved with a strategy of openness and transparency

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Abstract: In a number of European countries, public and private research institutions have adopted new practices and policies to engage with the public on the benefits of using animals in scientific and biomedical research. The European Animal Research Association (EARA),

supported by the Society for Neuroscience (SFN) and the Federation of European Neuroscience Societies (FENS), have been encouraging greater openness from institutions in the neuroscience community. Since 2018, EARA has assessed websites of EU institutions that carry out biomedical research using animals, and results show that a more consistent approach to openness is still required. Our 2020 study reveals, in many European countries, the research community is still reluctant to provide suitable information for the public on its research. Only just over half (59%) of EU institutions conducting animal research carry a statement on their websites to explain this, fewer than one third of websites (31%) carry any more detailed information. Under half (42%) of EU websites display imagery related to animal research, and a significant proportion of these were stock images, not necessarily reflective of the research carried out at these institutions. To improve transparency about animal research, for the past three years EARA, with SFN and FENS, has held events to encourage better communication. Over 1000 researchers and communications staff in eight countries attended expert panel discussions to understand the need for transparency, and media training aimed to equip them with the skills to engage with the public. There are now transparency agreements in Spain, Portugal, Belgium and the UK, where institutions have made a commitment to a strategy of openness on animal research. These commitments are that institutions will be proactive in seeking opportunities to explain when, how and why they use animals in research; will provide information to the media and the public about animal research; will explain the benefits obtained from using them compared to other models of research; will develop initiatives that generate greater public knowledge about the use of animals in research, will place a policy statement on their institution's website and will report annually to the Agreement on their progress on the commitments. **This workshop will outline learnings from these SFN/FENS events, demonstrating the valuable nature of these workshops in initiating and continuing transparency around animal research, and develop best practice to help the wider community embrace openness. We will also highlight experience from the transparency agreements, and outline how institutions in any country could adopt the same approach.**

Disclosures: B. Jones: None. **A. Barros:** None. **P. Janssen:** None.